



FINAL REPORT

Test Facility Study No. 172891, Report No. 38255

Sponsor Reference No. 2016MET-FYF2662

**[¹⁴C]-Malathion: The Pharmacokinetics of [¹⁴C]-Malathion in the Rat
Following Single Oral and Intravenous Administration**

Test Guidelines:

OECD Guideline for Testing of Chemicals: Toxicokinetics, No. 417, July 2010.

United States Environmental Protection Agency, Health Effects Test Guidelines (August 1998) OPPTS 870.7485 (Metabolism and Pharmacokinetics).

Author:

Mhairi Libberton, BSc
Oleg Chepelin, PhD

Study Completion Date:

15 May 2017

TEST FACILITY:

Charles River Laboratories Edinburgh Ltd
Elphinstone Research Centre
Tranent, East Lothian
EH33 2NE, UK

SPONSOR:

Cheminova A/S
P.O. Box 9
DK-7620 Lemvig
Denmark

1 STATEMENT OF NO CLAIM OF CONFIDENTIALITY

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2 COMPLIANCE STATEMENT

[¹⁴C]-Malathion: The Pharmacokinetics of [¹⁴C]-Malathion in the Rat Following Single Oral and Intravenous Administration

I, the undersigned, hereby declare that this study was performed in accordance with the OECD Principles of Good Laboratory Practice as incorporated into the United Kingdom Statutory Instrument for GLP and as accepted by Regulatory Authorities throughout the European Union, United States of America (FDA and EPA) and Japan (MHLW, MAFF and METI), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

The study was conducted according to the procedures herein described and this report represents a true and accurate record of the results obtained.



Mhairi Libberton, BSc
Study Director

15 May 2017

Date

A copy of the relevant Charles River Laboratories GLP certificate can be found in Appendix 1.

Submitter: Date:

3 QUALITY ASSURANCE STATEMENT

[¹⁴C]-Malathion: The Pharmacokinetics of [¹⁴C]-Malathion in the Rat Following Single Oral and Intravenous Administration

The Charles River Quality Assurance Unit conducted a protocol review, protocol amendment review, study-based inspections and report audits on this study (172891), as detailed below.

<u>Date of QA Activity</u>	<u>Activity</u>	<u>Date of Report to Management and Study Director</u>	
21-Jul-2016	Final Protocol	25-Jul-2016	25-Jul-2016
25-Aug-2016	Dose Administration	25-Aug-2016	25-Aug-2016
25-Aug-2016	Dose Preparation Review	26-Aug-2016	26-Aug-2016
26-Aug-2016	LSC Sample Preparation	26-Aug-2016	26-Aug-2016
26-Aug-2016	LSC Logging on/off	26-Aug-2016	26-Aug-2016
15-Sep-2016	Protocol Amendment 1	15-Sep-2016	15-Sep-2016
20-Oct-2016	Protocol Amendment 2	20-Oct-2016	20-Oct-2016
20-Oct-2016	Protocol Amendment 3	20-Oct-2016	20-Oct-2016
15-Nov-2016	Chromatography - LC-MS (MS-MS)	16-Nov-2016	16-Nov-2016
13-Jan-2017	Protocol Amendment 4	13-Jan-2017	13-Jan-2017
10-Feb-2017 - 11-Feb-2017	Draft Report	17-Feb-2017	17-Feb-2017
13-Feb-2017 - 17-Feb-2017			
21-Feb-2017 - 24-Feb-2017	Data Review - Chemistry	24-Feb-2017	24-Feb-2017
11-May-2017	Final Report	11-May-2017	11-May-2017

Process-based inspections relevant to this study are scheduled once every quarter. The outcome of each inspection is reported to Management and, where relevant, the Study Director.

Facilities relevant to this study are included in Charles River's annual facility inspection programme. The outcome of each inspection is reported to Management.

This report is considered to describe accurately and completely the procedures used in the study and the results obtained.

Jane P Dunsire
Jane P Dunsire, CBiol MRSB
Quality Assurance

15 May 2017
Date

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4 RESPONSIBLE PERSONNEL

Study Director:

Mhairi Libberton, BSc

Principal Analyst:

Martha A Green, BSc PhD

5 SUMMARY

This study was designed to investigate the whole blood and plasma kinetics of total radioactivity following an oral administration of [^{14}C]-malathion at either 40, 800 or 1200 mg/kg or an intravenous administration at 4 mg/kg to male rats. The nature of the radioactivity present in plasma and red blood cells was also examined.

Following a single oral administration of [^{14}C]-malathion at 40 mg/kg to male rats, measured mean peak blood (15.7 $\mu\text{g equiv/g}$) and plasma (27.3 $\mu\text{g equiv/mL}$) concentrations of total radioactivity were both attained at 1 h, gradually declining until no longer reliably detectable at 30 h or 72 h post dose, respectively. The absolute bioavailability was estimated to be in excess of 105%, suggesting absorption was complete.

Following a single oral administration of [^{14}C]-malathion at 800 mg/kg to male rats, measured mean blood (75 $\mu\text{g equiv/g}$) and plasma (152 $\mu\text{g equiv/mL}$) concentrations of total radioactivity initially peaked at 0.5 h then appeared to plateau until 4 h. Thereafter concentrations declined, falling below the limit of detection at 96 h in blood but remaining detectable in plasma. In comparison to the 40 mg/kg group, systemic exposure (as determined by $\text{AUC}_{(0-\text{inf})}$ values) increased in a proportional manner in blood and a sub-proportional manner in plasma. The absolute bioavailability was estimated to be in excess of 98%, suggesting absorption was complete.

Following a single oral administration of [^{14}C]-malathion at 1200 mg/kg to male rats, measured mean blood (146 $\mu\text{g equiv/g}$) and plasma (249 $\mu\text{g equiv/mL}$) concentrations of total radioactivity initially peaked at 0.5 h then formed a plateau until 8 h. Thereafter, concentrations declined to the final sampling timepoint of 96 h. In comparison to the 40 mg/kg group, (as determined by $\text{AUC}_{(0-\text{inf})}$ values), systemic exposure increased in a broadly proportional manner in blood and plasma. In comparison to the 800 mg/kg group, systemic exposure increased in a proportional manner in blood and a sub-proportional manner in plasma. The absolute bioavailability was estimated to be in excess of 94%, suggesting absorption was complete.

Text Table 1: Mean Pharmacokinetic Parameters of Total Radioactivity in Whole Blood and Plasma Following a Single Oral Administration of [^{14}C]-Malathion

Dose level (mg/kg)	Matrix	T_{max} (h)*	$T_{1/2}$ (h)	$\text{AUC}_{(0-\text{inf})}$ ($\mu\text{g equiv.h/g}$ or mL)	Bioavailability (F) (%)
40	Whole blood	1	5.35	84.8	102
	Plasma	1	21.7	178	117
800	Whole blood	1.5	29.0	1850	121
	Plasma	3	30.8	2750	98
1200	Whole blood	0.5	37.7	2460	118
	Plasma	2.25	25.7	3610	94

*Median reported

Following a single intravenous administration of [^{14}C]-malathion at 4 mg/kg to male rats, the theoretical concentration of radioactivity in blood and plasma at time zero (C_0) was 12.5 $\mu\text{g equiv/g}$ and 23.7 $\mu\text{g equiv/mL}$, respectively. Concentrations then rapidly declined to 0.03 $\mu\text{g equiv/g}$ at 24 h in blood and 0.02 $\mu\text{g equiv/mL}$ at 48 h in plasma, then were below the limit of reliable measurement thereafter.

Text Table 2: Mean Pharmacokinetic Parameters of Total Radioactivity in Whole Blood and Plasma Following a Single Intravenous Administration of [^{14}C]-Malathion

Dose level (mg/kg)	Matrix	C_0 ($\mu\text{g equiv/g}$ or mL)	$\text{AUC}_{(0-\text{inf})}$ ($\mu\text{g equiv.h/g}$ or mL)	$T_{1/2}$ (h)
4	Whole blood	12.5	7.45	11.0
	Plasma	23.7	13.7	27.0

The metabolism of malathion in plasma and red blood cells following oral administration of 800 and 1200 mg/kg [^{14}C]-malathion to male rats was investigated. Where possible, radiolabelled components were quantified and structures postulated for significant metabolites.

Up to 16 radiolabelled components were detected in the plasma and red blood cells in rats.

Malathion dicarboxylic acid was the major component in both the 800 and 1200 mg/kg dose groups in plasma and red blood cells, measuring between 33.262-121.264 $\mu\text{g equiv/g}$. Two structural isomers for desmethyl malathion monocarboxylic acid were observed, with one (labelled isomer 2) present at much greater concentrations (3.403-21.065 $\mu\text{g equiv/g}$). Malathion monocarboxylic acid was also observed and measured at a greater concentration in the 800 mg/kg dose group (10.018-12.270 $\mu\text{g equiv/g}$) relative to the 1200 mg/kg dose group (3.878-5.436 $\mu\text{g equiv/g}$). A sulphur substituted malathion monocarboxylic acid (metabolite 1) was also identified present at concentrations (<1.092-2.230 $\mu\text{g equiv/g}$). All remaining components were unassigned and were individually measured at a concentration <3 $\mu\text{g equiv/g}$.

6 INTRODUCTION

Malathion is an insecticide. This study was designed to investigate the whole blood and plasma kinetics of total radioactivity following an oral administration of [^{14}C]-malathion at either 40, 800 or 1200 mg/kg or an intravenous administration at 4 mg/kg. The nature of the radioactivity present in plasma and red blood cells was also examined.

6.1 Study Location

The study was carried out at Charles River Laboratories Edinburgh Ltd, Tranent, East Lothian, EH33 2NE, UK according to Study Plan No 172891 and Amendments 1-4 (Appendix 2).

6.2 Study Dates

Study initiation date: 15 July 2016

Experimental start date: 29 July 2016

Experimental completion date: 20 January 2017

Study completion date: See Compliance Page for date of Study Director's signature.

6.3 Test Guidelines

The study was conducted according to the following Regulatory Guidelines and guidance documents.

OECD Guideline for Testing of Chemicals: Toxicokinetics, No. 417, July 2010.

United States Environmental Protection Agency, Health Effects Test Guidelines (August 1998) OPPTS 870.7485 (Metabolism and Pharmacokinetics).

Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market.

Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.

Japanese Ministry of Agriculture, Forestry and Fisheries, Test Data for Registration of Agricultural Chemicals, 12 Nohsan No 8147, Agricultural Production Bureau, November 24, 2000.

6.4 Justification for Test System Selection

The Sprague Dawley strain of rat was chosen as this is a species that has been used in the toxicological evaluation of the test item. The study was conducted using male rats only as there is no sex difference apparent from existing ADME and toxicity data available for malathion.

The oral route was used as this represents a possible route of exposure and has been used in the toxicological evaluation of the test item. The intravenous route was used as a reference route for pharmacokinetic evaluation.

The number of rats chosen for this study satisfies the regulatory requirement for this type of study.

6.5 Dose Level Selection

Two oral dose levels (40 mg/kg and 800 mg/kg) were selected for consistency with previous metabolism studies. A third oral dose level (1200 mg/kg) was selected for consistency with a planned dietary study with the test item. The intravenous dose level (4 mg/kg) was selected as a factor of 10 lower than the oral dose level as no tolerability information was available. Corn oil was selected as the oral dose vehicle for consistency with previous studies. Ethanol:10% aqueous Captisol (sulfobutyl ether- β -cyclodextrin) (5:95)) was selected as a suitable non-irritant intravenous dose vehicle following trial assessment.

6.6 Archiving Information

All raw data generated and recorded during this study will be stored in the Scientific Archive of Charles River for 2 years after issue of the final report. At the end of the two year period, the Sponsor will be contacted regarding the disposal, transfer or continued storage of raw data.

The original signed copy of the final report will be stored indefinitely in the Scientific Archive of Charles River Laboratories Edinburgh Ltd.

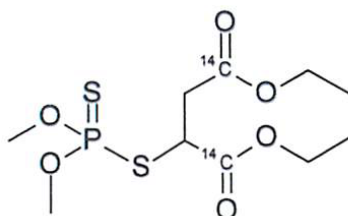
6.7 Retention of Biological Samples

Biological samples generated during the course of this study will be held deep frozen until issue of the final report. Charles River will contact the Sponsor to discuss the fate of the samples (disposal, return or retain at Charles River) on issue of the final report. Samples will be disposed of unless Charles River receives prior written instructions regarding shipment of samples to the Sponsor or continued storage at Charles River, at the Sponsor's request and expense.

7 MATERIALS AND METHODS

7.1 Test Item

Carbon 14 labelled malathion (Batch 9587CEO001-1), manufactured and supplied by Selcia, was stored in a freezer set to maintain a temperature of -20°C. The radiolabelled material was supplied as a solution in ethanol with a stated specific activity of 6.47 MBq/mg and a radioactive concentration of 37.3 MBq/mL. The Certificate of Analysis is presented in Appendix 3. The structure and site of labelling (^{14}C) of the radiolabelled material are shown below:



^{14}C label shared between each carbonyl

Non-radiolabelled malathion (Batch P2168-lbW-05) was supplied by the Sponsor as a liquid and was stored in a freezer set to maintain a temperature of -20°C. Non-radiolabelled malathion was used as a reference for chromatographic purposes and for the radiodilution of [^{14}C]-malathion in the dose formulations. The Certificate of Analysis is presented in Appendix 4.

The following reference standards were supplied by the Sponsor to aid metabolite identification. Copies of each certificate of analysis are presented in Appendix 5 to Appendix 25.

Reference Standard	Batch	Expiry Date	Storage
Diethyl Mercaptosuccinate	849-BSe-31B	27 Aug 2018	-20°C
O,O-Dimethyldithiophosphoric acid	291-BSe-62A	11 Apr 2018	Ambient
Desmethyl malaoxon dicarboxylic acid, trisodium salt	P1334-CSO-15-filtered	26 May 2017	-20°C
Tetra ethyl dithiosuccinate	195-ABB-79-1	16 Nov 2017	-80°C
Monoethyl fumarate	43-IA-167	16 Nov 2017	Ambient
Mercaptosuccinic acid	STBF9504V	Unknown	Ambient
Fumaric acid	BCBP4470V	Unknown	Ambient
Succinic acid	BCBR9019V	Unknown	Ambient
Maleic acid	SLBL3661V	Oct 2022 (retest date)	Ambient
Monoethyl maleate, potassium salt	91-FVL-122-2	05 Nov 2019	-20°C
Malathion Monocarboxylic Acid	1017-BSe-56B	31 Jan 2017	-20°C
Malathion Dicarboxylic Acid	621-BSe-81A	11 Feb 2026	-20°C
Desmethyl Malathion Monocarboxylic Acid, Potassium Salt	676-BSe-16A	02 Feb 2018	-20°C
Desmethyl Malathion Dicarboxylic Acid, Dicyclohexylammonium Salt	676-BSe-12A	21 Aug 2023	-20°C
S-(1,2-di(carbethoxy)ethyl)-O-methyl hydrogen phosphorodithioate Dicyclohexylammonium salt	924-BSe-15A	04 Apr 2019	-20°C
Malaoxon	676-BSe-89A	02 Feb 2021	-20°C
Isomalathion	924-BSe-56A	10 Mar 2018	-20°C
Diethyl Maleate	021281 IA	02 Apr 2023	-20°C
Diethyl Fumarate	Aldrich S13344-424	25 Mar 2018	-20°C
Diethylmethylthiosuccinate	D2014-BSe-MLT-O7B	05 July 2022	-20°C
O,O-dimethyl-thiophosphoric acid, dicyclohexylammonium salt	267-OSJ-54B	04 Feb 2021	-20°C

7.2 General Materials

Corn oil was obtained from Sigma Aldrich, UK.

Captisol (SBE- β -CD) was obtained from Cydex.

Sterile water for injection was obtained from Baxter.

Aquasafe 500 Plus[®] liquid scintillation fluid was obtained from Zinsser Analytic, Maidenhead, UK.

Carbo-Sorb® CO₂ absorbing solution and Permafluor® E⁺ scintillation fluid were used in conjunction with the PerkinElmer Model 307 Sample Oxidiser and were supplied by PerkinElmer Life Science and Analytical Instruments Inc, Sears Green, UK. Spec-Check™-[¹⁴C] was used to estimate efficiencies of combustion and was also obtained from PerkinElmer.

FlowLogic™-M scintillant was obtained from PerkinElmer Analytical Instruments, UK.

All other materials and chemicals used were of analytical grade where available.

7.3 Animals and Husbandry

Twenty male Sprague Dawley rats, age approximately 8-9 weeks at dosing (body weights 301-373 g) were supplied by Charles River (UK) Limited. The animals were acclimatised to the experimental unit for at least 5 days before use on the study and were carefully observed during this time to ensure that they were in good health and suitable for inclusion in the study.

During the pre-trial holding period, rats were multiply housed in solid floored polycarbonate and stainless steel caging with bedding material. During on-study periods, animals were multiply housed in polycarbonate and stainless steel cages with raised wire mesh floors.

A standard laboratory diet of known formulation (SDS Rat and Mouse Diet No.1 SDS, Special Diet Services, 1 Stepfield, Witham, UK) and domestic mains tap water, were available *ad libitum*. Each batch of diet is routinely analysed for composition and for the presence of contaminants. No contaminants were found to be present in the diet or water at levels considered to be capable of interfering with the purpose or outcome of the study. Representative analytical data for typical diet and water available in the study are retained in the study data.

The study room had automatic control of light cycle (alternating 12-hour light and dark cycles) and temperature. Ranges of temperature and relative humidity measured during the study were 18-22°C and 40-67%, respectively.

On-study, the appearance and behaviour of the animals were assessed at least twice daily and details recorded.

7.4 Animal Identification and Study Design

Phase	Dose Route	Dose Level (mg/kg)	Animal ID
1	Oral	40	001M-004M
2	Oral	800	005M-008M
3	Oral	1200	009M-012M
4	Intravenous	4	013M-016M
5	Oral	800	017M-018M
6	Oral	1200	019M-020M

The whole blood and plasma kinetics of total radioactivity was determined at either an oral dose at 40, 800 or 1200 mg/kg (Phase 1, 2 and 3, respectively) or an intravenous dose at 4 mg/kg (Phase 4) of [^{14}C]-malathion. In Phases 5 and 6, terminal blood samples were collected at *ca* C_{max} (determined from Phases 2 and 3) after oral administration at 800 or 1200 mg/kg [^{14}C]-malathion, for chromatographic analysis.

7.5 Dose Preparation and Stability

7.5.1 Radiochemical Purity

The radiochemical purity of stock [^{14}C]-malathion was assessed prior to dose preparation by HPLC.

The radiochemical purity of [^{14}C]-malathion was also assessed in the trial oral dose formulations (40, 800 and 1200 mg/kg) at 24 and 48 h post preparation, and the trial intravenous dose formulation (4 mg/kg) at 3 and 24 h post preparation, in order to determine the stability of [^{14}C]-malathion in the intended dose vehicles. A pre-dose and post-dose purity assessment was also carried out on each final dose formulation.

The HPLC method used is presented below:

Equipment

HPLC model: Agilent 1100/1260
Radiodetector model: Beta Ram 4
Data handling: Laura (LabLogic) version 4.2

Conditions

Column: Phenomenex Luna C18(2) (150 x 4.6 mm, 3 µm)
Column temperature: 30°C
Autosampler temperature: 15°C
Mobile Phase: A: Milli-Q H₂O: Acetonitrile (45:55 v/v)
Mobile Phase Conditions: Isocratic
Gradient:

Time (min)	% A
0	100
20	100

UV detector wavelength: 210 nm

7.5.2 Trial Formulations

Trial oral formulations were prepared at 8, 160 and 240 mg/mL and a trial intravenous formulation at 0.8 mg/mL in order to assess the suitability of the dose formulation procedures and also the radiochemical stability of [¹⁴C]-malathion in the dose preparation. The trial dose preparations mimicked the procedures required for the actual dose preparations but were prepared using the minimum practicable quantities of test item.

7.5.3 Dose Formulations

For each oral dosing occasion, an appropriate volume of [¹⁴C]-malathion was accurately dispensed into a volumetric flask containing a pre-weighed amount of non-radiolabelled malathion. The volumetric flask was made up to volume with ethanol and aliquots analysed by liquid scintillation counting (LSC) to determine the specific activity of the radio-diluted solution. The contents of the volumetric flask were transferred to a dose container with sequential washings using ethanol. The ethanol was evaporated to dryness under a steady stream of nitrogen and an appropriate volume of dose vehicle (corn oil) added in order to achieve the required target dose concentration.

Whenever possible, each dose formulation was stirred with a magnetic stirrer until dose formulation and dosing were complete. The dose formulations were stored in a fridge set to maintain a temperature of 4°C.

For the intravenous dose preparation, an appropriate amount of non-radiolabelled malathion was weighed into a volumetric flask and made up to volume with ethanol to form a stock

solution. An appropriate volume of [^{14}C]-malathion was then accurately dispensed into a dose jar and the relevant volume of unlabelled stock solution added. Ethanol was then added to the dose jar to make up 5% of the total dose volume. The solution was mixed and 10% aqueous sulfobutyl ether- β -cyclodextrin added to make up the remaining 95% dose volume.

Details of each individual dose preparation are listed below:

Phase	Concentration	Radioactive Concentration	Calculated Weight of Radiodiluted [^{14}C]-malathion (mg)	Weight of Dose (g)	Specific Activity (MBq/mg)
	mg/g	MBq/g			
1	9.05	1.05	99.6	11.0	0.116
2	143	1.73	1844	12.9	0.0121
3	222	1.86	2673	12.0	0.00835
4	0.804	0.936	9.52	11.8	1.16
5	181	2.14	1211	6.68	0.0118
6	253	2.08	1721	6.80	0.00822

7.6 Dose Administration

The oral formulations were administered by gastric gavage at a target dose volume of 5 mL/kg to achieve the target dose levels for each oral phase. The intravenous formulation was administered *via* tail vein as a slow bolus over *ca* 30 seconds at a target dose volume of 5 mL/kg to achieve the target dose level.

Each animal was accurately weighed prior to dosing. The syringes were weighed prior to and following each individual dose administration. The actual dose received by each animal was determined with reference to the radioactive concentration, the weight of dose administered and the calculated specific activity of the dose formulation. Any undosed residue, where appropriate, was also taken into account.

The dose received by each animal is presented in Appendix 26.

7.7 Sample Collection

This study was conducted in 6 Phases. In Phases 1, 2 and 3, 1 group of 4 male rats each received an oral administration of [^{14}C]-malathion at a target dose level of 40, 800 or 1200 mg/kg, respectively. In Phase 4, a group of 4 male rats each received an intravenous administration of [^{14}C]-malathion at a target dose level of 4 mg/kg. Blood samples (*ca* 0.2 mL) were collected by venepuncture of a tail or jugular vein into tubes containing lithium heparin anti-coagulant at the following time points:

5 min (Phase 4 only), 15 min, 30 min, 1, 2, 4, 8, 12, 24, 30, 48, 72, and 96 h post dose.

Duplicate aliquots of blood were removed for analysis and plasma was separated from the remaining blood by centrifugation. Levels of radioactivity were determined in each sample of whole blood and plasma collected.

In Phases 5 and 6, 1 group of 2 male rats each received an oral administration of [^{14}C]-malathion at a target dose level of 800 or 1200 mg/kg. All rats were humanely killed by CO_2 narcosis at 1.5 h post dose (*ca* T_{max} determined from Phase 2 and 3).

A terminal blood sample (*ca* 3-10 mL) was collected by cardiac puncture into tubes containing lithium heparin anti-coagulant. A small volume (*ca* 0.5 mL) was retained separately for radioanalysis. Plasma was separated from the remaining blood by centrifugation and the red blood cells retained. Levels of radioactivity were determined in each sample of whole blood, red blood cells and plasma collected. Remaining plasma and red blood cell samples were subjected to chromatographic analysis.

7.8 Sample Storage

All samples not analysed immediately were stored in a freezer set to maintain a temperature of -20°C until taken for analysis. Any remaining samples were returned to storage in a freezer set to maintain a temperature of -20°C .

7.9 Preparation of Samples for Total Radioactivity Analysis

Duplicate samples of plasma were made up to 1 mL with water and mixed with AquaSafe 500 Plus scintillation fluid (10 mL).

Duplicate aliquots of whole blood and red blood cells were combusted using a PerkinElmer 307 Sample Oxidiser. The resultant $^{14}\text{CO}_2$ generated was absorbed in Carbosorb[®] (8 mL) to which Permafluor[®] E⁺ scintillation fluid (10 mL) was added. Combustion of standards showed that recovery efficiencies were in excess of 97% throughout.

7.10 Preparation of Samples for Chromatographic Analysis

The following samples were used for metabolite profiling and identification:

Sample Matrix	Animals	Dose Route	Dose Level (mg/kg)	Timepoint (h)
Plasma	017M-018M	Oral	800	1.5
Red Blood Cells	017M-018M			
Plasma	019M-020M	Oral	1200	1.5
Red Blood Cells	019M-020M			

7.11 Quantification of Radioactivity

All samples prepared in scintillation fluid were subjected to LSC for 5 minutes, together with representative blank and standard vials, using a Packard Tri-carb 2100TR liquid scintillation analyser with automatic quench correction by an external method. Samples were allowed to heat and light stabilise prior to analysis. Prior to calculation of each result, a background count was determined and subtracted from each sample count rate.

For liquid scintillation counting, a limit of reliable determination of 30 d.p.m. above background has been instituted in these laboratories. The following table summarises the mean limit of reliable measurement values in this study (based on mean aliquot size):

Phase	Mean limit of reliable measurement ($\mu\text{g equiv/g}$ or mL)
1	0.14 (0.03)
2	1.4 (0.03)
3	1.5 (0.04)
4	0.01 (0.03)
5	1.0 (0.05)
6	1.2 (0.05)

Where results have arisen from data below the limit of reliable determination, the fact is noted.

7.11.1 Data Presentation

Data presented in results tables were computer generated in DEBRA (and rounded appropriately for inclusion in the report). As a consequence, calculation of individual and mean values from data presented will, in some instances, yield slight differences from the results presented.

7.12 Pharmacokinetic Evaluation

Pharmacokinetic parameters were estimated using Phoenix pharmacokinetic software (Certara, Version 1.4) using a non-compartmental approach consistent with the oral and intravenous dose routes of administration. All parameters were generated from [^{14}C]-malathion total radioactivity individual concentrations in whole blood and plasma from rats after single doses of radiolabel test material. Parameters were estimated using the mean actual dose received for each group and nominal sampling times relative to the start of each dose administration. All concentrations calculated from less than 30 dpm above background were excluded from the pharmacokinetic analysis.

The area under the [^{14}C]-malathion total radioactivity concentration versus time curve (AUC) was calculated using the linear trapezoidal method with linear interpolation. When practical, the terminal elimination phase of each concentration versus time curve was identified using at least the final three observed concentration values. The slope of the terminal elimination phase was determined using log linear regression on unweighted concentration data. Parameters relying on the determination of the terminal elimination phase were not reported (NR) if the coefficient of determination was less than 0.800 and/or if the extrapolation of the AUC to infinity represented more than 20% of the total area. The parameters described below were reported to 3 significant figures, with the exception of T_{\max} which has been reported to no more significant figures than needed to explain time. Additional parameters automatically generated by Phoenix, but not required by the Protocol, are maintained in the raw data.

Parameter	Description of Parameter
T_{\max}	The time after dosing at which the maximum concentration was observed.
C_0	The theoretical concentration at time zero after intravenous bolus dosing only.
C_{\max}	The maximum observed concentration measured after dosing.
C_{\max}/D	The C_{\max} divided by the dose administered.
$AUC_{(0-t)}$	The area under the concentration versus time curve from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed, using the linear or linear/log trapezoidal method.
$AUC_{(0-t)}/D$	The $AUC_{(0-t)}$ divided by the dose administered.
$AUC_{(0-\infty)}$	The area under the concentration versus time curve from time zero to infinity.
$AUC_{(0-\infty)}/D$	The $AUC_{(0-\infty)}$ divided by the dose administered.
$T_{1/2}$	The apparent terminal elimination half life.
CL	The apparent clearance rate of total radioactivity from the analysed test matrix following intravenous dosing only.
Vd	The apparent volume of distribution of total radioactivity in the test system.
F	Absolute bioavailability, calculated as the ratio of the dose-normalised AUC (oral) divided by dose normalised AUC (intravenous) x 100%.

7.13 Metabolite Profiling and Identification

7.13.1 Samples

The following pooled plasma and red blood cell samples were received for analysis.

Matrix	Animal ID	Sex	Route	Dose (mg/kg)	Timepoint (h)	Sample Code
Plasma	017M-018M	Male	Oral	800	1.5	P1
	019M-020M	Male	Oral	1200	1.5	P2
Red Blood Cells	017M-018M	Male	Oral	800	1.5	R1
	019M-020M	Male	Oral	1200	1.5	R2

7.13.2 Preparation of Samples

7.13.2.1 Plasma

A subsample (1 mL) of each plasma pool was extracted with acetonitrile (3 volumes of solvent/mL of sample), chilled in a freezer for 20 min, immediately centrifuged for 10 min at 4000 rpm and the supernatant separated from the pellet. The pellet was extracted a further two times with acetonitrile as above and the supernatants combined. The pellet was further extracted with water/acetonitrile (3:7, 3 volumes of solvent/mL of sample), centrifuged for 10 min at 4000 rpm and the supernatant separated from the pellet. Extracts for each plasma pool were combined and the centrifuge tubes that were used for extractions were washed with water (0.5-1 mL) and acetonitrile (0.5-1 mL), twice for P2 and four times for P1, as required to achieve sufficient recovery. The washes were combined with the extract and the sample concentrated to *ca* 200 μ L. The volume for the concentrated extract was adjusted to 1 mL (water/acetonitrile, 9:1 v/v), ready for LC-MS analysis. The remaining pellets were resuspended in Milli-Q H₂O (2 mL) and analysed in their entirety by LSC.

7.13.2.2 Red Blood Cells

A subsample (*ca* 1 g) of each red blood cell pool was extracted with acetonitrile (3 volumes of solvent/g of sample), chilled in a freezer for 20 min, immediately centrifuged for 10 min at 4000 rpm and the supernatant separated from the pellet. The pellet was extracted a further two times with acetonitrile as above and the supernatants combined. The pellet was further extracted with water/acetonitrile (3:7, 3 volumes of solvent/g of sample) and water/acetonitrile (1:1, 3 volumes of solvent/g of sample), each time the extracts were centrifuged for 10 min at 4000 rpm and the supernatant separated from the pellet. Extracts for each red blood cell pool were combined the sample concentrated to *ca* 200 μ L. The volume for the concentrated extract was adjusted to 1 mL (water/acetonitrile, 9:1 v/v), ready for LC-MS analysis. The remaining pellets were resuspended in Milli-Q H₂O (4 mL) and analysed in their entirety by LSC.

7.13.3 Analytical Techniques

7.13.3.1 Chromatography (LC-MSn)

Equipment

Mass Spectrometer:	Shimadzu LC-MS IT-ToF
HPLC:	Shimadzu Prominence XR Modular HPLC System
	Solvent Mixer 1.7 mL
Radio Detector:	LabLogic β -RAM Model 5 (300 μ L flow cell)
Data Handling:	Laura Version 4.2
	Shimadzu LC-MS Solutions Version 3.6

Approximately 25% of the flow from the HPLC was diverted to the mass spectrometer using an inline splitter, the remainder was sent to the radiodetector.

7.13.3.2 LC Conditions

Pre-column: Phenomenex Security Guard C18
Column: Imtakt Scherzo SS-C18 150 x 4.6 mm, 3 µm
Flow Rate: 1 mL/min
PDA Detection Range: 190-800
Column temperature: 30°C
Autosampler temperature: 4°C
Mobile Phases: A: 0.1% Formic acid (aq)
B: 0.2% Trifluoroacetic acid in acetonitrile

Gradient:	Time (mins)	%A	%B
	0	95	5
	10.00	95	5
	30.00	80	20
	60.00	30	70
	65.00	10	90
	65.01	95	5
	70.00	95	5

7.13.3.3 MS Conditions

The mass spectrometer optics were optimised for the analysis of [¹⁴C]-malathion. General instrument parameters were also recorded. Compound specific parameters are shown below.

Interface: ESI
CDL Temperature: 200°C
Block Temperature: 200°C
Drying Gas (Nitrogen): On
Polarity: Positive/Negative Switching
Potential: 4.5kV/-3.5kV
Nebulising Gas (Nitrogen): 1.5 L/min
Acquisition: Data Dependant (DDA) Method in both positive and negative ion mode using automatic gain control (ASC 10e6).

Event	1	2	3
Polarity	Positive	Positive	Positive
MS Stage	1	2	3
Scan range	100-1200	50-1250	50-1250
Energy	NA	60	60
Gas	NA	100	100
Peak selection	NA	Automatic by intensity	Automatic by intensity
Precursor isolation width	NA	3	3
Q (Low mass cut off) value	NA	0.251	0.251

Event	4	5	6
Polarity	Negative	Negative	Negative
MS Stage	1	2	3
Scan range	100-1200	50-1250	50-1250
Energy	NA	60	60
Gas	NA	100	100
Peak selection	NA	Automatic by intensity	Automatic by intensity
Precursor isolation width	NA	3	3
Q (Low mass cut off) value	NA	0.251	0.251

7.13.4 Data Evaluation

Following analysis by radio-LC-MSⁿ, the concentration (µg/g) for each metabolite in plasma and red blood cells was determined from the % region of interest (%ROI) on the chromatogram as follows:

$$\mu\text{g/g} = \% \text{ ROI}/100 \times \mu\text{g/g} \text{ calculated in the analysed extract}$$

The reliable limit of detection following LSC was assumed to be 30 d.p.m. above background while the limit of detection for radio-chromatography using a flow scintillation analyser was assumed to be 200 d.p.m.

7.13.5 Column Recovery

A representative column recovery was performed on a plasma and red blood cell sample using LC conditions described in section 7.13.3.2. The radioactivity recovered in the post column eluent was calculated to be quantitative against the radioactivity injected.

7.13.6 Structural Elucidation

The nature and identity of metabolites of [¹⁴C]-malathion in rat plasma and red blood cells was investigated by radio-LC-MSⁿ using equipment and methods described in section 7.13.3.2. Metabolites were identified using comparative chromatography with standard reference compounds, accurate mass measurement and MSⁿ fragmentation.

7.13.6.1 Accurate Mass Measurement

Accurate mass measurement was used to differentiate between potential metabolites of [¹⁴C]-malathion and unrelated endogenous components and to determine metabolite structure. The accurate mass error (ppm) was calculated from a comparison of the measured mass with the theoretical mass for a suggested assignment. A low accurate mass error (<5 ppm or <5 mDa for low level ions) supports the assignment. Conversely, a large accurate mass error would refute the assignment:

$$\text{Accurate mass error (ppm)} = \left(\frac{\text{Measured mass} - \text{Theoretical mass}}{\text{Theoretical mass}} \right) \times 1000000$$

7.13.6.2 Isotope Pattern

The presence of a radio label and sulphur atoms in [¹⁴C]-malathion resulted in ions with elevated mass. These afford a distinctive isotope pattern i.e. [M], [M+2]... corresponding to the number of ¹⁴C atoms and/or sulphur atoms incorporated into the structure. This was typically conserved among metabolites of malathion that contained the ¹⁴C label and sulphur atoms. The isotope pattern observed in full scan mass spectra was used to support a postulated identification as it demonstrated the presence of the radio label/sulphur atoms.

7.14 Protocol Deviation

On two occasions (30 September 2016 and 01 October 2016), the temperature in the animal room fell below the accepted range stated in the protocol of 19-23°C (18.2 and 17.7°C, respectively). These values were marginally below the accepted minimum and no impact on animal health was noted, therefore there was no study impact.

8 RESULTS

8.1 Radiochemical Purity and Stability

[¹⁴C]-malathion was shown, by co-chromatography with malathion, to be 98.9% pure. [¹⁴C]-malathion was also shown to be stable in the 8, 160 and 240 mg/mL trial oral formulations at 24 and 48 hours post preparation, and in the 0.8 mg/mL trial intravenous formulation at 3 and 24 hours post preparation, therefore confirming its stability for the duration of the dosing periods. The purity of [¹⁴C]-malathion in each formulation prior to and following dose administration was also assessed. Purity results are summarised in the following table:

Phase	Target Concentration (mg/mL)	Target Dose Level (mg/kg)	Measurement Time	HPLC Purity %
Initial stock purity	NA	NA	NA	98.9
Trial Oral	8		24 h	99.4
	160		48 h	98.5
			24 h	98.9
			48 h	98.7
			24 h	98.0
	48 h		98.7	
Trial Intravenous	0.8		3 h	99.5
			24 h	98.5
1	8	40	Pre-dose	98.0
			Post-dose	99.6
2	160	800	Pre-dose	98.2
			Post-dose	100
3	240	1200	Pre-dose	98.9
			Post-dose	98.6
4	0.8	4	Pre-dose	98.3
			Post-dose	98.8
5	160	800	Pre-dose	97.7
			Post-dose	98.4
6	240	1200	Pre-dose	98.1
			Post-dose	98.1

NA = Not applicable

Representative UV and radiochromatograms are presented in Appendix 27 to Appendix 30.

8.2 Dose Levels

The actual doses administered to the animals are detailed in Appendix 26. In Phase 1-3, the 40, 800 and 1200 mg/kg oral doses received by the animals were in the range 42.0-43.4 mg/kg, 746-804 mg/kg and 1053-1105 mg/kg, respectively. The 4 mg/kg intravenous doses received by the animals were in the range 3.73-3.97 mg/kg. In Phase 5 and

6, the 800 and 1200 mg/kg oral doses received by the animals were in the range 882-887 and 1240-1241 mg/kg.

8.3 Animal Observations

During the course of the study, no overt toxicological signs were observed in the test animals.

8.4 Pharmacokinetic Analysis

Mean concentrations of total radioactivity in whole blood and plasma are presented in Table 1 to Table 4, respectively. The mean blood to plasma ratios of radioactivity are presented in Table 5 and Table 6.

8.4.1 Whole Blood and Plasma Kinetics Following Oral Administration at 40 mg/kg (Phase 1)

Mean concentrations of total radioactivity in whole blood and plasma in male rats are presented graphically in Figure 1 and 2, respectively. Individual animal whole blood and plasma concentration data are presented in Appendix 31. Pharmacokinetic parameters are presented in Table 7.

Following a single oral administration of [^{14}C]-malathion at 40 mg/kg to male rats, blood concentrations of total radioactivity were detectable from the first sampling time of 0.25 h. Total radioactivity vs time profiles were consistent with the oral dose route whereby a post-dose increase was evident followed by a generally bi-phasic decline in concentrations. The measured mean peak concentrations of total radioactivity were attained at 1 h (15.7 $\mu\text{g equiv/g}$). Thereafter, blood concentrations gradually declined to 0.3 $\mu\text{g equiv/g}$ at 24 h and were below the limit of reliable measurement by 30 h post dose. The calculated elimination half-life ($T_{1/2}$) was 5.35 h and the $\text{AUC}_{(0-\text{inf})}$ was 84.8 $\mu\text{g equiv.h/g}$.

Plasma concentrations were also detectable from 0.25 h. The plasma measured mean maximum value was 27.3 $\mu\text{g equiv/mL}$ at 1 h post dose. Thereafter, plasma concentrations steadily declined to 0.2 $\mu\text{g equiv/mL}$ at 48 h then were below the limit of reliable measurement. The calculated elimination half-life ($T_{1/2}$) was 21.7 h and $\text{AUC}_{(0-\text{inf})}$ was 178 $\mu\text{g equiv.h/mL}$.

Over the 96 h sampling period, concentrations were quantifiable until *ca* 24 hours. Blood to plasma ratios were generally consistent and in the range 0.44-0.61 over this time, suggesting that the circulating radioactivity had a greater affinity with the plasma fraction.

The oral bioavailability (F) was 102% in whole blood and 117% in plasma.

8.4.2 Whole Blood and Plasma Kinetics Following Oral Administration at 800 mg/kg (Phase 2)

Mean concentrations of total radioactivity in whole blood and plasma in male rats are presented graphically in Figure 3 and Figure 4, respectively. Individual animal blood and plasma concentration data are presented in Appendix 32. Pharmacokinetic parameters are presented in Table 8.

Following a single oral administration of [^{14}C]-malathion at 800 mg/kg to male rats, blood concentrations of total radioactivity were detectable from the first sampling time of 0.25 h. Measured concentrations of total radioactivity initially reached a maximum at 0.5 h (75 $\mu\text{g equiv/g}$). Thereafter, mean blood concentrations appeared to plateau until 4 h (74 $\mu\text{g equiv/g}$). The concentration then gradually declined until 72 h (3 $\mu\text{g equiv/g}$) but had dropped below the limit of reliable measurement at the last sampling time of 96 h. The $T_{1/2}$ value was 29.0 h and $\text{AUC}_{(0-\text{inf})}$ was 1850 $\mu\text{g equiv.h/g}$.

Plasma concentrations were also detectable from 0.25 h. Plasma measured maximum concentration was attained at 0.5 h with a value of 152 $\mu\text{g equiv/mL}$. Thereafter, plasma concentrations appeared to plateau until 4 h (150 $\mu\text{g equiv/mL}$), then decreasing until 96 h (2 $\mu\text{g equiv/mL}$). The $T_{1/2}$ was 30.8 h and the $\text{AUC}_{(0-\text{inf})}$ was 2750 $\mu\text{g equiv.h/mL}$.

Where concentrations were quantifiable over the 96 h sampling period, blood to plasma ratios were all <1 , suggesting that the circulating radioactivity was more associated with the plasma fraction. Between 0.25 and 24 h, blood to plasma ratios were in a slightly lower range (0.48-0.63) compared to the period 30-72 h (0.74-0.85), suggesting that the circulating radioactivity may have been becoming less associated with the plasma fraction at the later sampling timepoints.

The oral bioavailability (F) was 121% in whole blood and 98% in plasma.

8.4.3 Whole Blood and Plasma Kinetics Following Oral Administration at 1200 mg/kg (Phase 3)

Mean concentrations of total radioactivity in whole blood and plasma in male rats are presented graphically in Figure 5 and Figure 6, respectively. Individual animal blood and plasma concentration data are presented in Appendix 33. Pharmacokinetic parameters are presented in Table 9.

Following a single oral administration of [^{14}C]-malathion at 1200 mg/kg to male rats, blood concentrations of total radioactivity were detectable at all sampling timepoints. Blood measured mean peak concentrations of total radioactivity were attained at 0.5 h (146 $\mu\text{g equiv/g}$), appearing to form a plateau until 8 h (96 $\mu\text{g equiv/g}$). Thereafter, the

concentrations gradually decreased to 5 µg equiv/g at 96 h, with a calculated elimination half-life ($T_{1/2}$) of 37.7 h. The $AUC_{(0-inf)}$ was 2460 µg equiv.h/g.

Plasma concentrations were also detectable from 0.25 h. Plasma measured mean peak concentration was attained at 0.5 h with a value of 249 µg equiv/mL. Thereafter, plasma concentrations plateaued until 8 h (167 µg equiv/mL). Concentrations then gradually decreased to 4 µg equiv/mL at the final sampling timepoint of 96 h. The calculated $T_{1/2}$ was 25.7 h and $AUC_{(0-inf)}$ was 3610 µg equiv.h/mL.

Between the sampling times 0.25 and 30 h (range 0.51-0.65) blood to plasma ratios were all <1, suggesting that the circulating radioactivity was more associated with the plasma fraction. At 72 h, the ratio increased to 0.83 then become >1 at 72 and 96 h post dose, indicating that the circulating radioactivity was becoming less associated with the plasma fraction at the later sampling timepoints.

The oral bioavailability (F) was 118% in whole blood and 94% in plasma.

8.4.4 Whole Blood and Plasma Kinetics Following Intravenous Administration at 4 mg/kg (Phase 4)

Mean concentrations of total radioactivity in whole blood and plasma in male rats are presented graphically in Figure 7 and Figure 8, respectively. Individual animal blood and plasma concentration data are presented in Appendix 34. Pharmacokinetic parameters are presented in Table 10.

Following a single intravenous administration of [^{14}C]-malathion at 4 mg/kg to male rats, the theoretical concentration of radioactivity in blood at time zero (C_0) was 12.5 µg equiv/g. Thereafter, blood concentrations rapidly declined to 0.07 µg equiv/g by 8 h and 0.03 µg equiv/g at 24 h then were below the limit of reliable measurement. The calculated elimination half-life ($T_{1/2}$) was 11.0 h and the $AUC_{(0-inf)}$ was 7.45 µg equiv.h/g.

The theoretical concentration of radioactivity in plasma at time zero (C_0) was 23.7 µg equiv/mL. Plasma measured mean concentrations then decreased to 0.11 µg equiv/mL at 8 h and 0.02 µg equiv/mL at 48 h then fell below the limit of reliable measurement. The calculated elimination half-life ($T_{1/2}$) was 27.0 h and the $AUC_{(0-inf)}$ was 13.7 µg equiv.h/mL.

Over the 96 h sampling period, concentrations were quantifiable until 24 h. Blood to plasma ratios were in the range 0.53-0.79 over this time and generally increased at each time point, suggesting that the circulating radioactivity was becoming less associated with the plasma fraction.

8.4.5 Dose Proportionality

Following an increased oral dose from 40 mg/kg to 800 mg/kg, systemic exposure (as determined by $AUC_{(0-inf)}$ values) increased in a proportional manner in blood and a sub-proportional manner in plasma. Comparing the 40 mg/kg to the 1200 mg/kg group, systemic exposure increased in a broadly proportional manner in both blood and plasma. In comparison to the 800 mg/kg group, systemic exposure increased in the 1200 mg/kg group in a proportional manner in blood and a sub-proportional manner in plasma. Estimates of bioavailability indicated absorption was complete in all groups.

8.5 Chromatographic Analysis

8.5.1 Collection of Plasma and Red Blood Cells for Chromatographic Analysis

A terminal blood collection was taken from the pairs of male rats given a single 800 mg/kg or 1200 mg/kg oral dose of [^{14}C]-malathion and terminated at the approximate T_{max} (1.5 h). These samples were used to enable the profiling of metabolites in plasma and red blood cells. The blood and plasma concentrations of radioactivity for these animals, (Appendix 35), were consistent with concentrations in similarly treated rats dosed in Phases 2 and 3.

8.5.2 Extraction of Radioactivity in Pooled Samples

In plasma, the majority of the radioactivity was recovered in the acetonitrile and acetonitrile/water extracts. These extracts accounted for a total of 83.2% total radioactivity (168.574 μg equiv/g) for P1 and 93.4% total radioactivity (149.921 μg equiv/g) for P2. Between 95-105% of the radioactivity was recovered on concentration and reconstitution in preparation of plasma samples for chromatographic analysis.

In red blood cells, the majority of the radioactivity was recovered in the acetonitrile and acetonitrile/water extracts. These extracts accounted for 97.8% total radioactivity (83.002 μg equiv/g) for R1 and 99.4% total radioactivity (53.381 μg equiv/g) for R2. Between 85-90% of the radioactivity was recovered on concentration and reconstitution in preparation of red blood cell samples for chromatographic analysis. The recoveries on concentration were lower than for plasma due to the nature of the red blood cell samples.

8.5.3 Quantification of Radiolabelled Metabolites

The radiochromatograms for plasma are presented in Figure 14 and Figure 15 and red blood cells in Figure 16 and Figure 17. Up to 16 radiolabelled components were detected (Table 11).

Malathion dicarboxylic acid was the major component in plasma and was measured at a concentration of 117.342 and 121.264 μg equiv/g in the 1200 mg/kg and 800 mg/kg dose

samples, respectively. Two structural isomers for desmethyl malathion monocarboxylic acid were observed, labelled 1 and 2, measuring at 2.290-4.737 $\mu\text{g equiv/g}$ and 16.449-21.065 $\mu\text{g equiv/g}$, respectively (Table 12). Due to LC-MSⁿ limitations it was not possible to differentiate between isomers, however isomer 2 was observed to be present at a much greater concentration. Malathion monocarboxylic acid was also present in plasma (5.436-12.270 $\mu\text{g equiv/g}$). Sulphur substituted malathion monocarboxylic acid, labelled metabolite 1, was identified at a concentration of 1.795-2.230 $\mu\text{g equiv/g}$. All remaining components were unassigned and were individually measured at a concentration <3 $\mu\text{g equiv/g}$.

Malathion dicarboxylic acid was also the major component in red blood cells and was measured at a concentration of 33.262-47.368 $\mu\text{g equiv/g}$ in the 1200 mg/kg and 800 mg/kg dose samples, respectively. Two structural isomers for desmethyl malathion monocarboxylic acid were observed, labelled 1 and 2, measuring <3.288 $\mu\text{g equiv/g}$ and 3.403-5.673 $\mu\text{g equiv/g}$, respectively (Table 12). Due to LC-MSⁿ limitations it was not possible to determine which isomer was which, however isomer 2 was observed to be present at a much greater concentration. Malathion monocarboxylic acid was also present in red blood cells (3.878-10.018 $\mu\text{g equiv/g}$). Sulphur substituted malathion monocarboxylic acid, labelled metabolite 1, was identified at a concentration of <1.543 $\mu\text{g equiv/g}$. All remaining components were unassigned and were individually measured at a concentration <2 $\mu\text{g equiv/g}$.

No other reference standards were detected by radio LC/MS in either the plasma or red blood cells (see Table 15 for details).

8.6 Radio LC-MS & LC-MSMS Analysis

8.6.1 Reference Standards

Reference standards in Table 15 were analysed individually using LC conditions described in Section 7.13.3.2. Representative extracted ion chromatograms (EIC), mass spectra and postulated structure of observed ions for malathion dicarboxylic acid, malathion monocarboxylic acid and desmethyl malathion monocarboxylic acid are presented in Figure 9 to Figure 13, respectively.

Representative radio-chromatograms for Plasma and Red Blood Cell extracts are presented in Figure 14 to Figure 17.

Reference standards malathion dicarboxylic acid, malathion monocarboxylic acid and desmethyl malathion monocarboxylic acid were confirmed by comparison of acquired radio-LC-MSⁿ data with reference standard data and by observation of an isotope mass pattern

resulting from presence of the radiolabel/sulphur atoms. Data was broadly comparable between samples and reference standards.

Reference standard desmethyl malathion monocarboxylic acid displayed 4 peaks in the EIC for m/z 286.9818 ($[M-H]^-$) at 36.4, 42.2, 43.6 and 50.8 min (Figure 13). The 4 peaks represented 2 structural isomers for desmethyl malathion monocarboxylic acid, assigned desmethyl malathion monocarboxylic acid 1 and desmethyl malathion monocarboxylic acid 2 (structures for both in Table 13). Further splitting of 2 peaks into 4 is postulated to be due to the ionised and neutral forms when eluting through the LC column. Using LC-MS fragmentation data it was possible to assign the components at 36.4 and 42.2 min in the EIC for m/z 286.9818 ($[M-H]^-$) as desmethyl malathion monocarboxylic acid 1, and the peaks at 43.6 and 50.8 min as desmethyl malathion monocarboxylic acid 2. It was however not possible to assign which isomer corresponded to which set of peaks.

8.6.2 Confirmation of malathion dicarboxylic acid

The radiolabelled component with retention time *ca.* 39.8 min (Figure 15) was assigned as malathion dicarboxylic acid. This was supported by accurate mass measurement and by comparison of retention time and fragmentation pathway with an authentic reference standard for malathion dicarboxylic acid (Figure 9).

The EIC for the ion with m/z 272.9662 ($[M-H]^-$) showed a peak with retention time *ca.* 39.9 min (Figure 18A) which was consistent with the radio-peak observed in the radio chromatogram (Figure 15) and the retention time of *ca.* 39.5 min for reference standard malathion dicarboxylic acid (Figure 9A). Accurate mass measurement of the molecular ion (Figure 18B/C) returned a mass error of 2.20 ppm for the formula $C_6H_{11}O_6PS_2$. The presence of an $[M]$, $[M+2]$... isotope pattern confirmed the presence of the radio label/sulphur atoms (Figure 18C). Fragmentation of the deprotonated ion resulted in a diagnostic product ion with m/z 141 (Figure 18D/E), constant with that observed during fragmentation of malathion dicarboxylic acid reference standard (Figure 9D/E).

8.6.3 Confirmation of malathion monocarboxylic acid

The radiolabelled component with retention time *ca.* 48.0 min (Figure 14) was assigned as malathion monocarboxylic acid. This was supported by accurate mass measurement and by comparison of retention time and fragmentation pathway with an authentic reference standard for malathion monocarboxylic acid (Figure 10).

The EIC for the ion with m/z 324.9940 ($[M+Na]^+$) showed a peak with retention time *ca.* 48.2 min (Figure 19A) which was consistent with the radio-peak observed in the radio chromatogram (Figure 14) and the retention time of *ca.* 47.6 min for reference standard malathion monocarboxylic acid (Figure 10). Accurate mass measurement of the sodium

adduct (Figure 19B/C) returned a mass error of 1.85 ppm for the formula $C_8H_{14}O_6NaPS_2$. The presence of an $[M]$, $[M+2]$... isotope pattern confirmed the presence of the radio label/sulphur atoms (Figure 19C). Fragmentation of the sodium adduct ion resulted in diagnostic product ions with m/z 303, 285, 257 and 167 (Figure 19B/D/E), consistent with that observed during fragmentation of malathion monocarboxylic acid reference standard (Figure 10).

Two structural isomers for malathion monocarboxylic acid are possible. Using LC-MS data it was not possible to identify if just one or both were present.

8.6.4 Confirmation of desmethyl malathion monocarboxylic acid

The radiolabelled components with retention time *ca.* 36.5, 42.0, 43.3 and 50.5 were assigned as two desmethyl malathion monocarboxylic acid isomers (Figure 15). This assignment was supported by low accurate mass errors for the postulated structure and by comparison of the retention times and fragmentation pathways with an authentic reference standard for desmethyl malathion monocarboxylic acid.

The EIC for the ion with m/z 286.9818 ($[M-H]^-$) showed three peaks with retention time *ca.* 42.4, 44.0 and 50.7 min (Figure 20A, Figure 21A and Figure 22A), consistent with the radio-peaks at *ca.* 42.0, 43.3 and 50.5 min in the radiochromatogram (Figure 15) and the retention times of 42.2, 43.6 and 50.8 min for reference standard desmethyl malathion monocarboxylic acid (Figure 11A, Figure 12A, Figure 13A). Accurate mass measurement of the molecular ion (Figure 20C, Figure 21C and Figure 22C) returned mass errors of 1.39, 2.79, 1.74 ppm for the formula $C_7H_{13}O_6PS_2$, respectively. The presence of an $[M]$, $[M+2]$... isotope pattern confirmed the presence of the radio label/sulphur atoms (Figure 20C, Figure 21C and Figure 22C). Fragmentation of the deprotonated molecular ion for the component at 42.0 min resulted in diagnostic product ions with m/z 241 and 143 (Figure 20D/E). This was consistent with fragmentation of the 42.2 min component in desmethyl malathion monocarboxylic acid reference standard (Figure 11E/F). Fragmentation of the deprotonated molecular ion for the components at 43.3 and 50.5 min resulted in diagnostic product ions with m/z 253, 241, 143 and 127 (Figure 21D/E/F and Figure 22D/E/F). This fragmentation pathway was consistent with the 43.6 and 50.8 min component in desmethyl malathion monocarboxylic acid reference standard (Figure 12E/F/G/H, Figure 13E/F/G).

The radio component with retention time of 36.5 min (Figure 15) was assigned as desmethyl malathion monocarboxylic acid 1 by comparison of retention time with an authentic reference standard for desmethyl malathion monocarboxylic acid. The component at 36.5 min was however not detected by LC-MS analysis due to the low concentration levels.

The two peaks at 36.5 and 42.0 min in the radiochromatogram and EIC for m/z 286.9818 ($[M-H]^-$) were for one of the two possible structural isomers, assigned desmethyl malathion monocarboxylic acid 1, and the peaks at 43.3 and 50.5 for the other, assigned desmethyl

malathion monocarboxylic acid 2. Using LC-MS fragmentation data it was possible to confirm that components at 36.5 and 42.0 min were one isomer and 43.3 and 50.5 min were the other. The split in the peaks is postulated to be due to the ionised and neutral form for both isomers when eluting through the LC column.

9 CONCLUSIONS

Following a single oral dose of [^{14}C]-malathion at a target dose of 40, 800 or 1200 mg/kg to male rats, the dose was rapidly absorbed with whole blood and plasma measured maximum mean concentrations of total radioactivity achieved at 1 h, 0.5 h and 0.5 h, respectively.

In all dose groups, total systemic exposure was generally higher in plasma compared to whole blood. In the oral 40 mg/kg group, concentrations were quantifiable until *ca* 24 hours post dose. Within this period, blood to plasma ratios were generally consistent and <1 , suggesting circulating radioactivity was more associated with the plasma fraction. In the oral 800 and 1200 mg/kg groups, total radioactivity became less associated with the plasma fraction at the later sampling times. This trend was especially evident at the 1200 mg/kg dose where the blood to plasma ratio increased to >1 at 72 and 96 h post dose.

As oral doses of [^{14}C]-malathion increased from 40 mg/kg to 800 mg/kg and 1200 mg/kg, systemic exposure to total radioactivity increased in both whole blood and plasma. In blood, the increase was consistently proportional. In plasma, the increase was sub proportional with the exception of the increase from 800 to 1200 mg/kg where the relationship was proportional. At the 800 and 1200 mg/kg doses, concentrations remained close to the maximum measured concentration between 2-8 h before decreasing, suggesting prolonged absorption and/or saturation in metabolic/elimination processes following increased doses. A comparison of AUC values between intravenous and oral doses gave oral bioavailability, F , values of approximately 100%, confirming quantitative oral absorption. In a couple of instances F was calculated to be $>100\%$ and this was attributed to the differences due to extrapolation of the intravenous dose level to higher oral dose levels.

Following a single intravenous dose of [^{14}C]-malathion at a target dose of 4 mg/kg, the concentration of radioactivity in whole blood and plasma steadily declined to 24 and 48 h post dose, respectively, then falling below the limit of detection.

Up to 16 radiolabelled components were detected in the plasma and red blood cells in rats, with the metabolites detected qualitatively similar at both dose levels.

Malathion dicarboxylic acid was the major component in both the low and high dose plasma and red blood cells, measured between 33.262-121.264 $\mu\text{g equiv/g}$. Two structural isomers for desmethyl malathion monocarboxylic acid were observed, with one (labelled isomer 2) present at much greater concentrations (3.403-21.065 $\mu\text{g equiv/g}$). Malathion monocarboxylic acid was also observed and measured at a greater concentration in the 800 mg/kg dose group (10.018-12.270 $\mu\text{g equiv/g}$) relative to the 1200 mg/kg dose group (3.878-5.436 $\mu\text{g equiv/g}$). A sulphur substituted malathion monocarboxylic acid (metabolite 1) was also identified present at concentrations (<1.092 -2.230 $\mu\text{g equiv/g}$). All

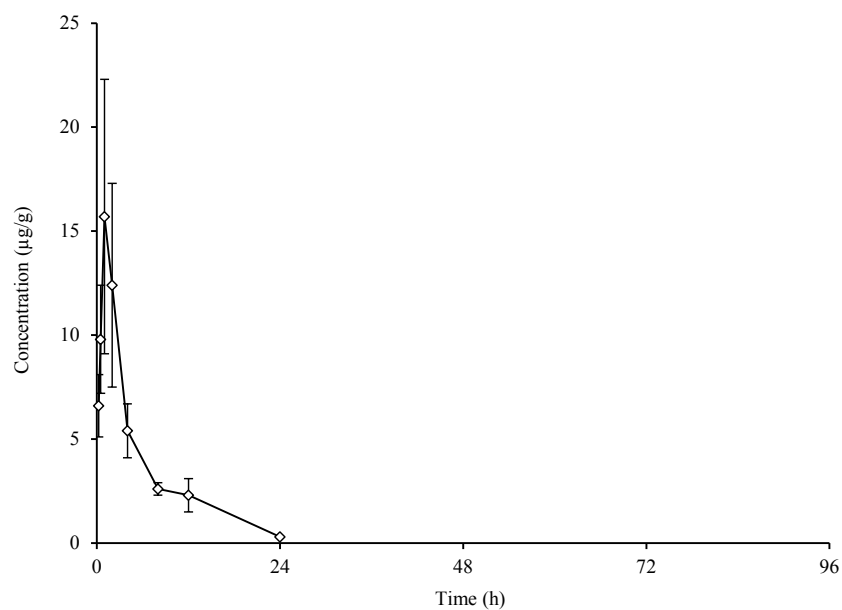
remaining components were unassigned and were individually measured at a concentration <3 µg equiv/g.

See Table 11 and Table 12 for quantification of identified metabolites and Table 13 and Table 14 for a summary of identified metabolites. Table 15 summarises the analysed reference standards which were not detected.

10 FIGURES

Figure 1 **Mean Concentration of Total Radioactivity in Whole Blood Following a Single Oral Administration of [¹⁴C]-Malathion to Male Rats at a Target Dose Level of 40 mg/kg**

Linear



Semi log

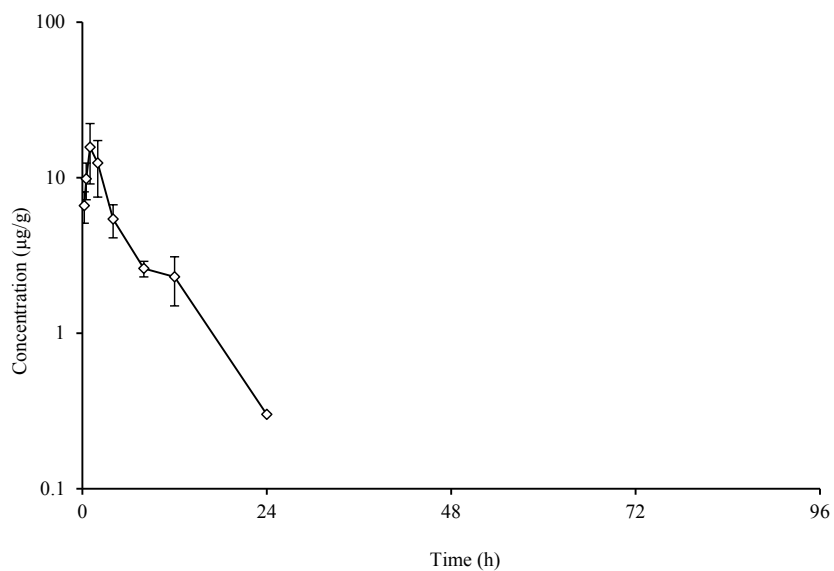
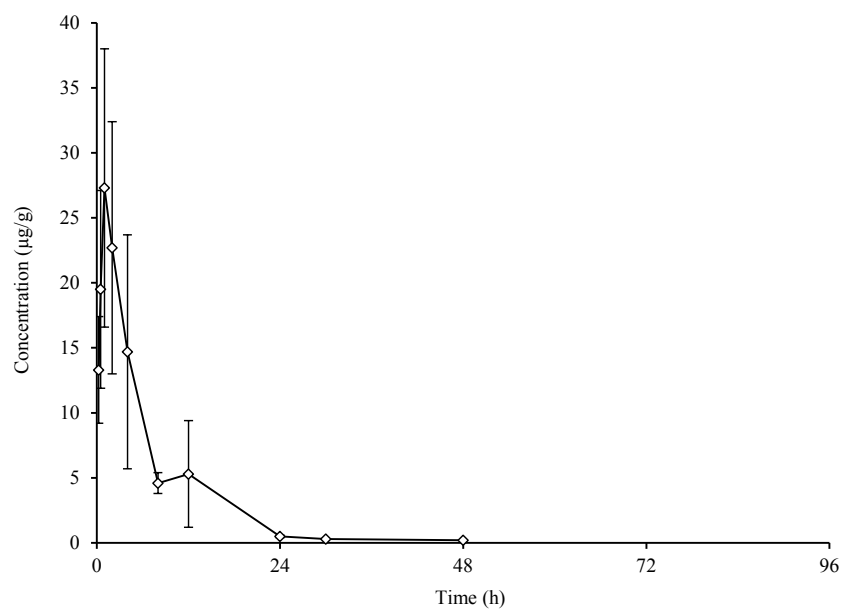


Figure 2 **Mean Concentration of Total Radioactivity in Plasma Following a Single Oral Administration of [¹⁴C]-Malathion to Male Rats at a Target Dose Level of 40 mg/kg**

Linear



Semi log

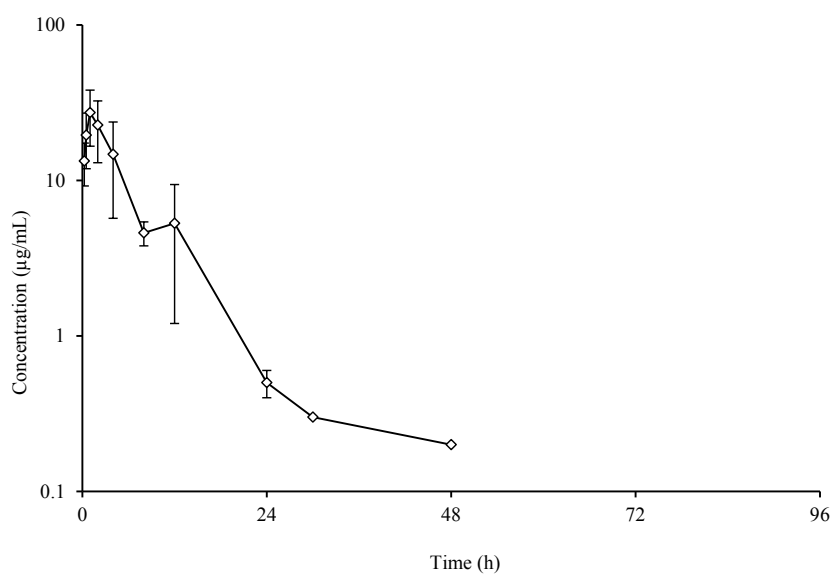
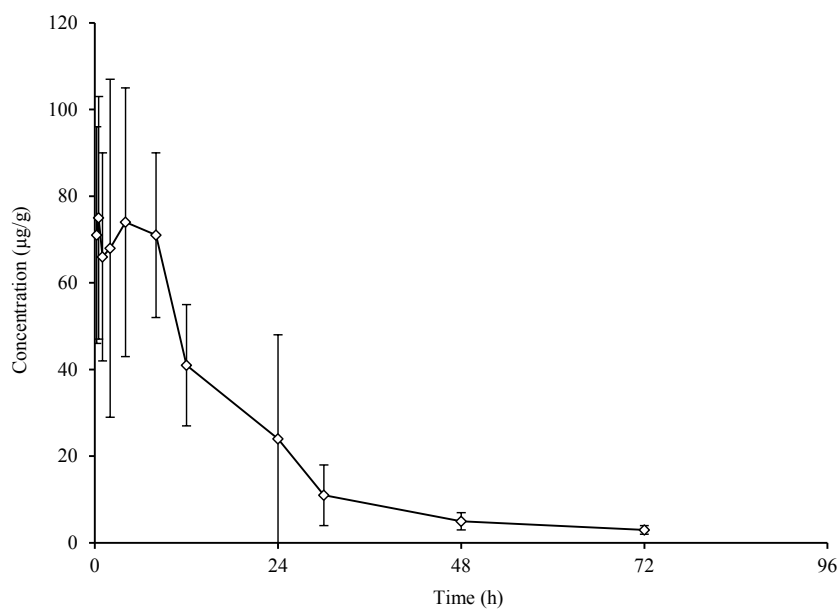


Figure 3 **Mean Concentration of Total Radioactivity in Whole Blood Following a Single Oral Administration of [¹⁴C]-Malathion to Male Rats at a Target Dose Level of 800 mg/kg**

Linear



Semi log

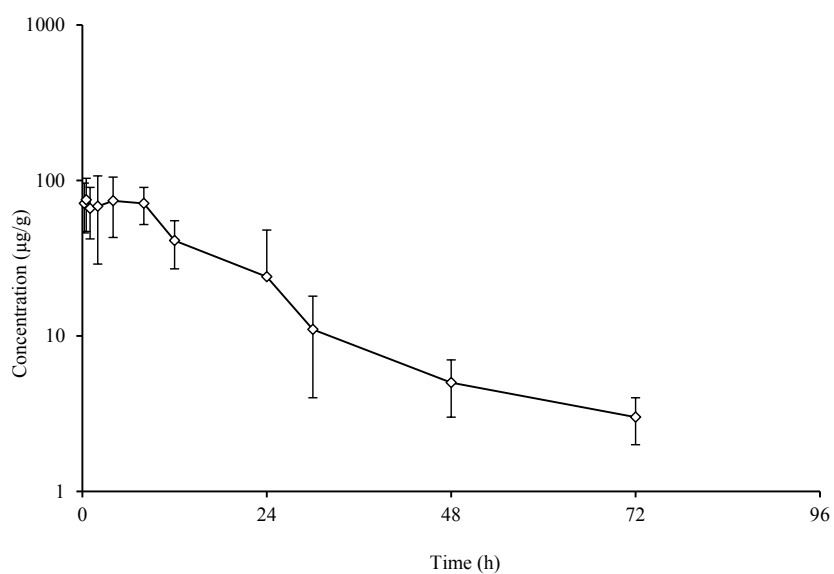
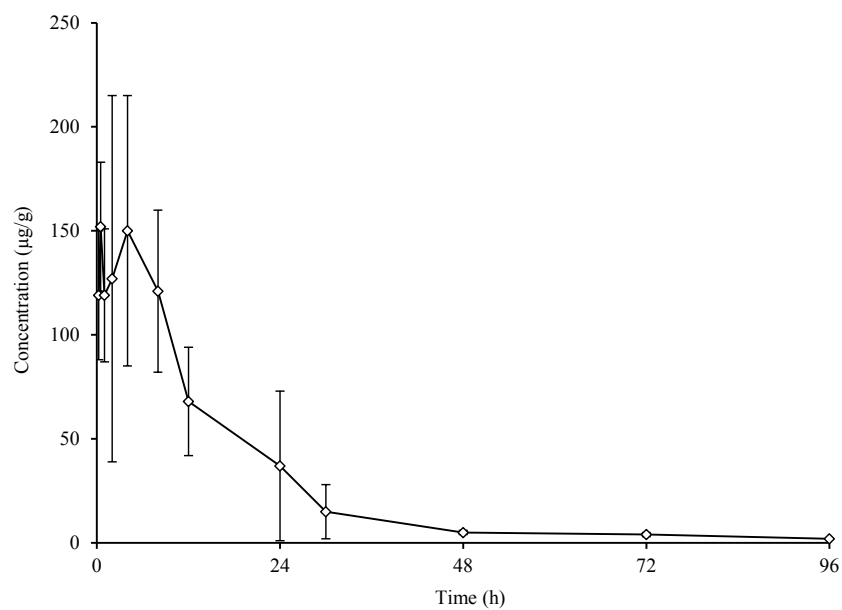


Figure 4 **Mean Concentration of Total Radioactivity in Plasma Following a Single Oral Administration of [¹⁴C]-Malathion to Male Rats at a Target Dose Level of 800 mg/kg**

Linear



Semi log

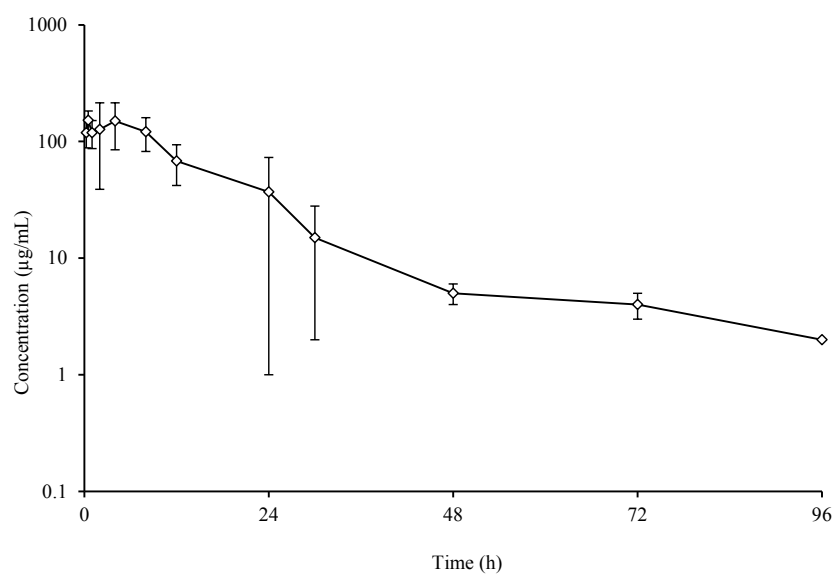
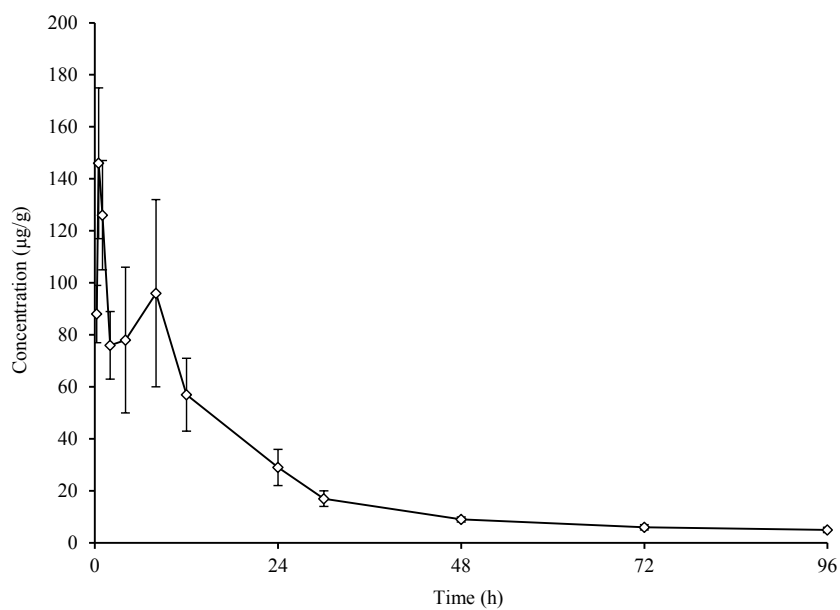


Figure 5 **Mean Concentration of Total Radioactivity in Whole Blood Following a Single Oral Administration of [¹⁴C]-Malathion to Male Rats at a Target Dose Level of 1200 mg/kg**

Linear



Semi log

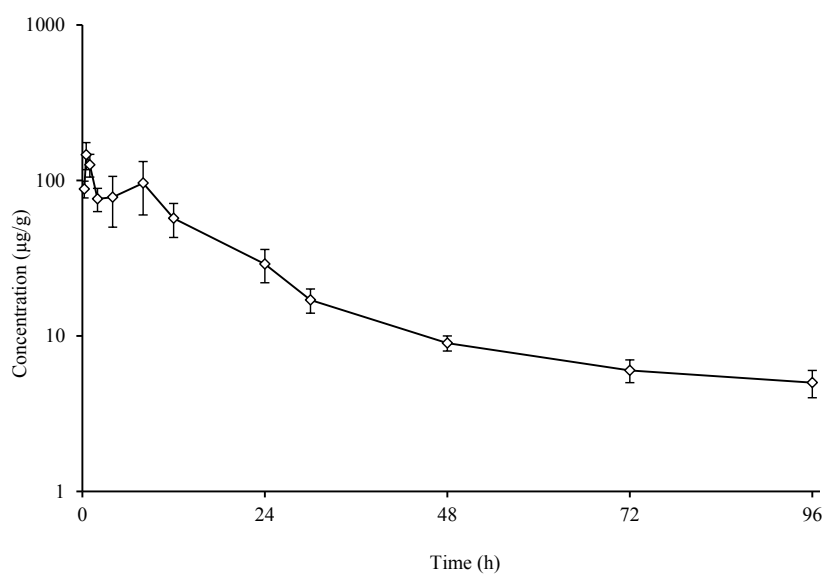
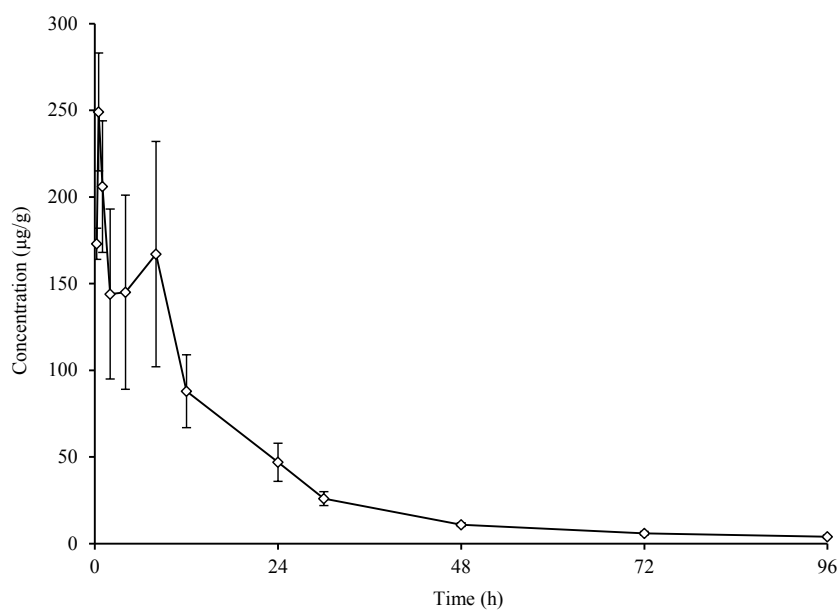


Figure 6 **Mean Concentration of Total Radioactivity in Plasma Following a Single Oral Administration of [¹⁴C]-Malathion to Male Rats at a Target Dose Level of 1200 mg/kg**

Linear



Semi log

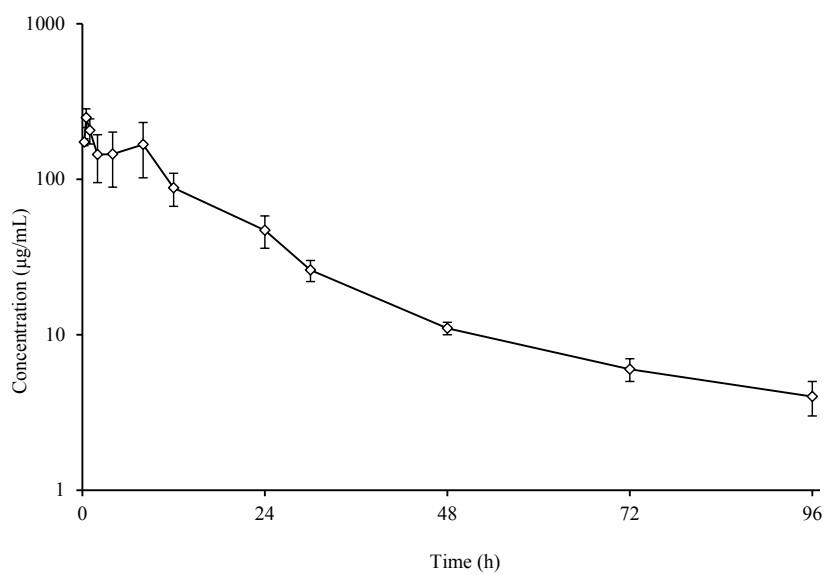
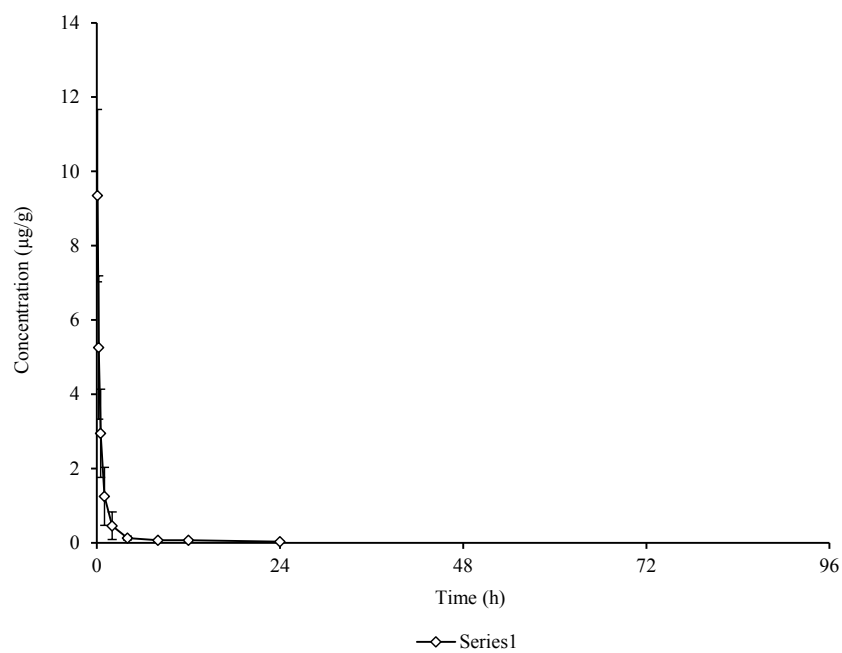


Figure 7 **Mean Concentration of Total Radioactivity in Whole Blood Following a Single Intravenous Administration of [^{14}C]-Malathion to Male Rats at a Target Dose Level of 4 mg/kg**

Linear



Semi log

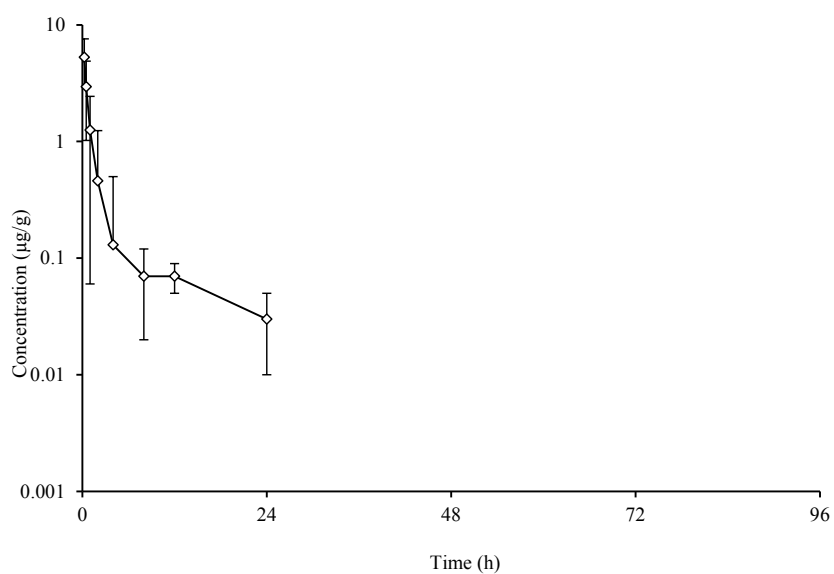
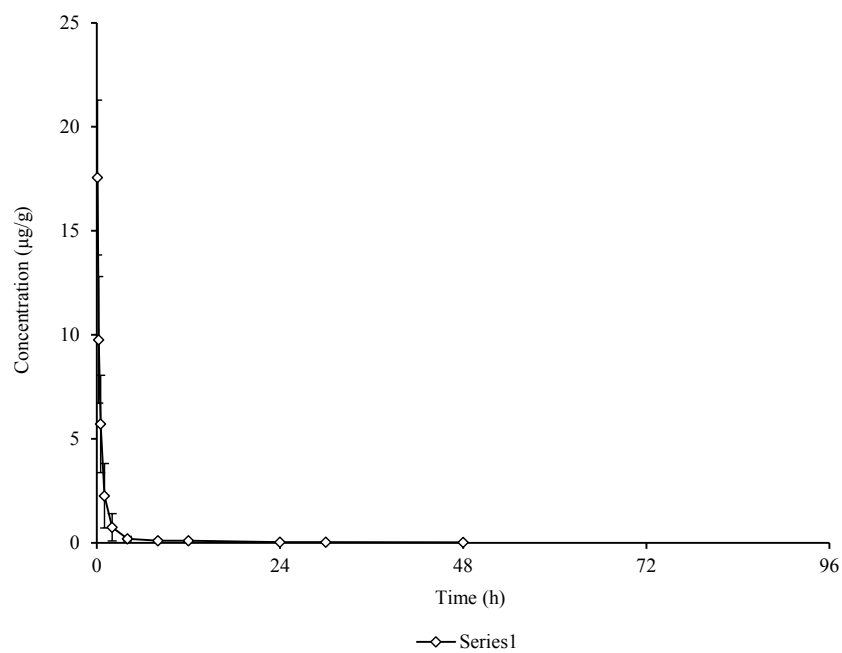


Figure 8 **Mean Concentration of Total Radioactivity in Plasma Following a Single Intravenous Administration of [¹⁴C]-Malathion to Male Rats at a Target Dose Level of 4 mg/kg**

Linear



Semi log

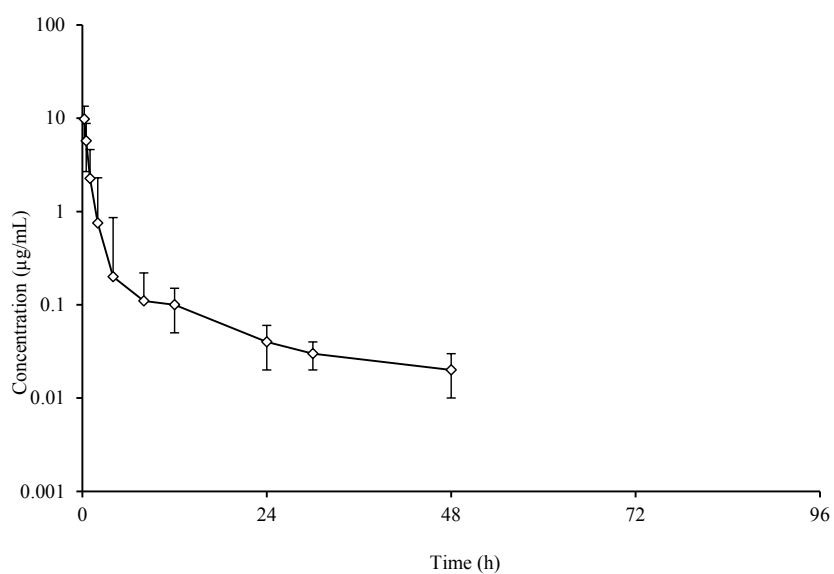
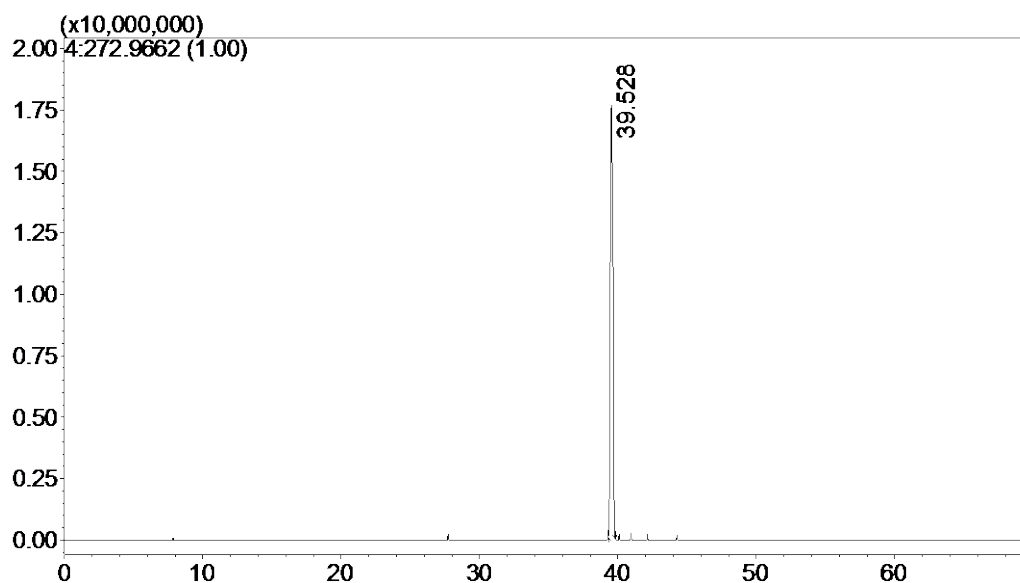


Figure 9 **Representative LC-MS Analysis of Reference Standard Malathion Dicarboxylic Acid**

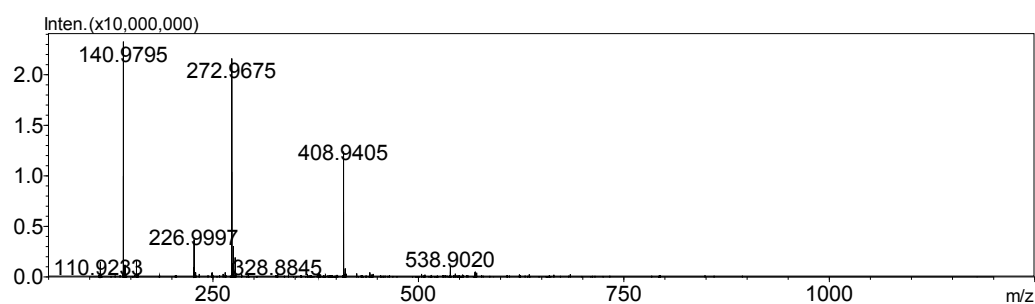
A. Extracted Ion Chromatogram for m/z 272.9662 ($[M-H]^-$)

MS File: CHE018012



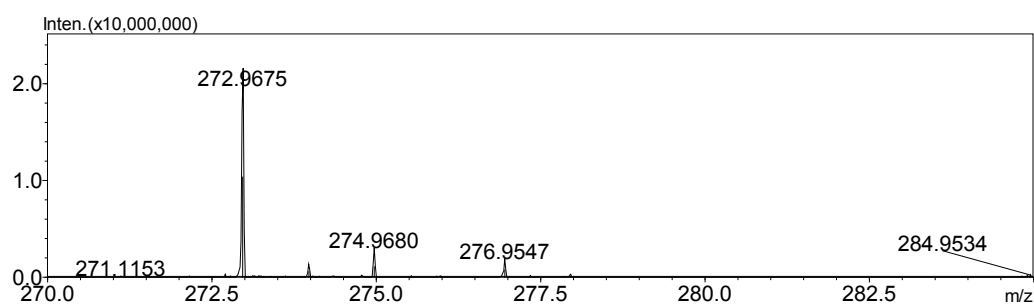
B. Full Scan Mass Spectrum

Event#: 4 MS(E-) Ret. Time : 39.380 -> 39.760 - 34.075 -> 39.202 Scan# : 7276 -> 7348 - 6291 -> 7240



**Figure 9 Representative LC-MS Analysis of Reference Standard
(continued) Malathion Dicarboxylic Acid****C. Expanded Full Scan Mass Spectrum**

Event#: 4 MS(E-) Ret. Time : 39.380 -> 39.760 - 34.075 -> 39.202 Scan# : 7276 -> 7348 - 6291 -> 7240

**D. Expanded MS/MS Fragmentation Spectrum**

Event#: 5 MS/MS(E-) Ret. Time : 39.443 -> 39.507 Scan# : 7289 -> 7301 Precursor : 272.9695 Cutoff : 75

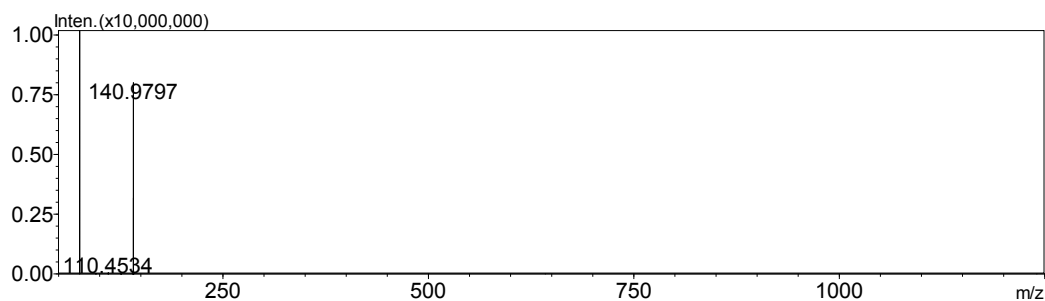


Figure 9 **Representative LC-MS Analysis of Reference Standard Malathion**
(continued) **Dicarboxylic Acid**

E. Postulated Structures of Observed Ions

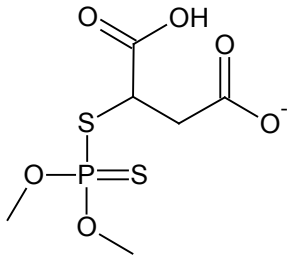
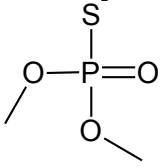
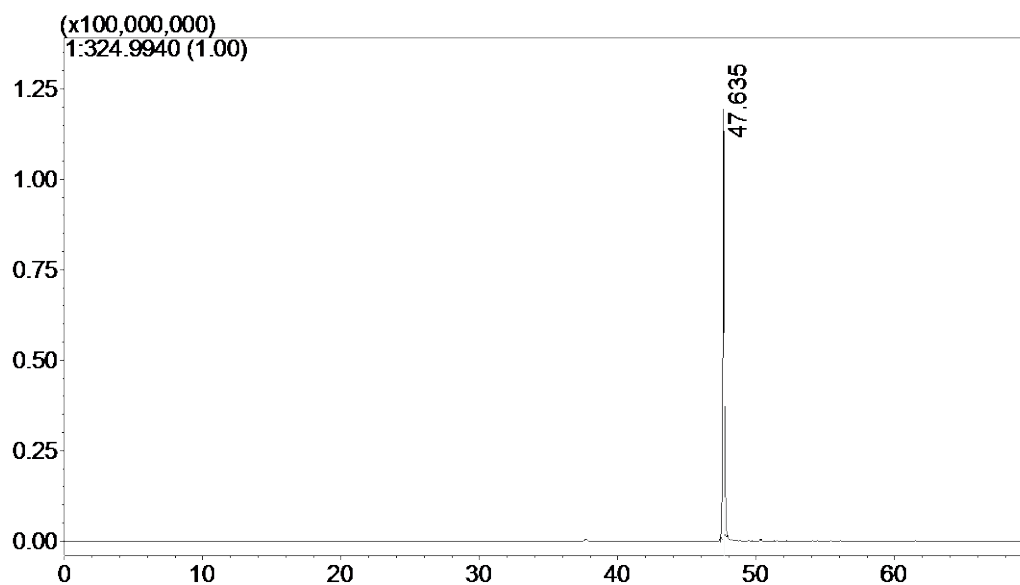
Observed Mass	Calculated Mass	Chemical Formula	Postulated Structure
272.9675	272.9662	$C_6H_{10}O_6PS_2$	
140.9797	140.9781	$C_2H_6O_3PS$	

Figure 10 **Representative LC-MS Analysis of Reference Standard Malathion Monocarboxylic Acid**

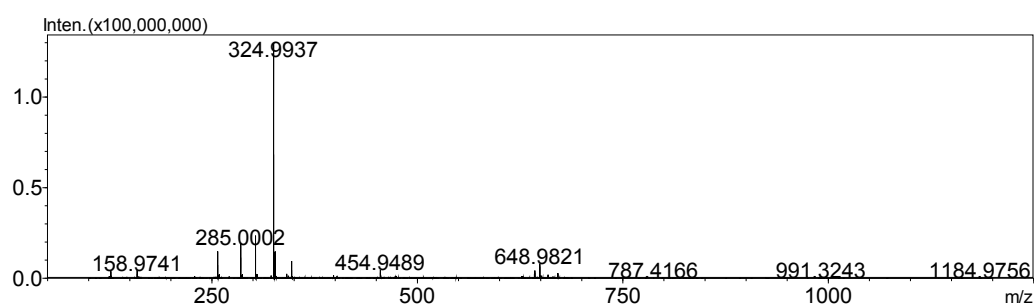
A. Extracted Ion Chromatogram for m/z 324.9940 ($[M+Na]^+$)

MS File: CHE018018



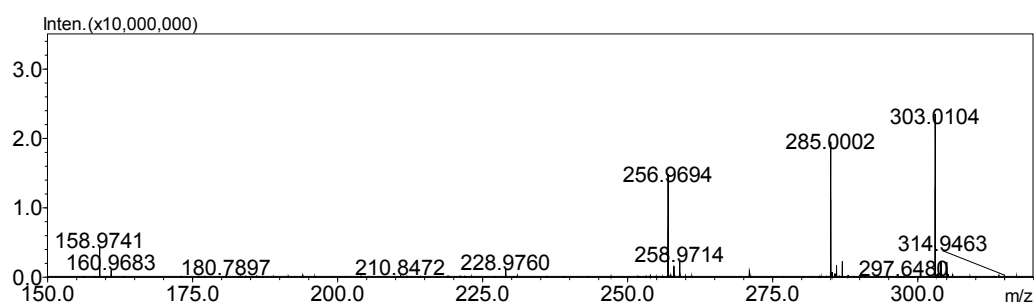
B. Full Scan Mass Spectrum

Event#: 1 MS(E+) Ret. Time : 47.535 -> 47.783 - 42.158 -> 47.392 Scan# : 8683 -> 8731 - 7692 -> 8653

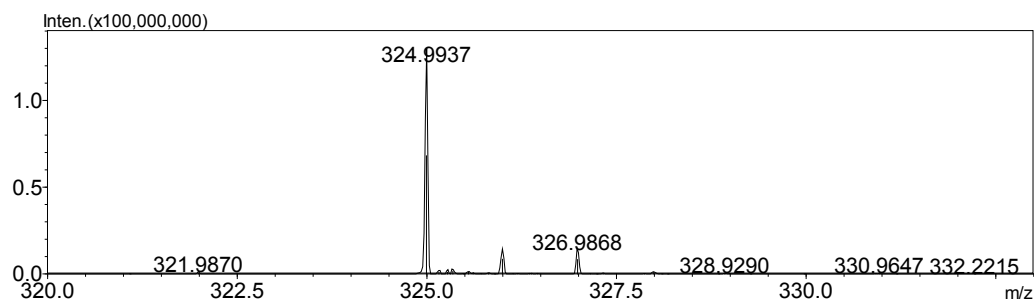


**Figure 10 Representative LC-MS Analysis of Reference Standard Malathion
(continued) Monocarboxylic Acid****C. Expanded Full Scan Mass Spectrum**

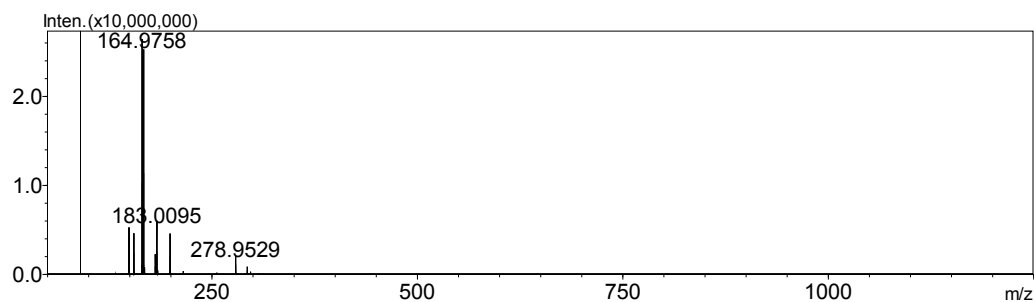
Event#: 1 MS(E+) Ret. Time : 47.535 -> 47.783 - 42.158 -> 47.380 Scan# : 8683 -> 8731 - 7692 -> 8653

**D. Expanded Full Scan Mass Spectrum**

Event#: 1 MS(E+) Ret. Time : 47.535 -> 47.783 - 42.158 -> 47.392 Scan# : 8683 -> 8731 - 7692 -> 8653

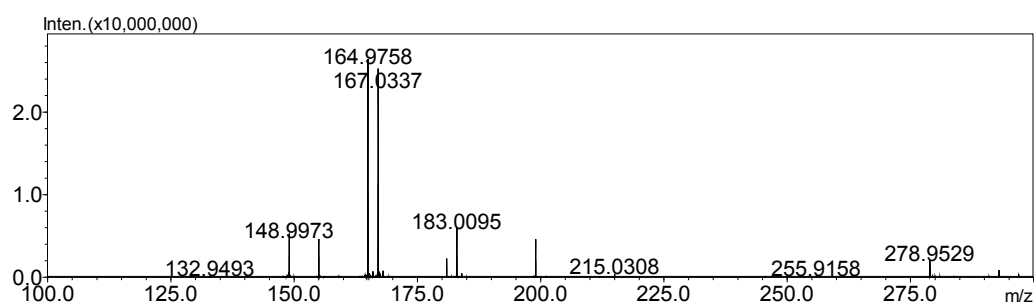
**E. MS/MS Fragmentation Spectrum**

Event#: 2 MS/MS(E+) Ret. Time : 47.535 -> 47.598 Scan# : 8684 -> 8696 Precursor : 324.9900 Cutoff : 89



**Figure 10 Representative LC-MS Analysis of Reference Standard Malathion
(continued) Monocarboxylic Acid****F. Expanded MS/MS Fragmentation Spectrum**

Event#: 2 MS/MS(E+) Ret. Time : 47.535 -> 47.598 Scan# : 8684 -> 8696 Precursor :
324.9900 Cutoff : 89



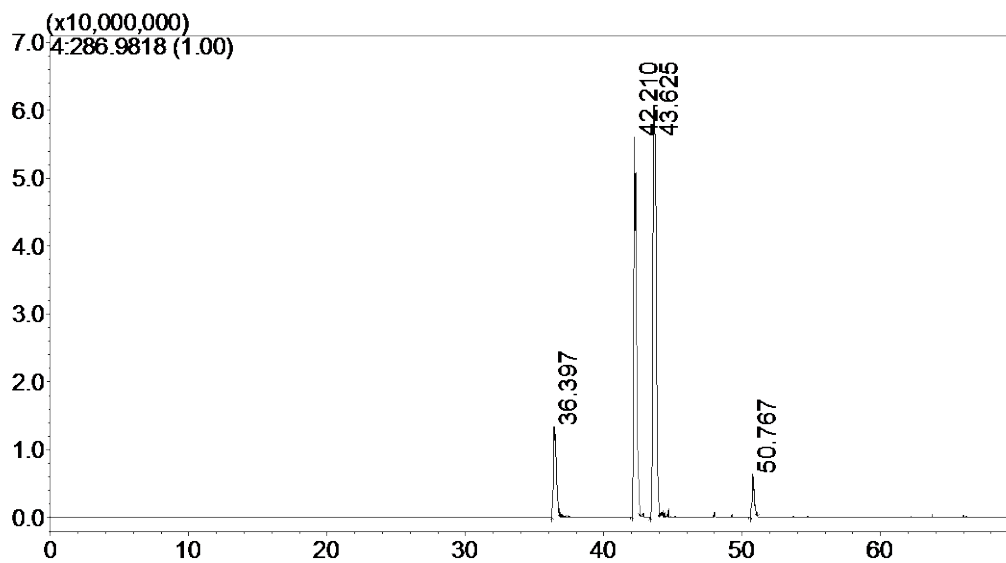
**Figure 10 Representative LC-MS Analysis of Reference Standard Malathion
(continued) Monocarboxylic Acid****G. Postulated Structures of Observed Ions**

Observed Mass	Calculated Mass	Chemical Formula	Postulated Structure
324.9937	324.9940	$C_8H_{15}NaO_6PS_2$	
303.0104	303.0120	$C_8H_{16}O_6PS_2$	
285.0002 (in-source)	285.0015	$C_8H_{14}O_5PS_2$	
256.9694 (in-source)	256.9702	$C_6H_{10}O_5PS_2$	
167.0337	167.0315	$C_6H_8NaO_4$	

Figure 11 **Representative LC-MS Analysis of Reference Standard Desmethyl Malathion Monocarboxylic Acid (42.2 min)**

A. Extracted Ion Chromatogram for m/z 286.9818 ($[M-H]^-$)

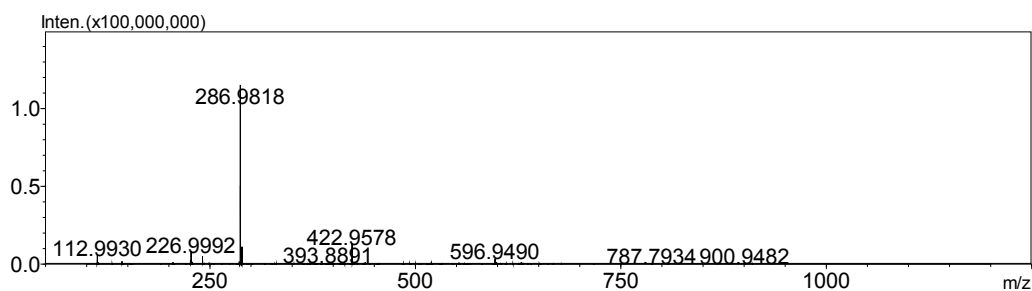
MS File: CHE018017



See explanation in section 8.6.1 for the reason why 4 peaks are observed in the EIC.

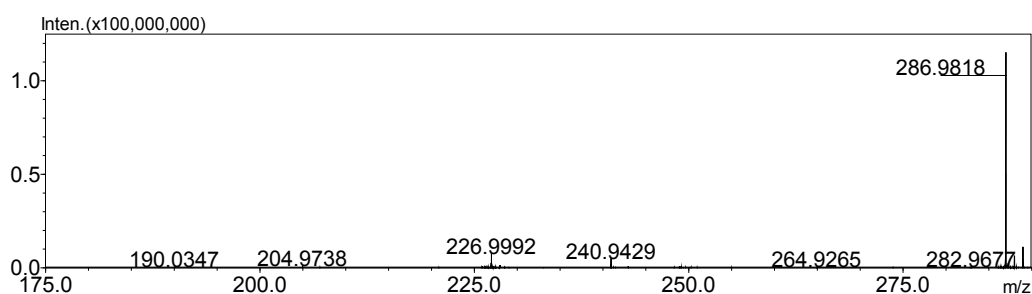
B. Full Scan Mass Spectrum (42.2 min)

Event#: 4 MS(E-) Ret. Time : 42.172 -> 42.362 - 38.510 -> 42.021 Scan# : 7697 -> 7733 - 7021 -> 7668

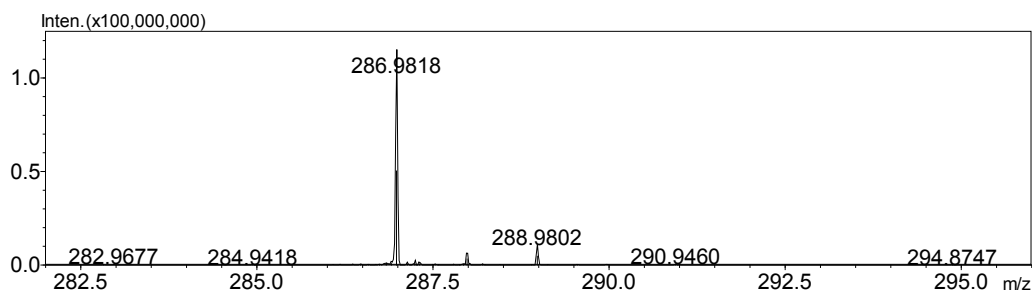


**Figure 11 Representative LC-MS Analysis of Reference Standard Desmethyl
(continued) Malathion Monocarboxylic Acid (42.2 min)****C. Expanded Full Scan Mass Spectrum (42.2 min)**

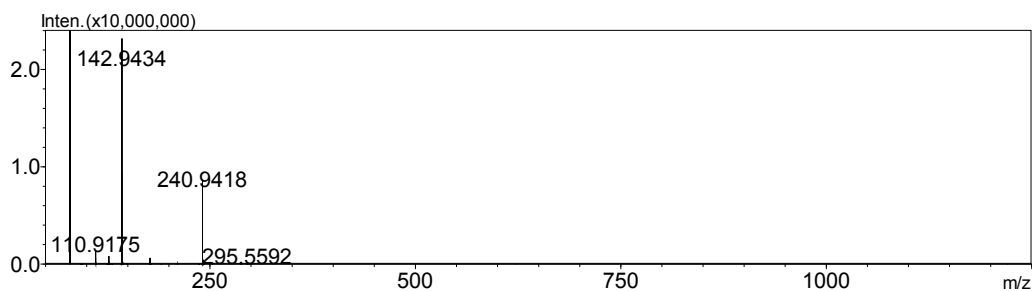
Event#: 4 MS(E-) Ret. Time : 42.172 -> 42.362 - 38.510 -> 42.021 Scan# : 7697 -> 7733 - 7021 -> 7668

**D. Expanded Full Scan Mass Spectrum (42.2 min)**

Event#: 4 MS(E-) Ret. Time : 42.172 -> 42.362 - 38.510 -> 42.021 Scan# : 7697 -> 7733 - 7021 -> 7668

**E. MS/MS Fragmentation Spectrum (42.2 min)**

Event#: 5 MS/MS(E-) Ret. Time : 42.362 -> 42.425 Scan# : 7734 -> 7746 Precursor : 286.9876 Cutoff : 79



**Figure 11 Representative LC-MS Analysis of Reference Standard Desmethyl
(continued) Malathion Monocarboxylic Acid (42.2 min)****F. Postulated Structures of Observed Ions**

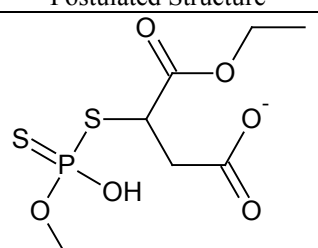
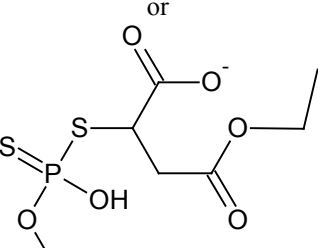
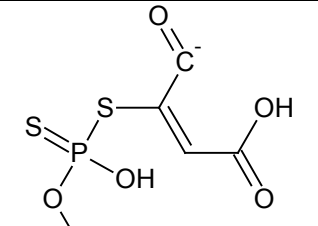
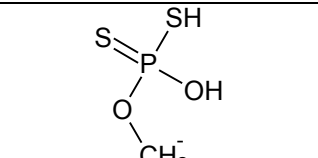
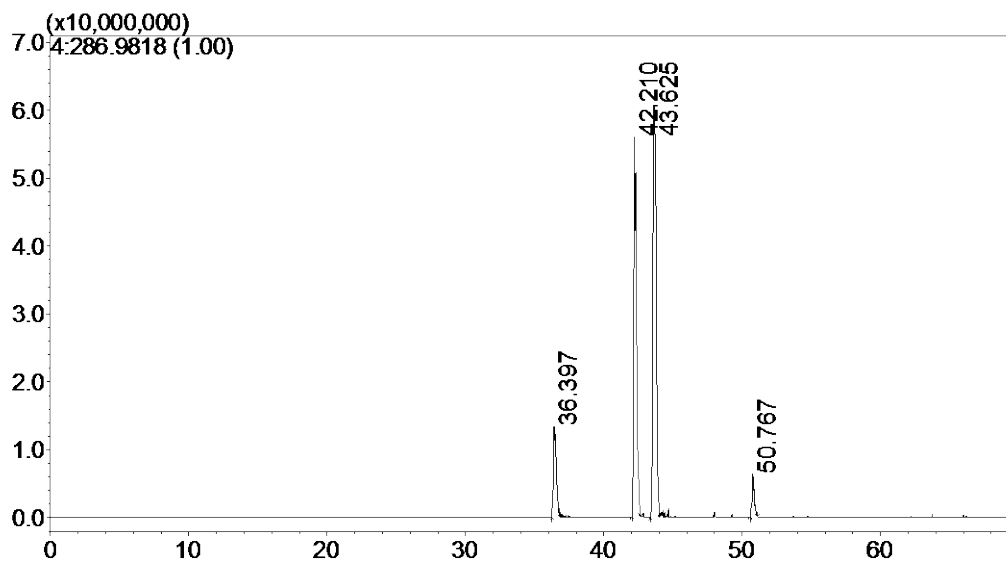
Observed Mass	Calculated Mass	Chemical Formula	Postulated Structure
286.9818	286.9818	$C_7H_{12}O_6PS_2$	 or 
240.9418	240.9400	$C_5H_6O_5PS_2$	
142.9434	142.9400	$CH_4O_2PS_2$	

Figure 12 **Representative LC-MS Analysis of Reference Standard Desmethyl Malathion Monocarboxylic Acid (43.6 min)**

A. Extracted Ion Chromatogram for m/z 286.9818 ($[M-H]^-$)

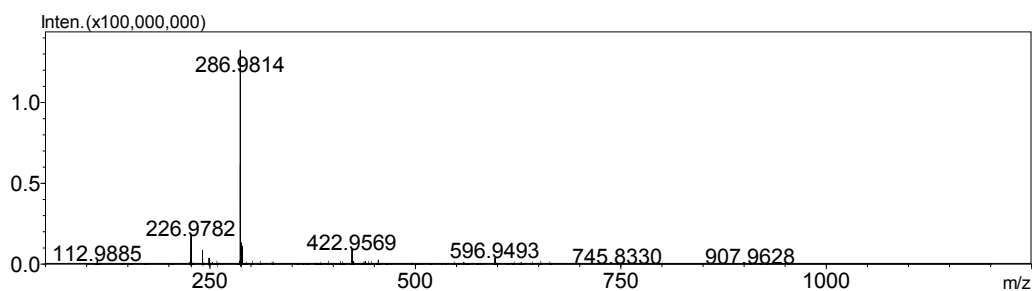
MS File: CHE018017



See explanation in section 8.6.1 for the reason why 4 peaks are observed in the EIC.

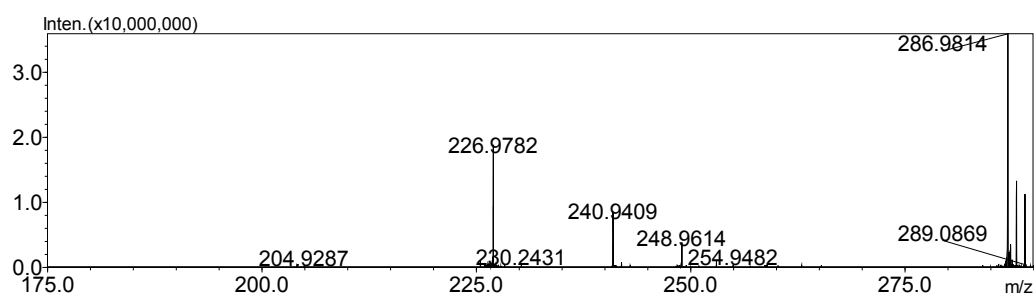
B. Full Scan Mass Spectrum (43.6 min)

Event#: 4 MS(E-) Ret. Time : 43.563 -> 43.770 - 44.135 -> 46.279 Scan# : 7956 -> 7994 - 8062 -> 8453

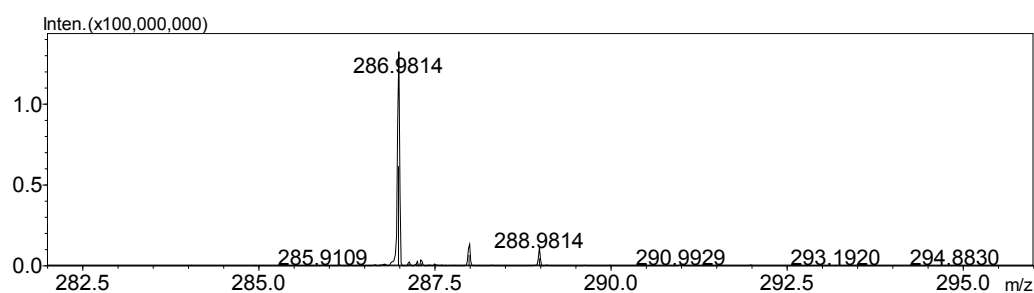


**Figure 12 Representative LC-MS Analysis of Reference Standard Desmethyl
(continued) Malathion Monocarboxylic Acid (43.6 min)****C. Expanded Full Scan Mass Spectrum (43.6 min)**

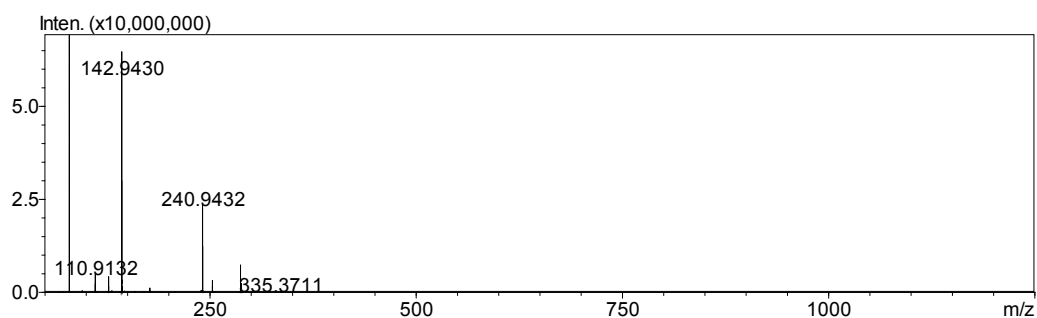
Event#: 4 MS(E-) Ret. Time : 43.563 -> 43.770 - 44.135 -> 46.279 Scan# : 7956 -> 7994 - 8062 -> 8453

**D. Expanded Full Scan Mass Spectrum (43.6 min)**

Event#: 4 MS(E-) Ret. Time : 43.563 -> 43.770 - 44.135 -> 46.279 Scan# : 7956 -> 7994 - 8062 -> 8453#

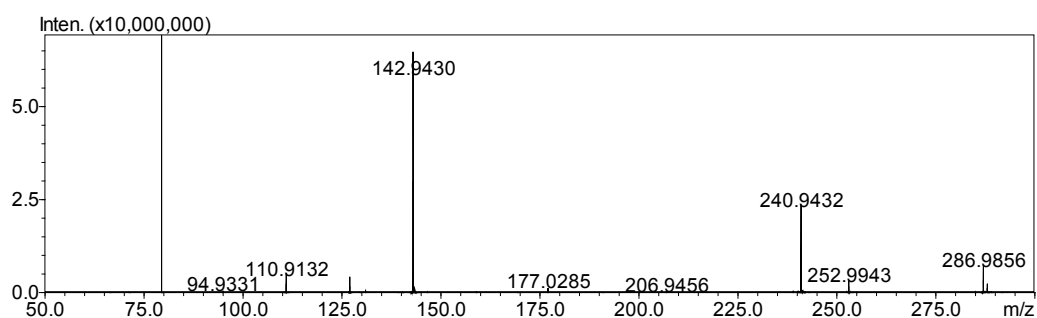
**E. MS/MS Fragmentation Spectrum (43.6 min)**

Event#: 5 MS/MS(E-) Ret. Time : 43.707 -> 43.770 Scan# : 7983 -> 7995 Precursor : 286.9839 Cutoff : 79

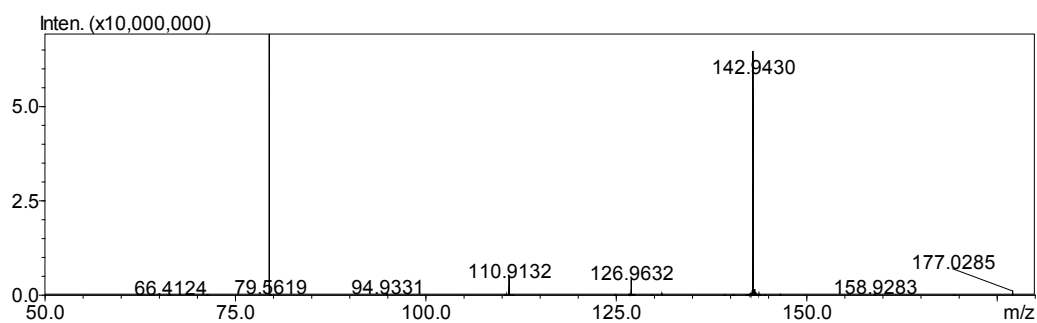


**Figure 12 Representative LC-MS Analysis of Reference Standard Desmethyl
(continued) Malathion Monocarboxylic Acid (43.6 min)****F. Expanded MS/MS Fragmentation Spectrum (43.6 min)**

Event#: 5 MS/MS(E-) Ret. Time : 43.707 -> 43.770 Scan# : 7983 -> 7995 Precursor :
286.9839 Cutoff : 79

**G. Expanded MS/MS Fragmentation Spectrum (43.6 min)**

Event#: 5 MS/MS(E-) Ret. Time : 43.707 -> 43.770 Scan# : 7983 -> 7995 Precursor :
286.9839 Cutoff : 79



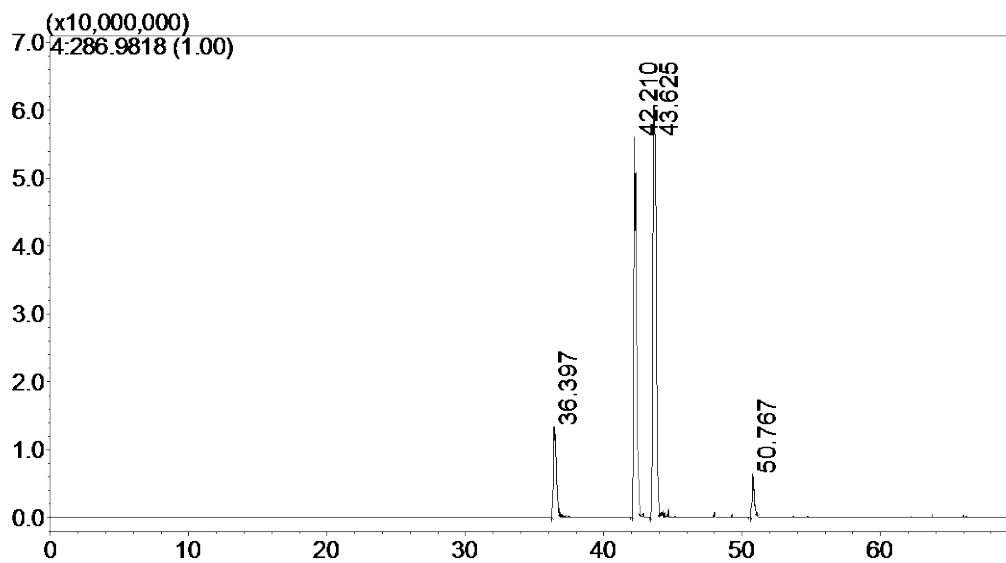
**Figure 12 Representative LC-MS Analysis of Reference Standard Desmethyl
(continued) Malathion Monocarboxylic Acid (43.6 min)****H. Postulated Structures of Observed Ions**

Observed Mass	Calculated Mass	Chemical Formula	Postulated Structure
286.9814	286.9818	$C_7H_{12}O_6PS_2$	
252.9943	252.9941	$C_7H_{10}O_6PS$	
240.9432	240.9400	$C_5H_6O_5PS_2$	
142.9430	142.9400	$CH_4O_2PS_2$	
126.9632	126.9624	CH_4O_3PS	

Figure 13 **Representative LC-MS Analysis of Reference Standard Desmethyl Malathion Monocarboxylic Acid (50.8 min)**

A. Extracted Ion Chromatogram for m/z 286.9818 ($[M-H]^-$)

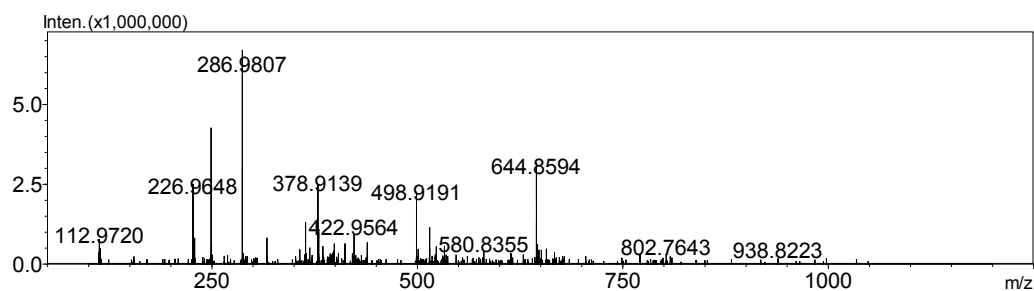
MS File: CHE018017



See explanation in section 8.6.1 for the reason why 4 peaks are observed in the EIC.

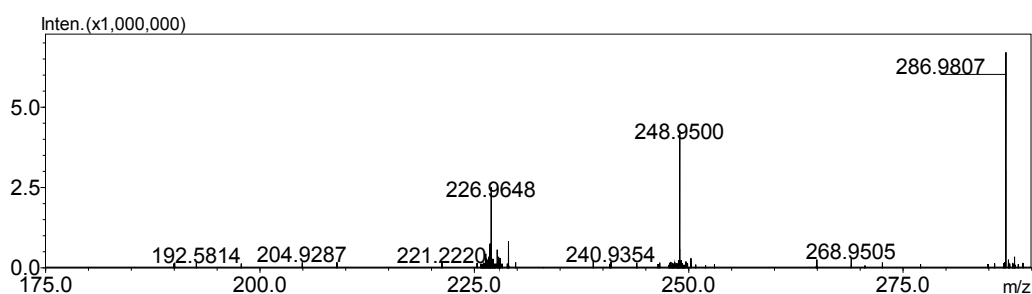
B. Full Scan Mass Spectrum (50.8 min)

Event#: 4 MS(E-) Ret. Time : 50.610 -> 50.977 - 48.587 -> 50.507 Scan# : 9255 -> 9323 - 8882 -> 9235

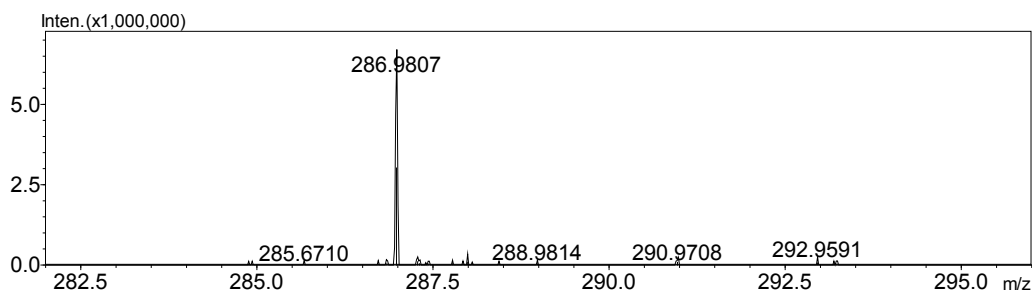


**Figure 13 Representative LC-MS Analysis of Reference Standard Desmethyl
(continued) Malathion Monocarboxylic Acid (50.8 min)****C. Expanded Full Scan Mass Spectrum (50.8 min)**

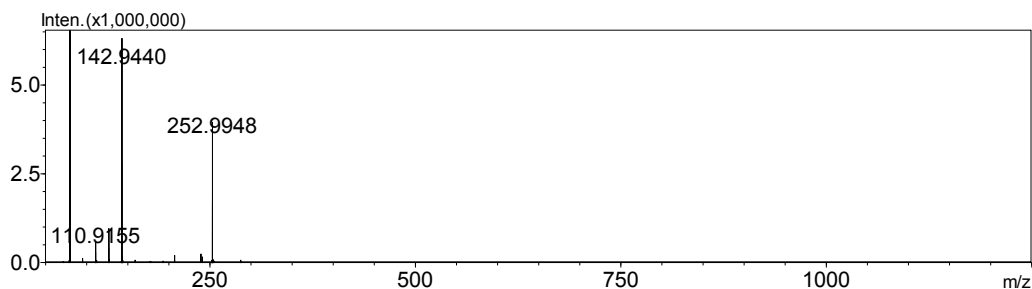
Event#: 4 MS(E-) Ret. Time : 50.610 -> 50.977 - 48.587 -> 50.507 Scan# : 9255 -> 9323 - 8882 -> 9235

**D. Expanded Full Scan Mass Spectrum (50.8 min)**

Event#: 4 MS(E-) Ret. Time : 50.610 -> 50.977 - 48.587 -> 50.507 Scan# : 9255 -> 9323 - 8882 -> 9235

**E. MS/MS Fragmentation Spectrum (50.8 min)**

Event#: 5 MS/MS(E-) Ret. Time : 50.702 -> 50.733 Scan# : 9274 -> 9280 Precursor : 286.9822 Cutoff : 79



**Figure 13 Representative LC-MS Analysis of Reference Standard Desmethyl
(continued) Malathion Monocarboxylic Acid (50.8 min)****F. Expanded MS/MS Fragmentation Spectrum (50.8 min)**

Event#: 5 MS/MS(E-) Ret. Time : 50.702 -> 50.733 Scan# : 9274 -> 9280 Precursor :
286.9822 Cutoff : 79

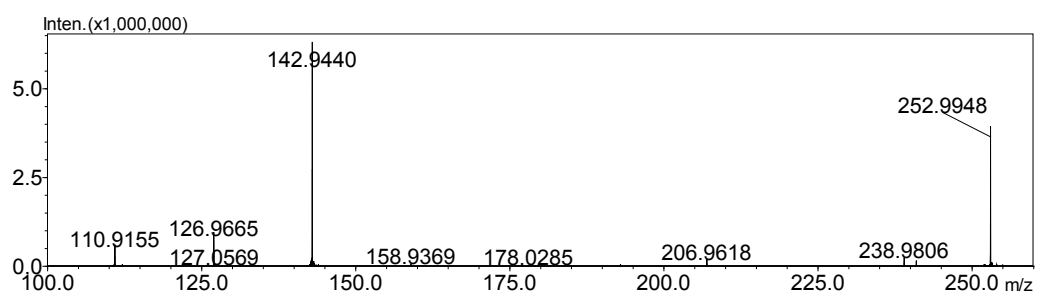


Figure 13 **Representative LC-MS Analysis of Reference Standard Desmethyl**
(continued) **Malathion Monocarboxylic Acid (50.8 min)****G. Postulated Structures of Observed Ions**

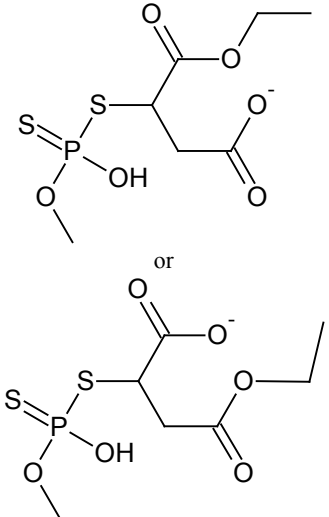
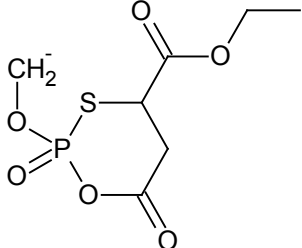
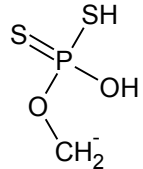
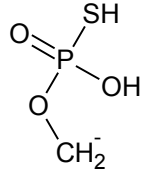
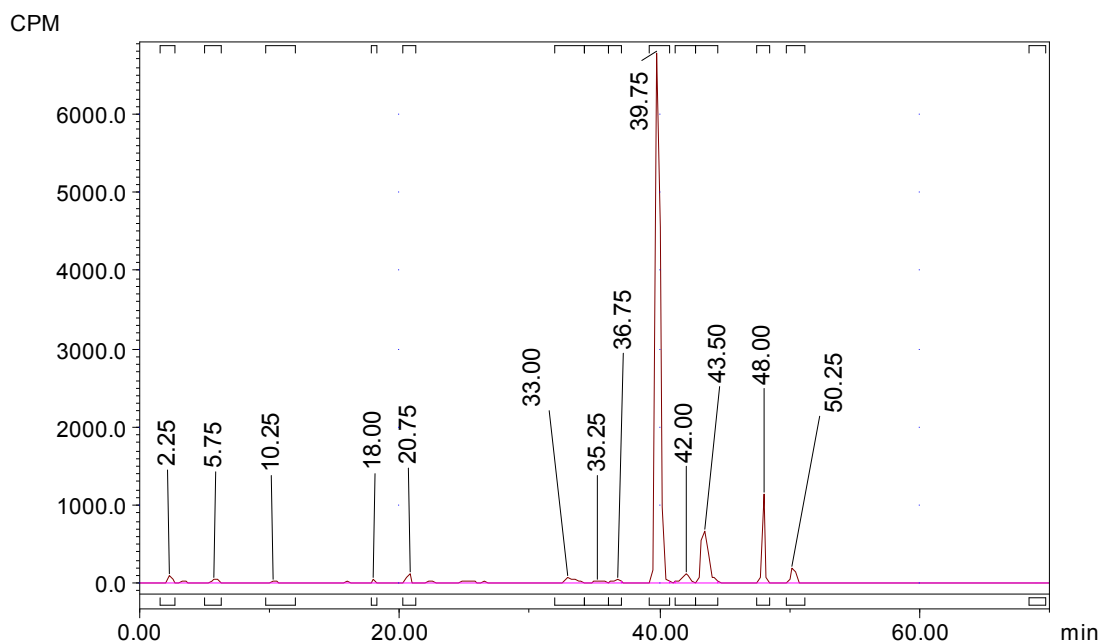
Observed Mass	Calculated Mass	Chemical Formula	Postulated Structure
286.9807	286.9818	$C_7H_{12}O_6PS_2$	
252.9948	252.9941	$C_7H_{10}O_6PS$	
142.9440	142.9400	$CH_4O_2PS_2$	
126.9665	126.9624	CH_4O_3PS	

Figure 14 Radiochromatogram of Plasma Collected from Male Rats 1.5 h after Oral Administration of [¹⁴C]-Malathion (800 mg/kg)

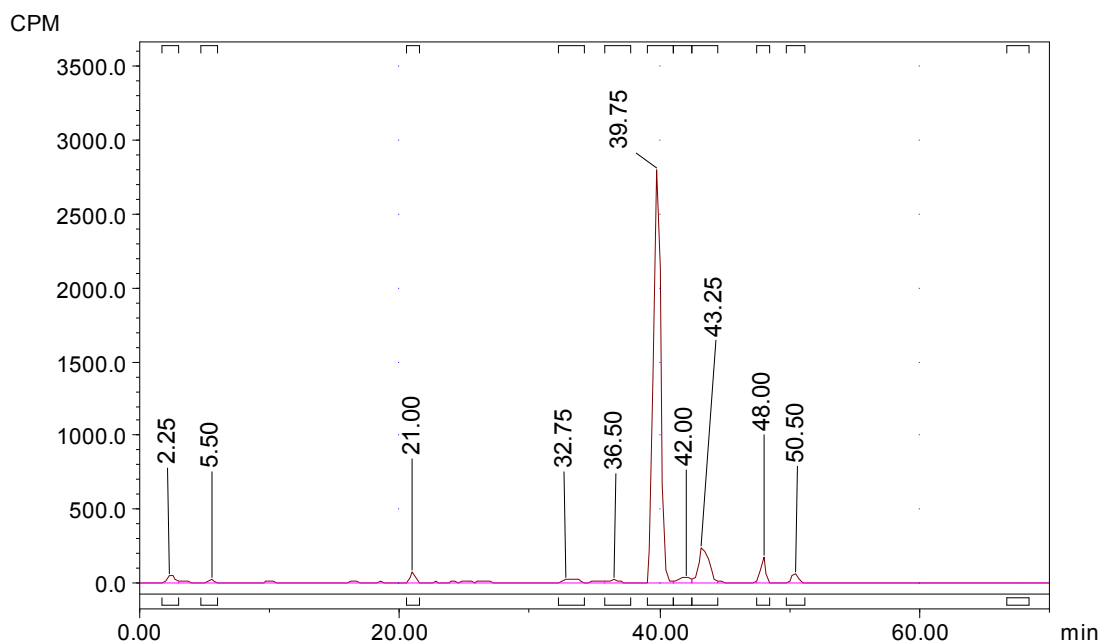
MS File Name: CHE018099
 Laura File Name: 11884 025 P1S.7E.Evaluation
 Sample Name: P1S.7E
 Concentration: 168.574 µg equiv/g



Name	Retention (min)	%ROI (%)	µg equiv/g
Region 1	2.25	0.81	1.365
Region 2	5.75	0.71	1.191
Region 3	10.25	0.52	0.875
Region 4	18.00	0.25	0.418
Metabolite 1 (m/z 259)	20.75	1.06	1.795
Region 6	33.00	1.67	2.811
Region 7	35.25	0.58	0.970
Desmethyl Malathion Monocarboxylic Acid 1	36.75	0.56	0.943
Malathion Dicarboxylic Acid	39.75	71.93	121.264
Desmethyl Malathion Monocarboxylic Acid 1	42.00	2.14	3.607
Desmethyl Malathion Monocarboxylic Acid 2	43.50	10.34	17.438
Malathion Monocarboxylic Acid	48.00	7.28	12.270
Desmethyl Malathion Monocarboxylic Acid 2	50.25	2.15	3.627
13 Peaks		100.00	

Figure 15 **Radiochromatogram of Plasma Collected from Male Rats 1.5 h after Oral Administration of [¹⁴C]-Malathion (1200 mg/kg)**

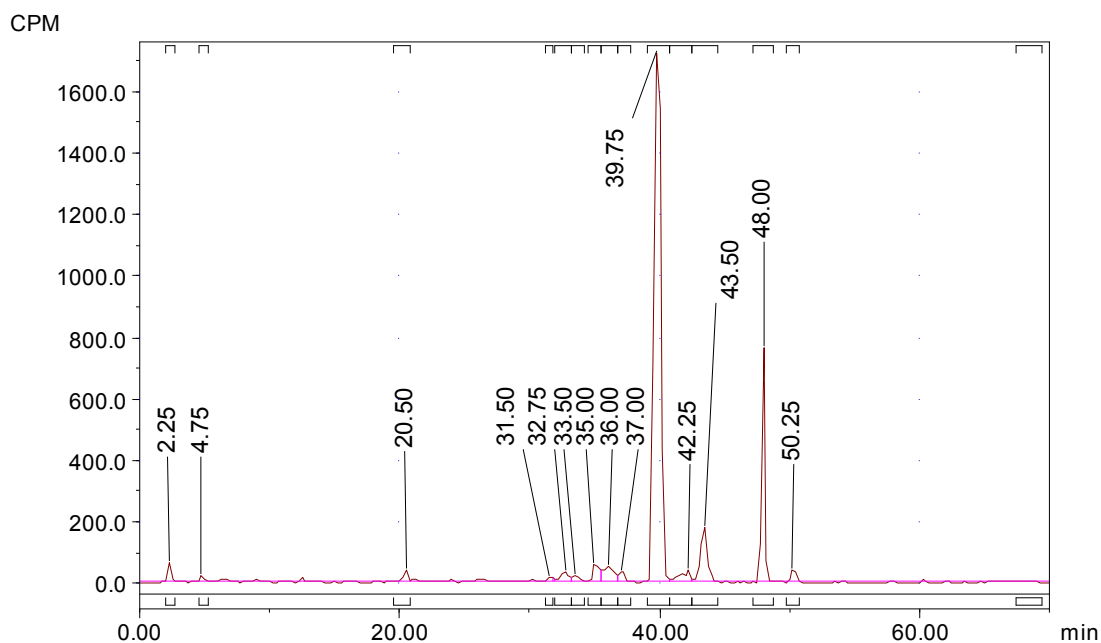
MS File Name: CHE018133
Laura File Name: 12015 031 P2S.5E re-run.Evaluation
Sample Name: P2S.5E
Concentration: 149.921 µg equiv/g



Name	Retention (min)	%ROI (%)	µg equiv/g
Region 1	2.25	1.40	2.099
Region 2	5.50	0.45	0.681
Metabolite 1 (m/z 259)	21.00	1.49	2.230
Region 4	32.75	1.57	2.350
Desmethyl Malathion Monocarboxylic Acid 1	36.50	0.70	1.045
Malathion Dicarboxylic Acid	39.75	78.27	117.342
Desmethyl Malathion Monocarboxylic Acid 1	42.00	1.53	2.290
Desmethyl Malathion Monocarboxylic Acid 2	43.25	9.47	14.191
Malathion Monocarboxylic Acid	48.00	3.63	5.436
Desmethyl Malathion Monocarboxylic Acid 2	50.50	1.51	2.258
10 Peaks		100.00	

Figure 16 Radiochromatogram of Red Blood Cells Collected from Male Rats 1.5 h after Oral Administration of [¹⁴C]-Malathion (800 mg/kg)

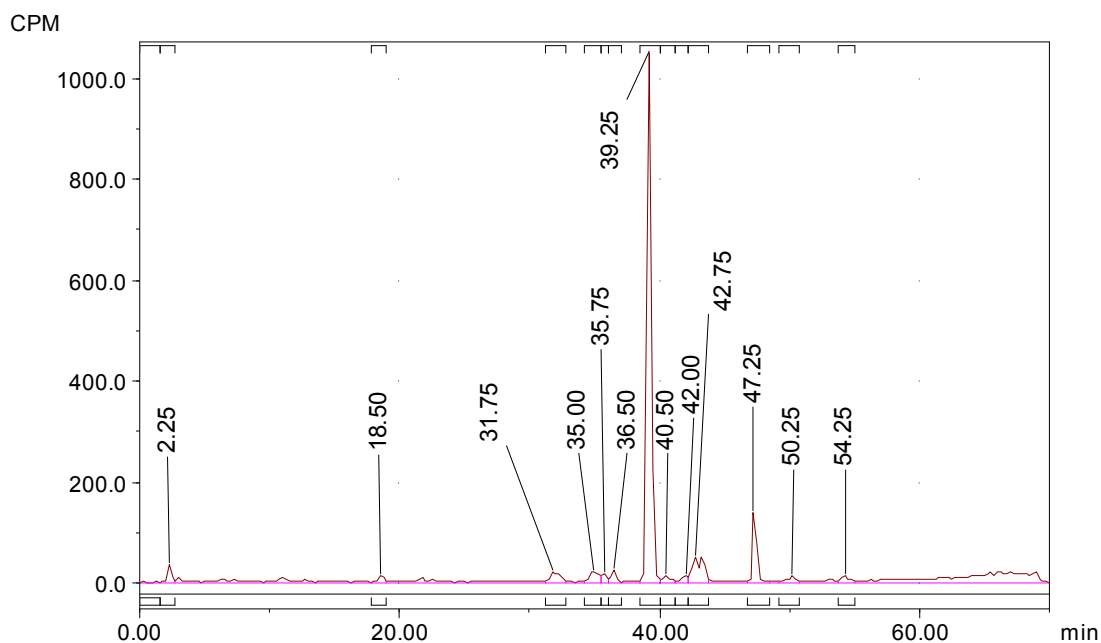
MS File Name: CHE018098
 Laura File Name: 11989 024 R1S.5E.Evaluation
 Sample Name: R1S.5E
 Concentration: 70.967 µg equiv/g



Name	Retention (min)	%ROI (%)	µg equiv/g
Region 1	2.25	0.96	0.680
Region 2	4.75	0.35	0.251
Metabolite 1 (m/z 259)	20.50	0.81	0.575
Region 3	31.50	0.15	0.110
Region 4	32.75	1.28	0.908
Region 5	33.50	0.67	0.477
Region 6	35.00	1.53	1.089
Desmethyl Malathion Monocarboxylic Acid 1	36.00	2.84	2.013
Region 8	37.00	1.22	0.864
Malathion Dicarboxylic Acid	39.75	66.75	47.368
Desmethyl Malathion Monocarboxylic Acid 1	42.25	1.80	1.275
Desmethyl Malathion Monocarboxylic Acid 2	43.50	6.46	4.581
Malathion Monocarboxylic Acid	48.00	14.12	10.018
Desmethyl Malathion Monocarboxylic Acid 2	50.25	1.07	0.756
14 Peaks		100.00	

Figure 17 **Radiochromatogram of Red Blood Cells Collected from Male Rats 1.5 h after Oral Administration of [¹⁴C]-Malathion (1200 mg/kg)**

MS File Name: CHE018020
 Laura File Name: 11854 020 R2S.5E.Evaluation
 Sample Name: R2.5E
 Concentration: 47.669 µg equiv/g

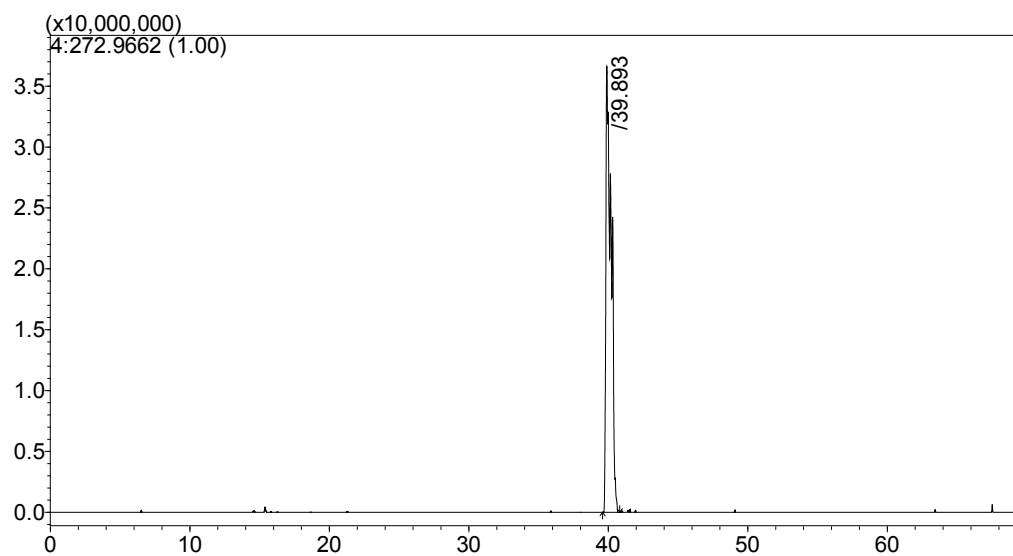


Name	Retention (min)	%ROI (%)	µg equiv/g
Region 1	2.25	1.82	0.869
Metabolite 1 (m/z 259)	18.50	1.06	0.503
Region 2	31.75	2.44	1.162
Region 3	35.00	2.38	1.134
Desmethyl Malathion Monocarboxylic Acid 1	35.75	1.02	0.487
Region 5	36.50	1.73	0.824
Malathion Dicarboxylic Acid	39.25	69.78	33.262
Region 6	40.50	1.16	0.553
Desmethyl Malathion Monocarboxylic Acid 1	42.00	1.03	0.492
Desmethyl Malathion Monocarboxylic Acid 2	42.75	7.14	3.403
Malathion Monocarboxylic Acid	47.25	8.14	3.878
Desmethyl Malathion Monocarboxylic Acid 2	50.25	1.22	0.581
Region 8	54.25	1.09	0.520
13 Peaks		100.00	

Figure 18 **Representative LC-MS Analysis of Malathion Dicarboxylic Acid**
confirmed at 39.8 min in Plasma (P2)

A. Extracted Ion Chromatogram for m/z 272.9662 ($[M-H]^-$)

MS File: CHE018133



B. Full Scan Mass Spectrum

Event#: 4 MS(E-) Ret. Time : 40.068 - 39.405 -> 39.649 Scan# : 7407 - 7283 -> 7331

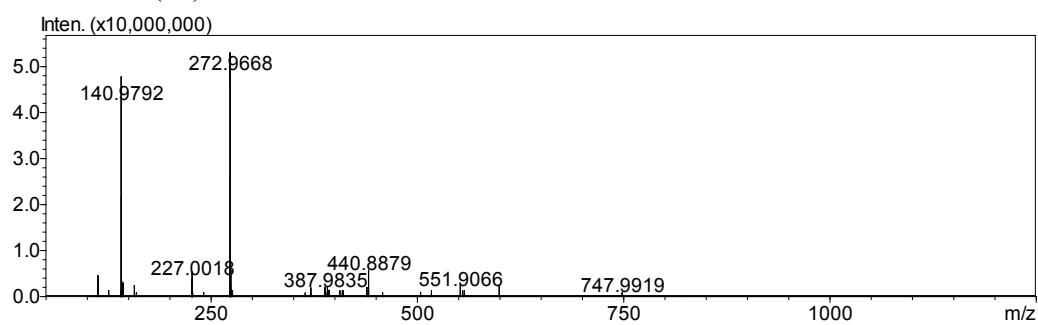
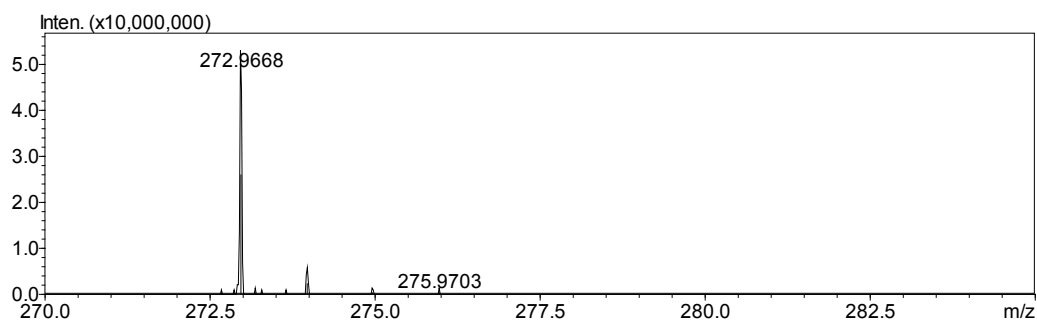


Figure 18 **Representative LC-MS Analysis of Malathion Dicarboxylic Acid**
(continued) **confirmed at 39.8 min in Plasma (P2)**

C. Expanded Full Scan Mass Spectrum

Event#: 4 MS(E-) Ret. Time : 40.068 - 39.405 -> 39.649 Scan# : 7407 - 7283 -> 7331



D. Expanded MS/MS Product Ion Spectrum

Event#: 5 MS/MS(E-) Ret. Time : 40.068 Scan# : 7408 Precursor : 272.9677 Cutoff : 75

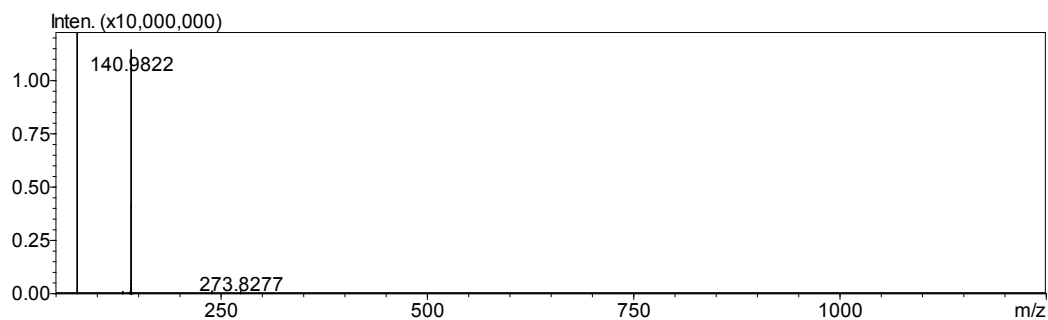


Figure 18 **Representative LC-MS Analysis of Malathion Dicarboxylic Acid**
(continued) **confirmed at 39.8 min in Plasma (P2)**

E. Postulated Structures of Observed Ions

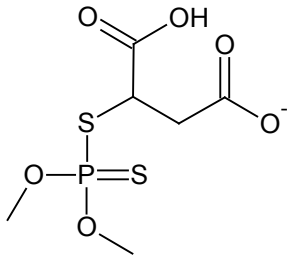
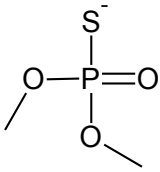
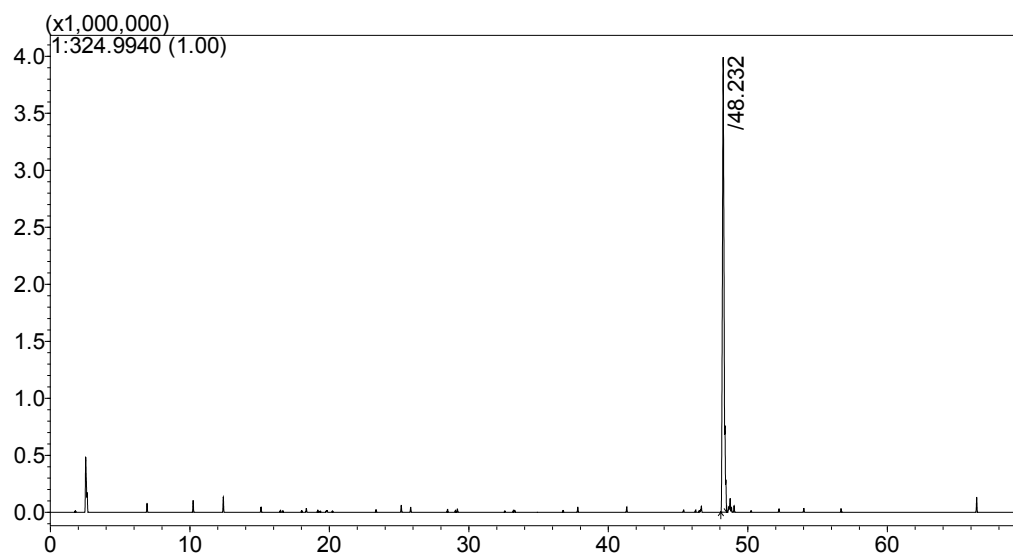
Observed Mass	Calculated Mass	Chemical Formula	Postulated Structure
272.9668	272.9662	$C_6H_{10}O_6PS_2$	 <p>The structure shows a central carbon atom bonded to a carboxylic acid group (COOH) and a carboxylate group (COO⁻). This central carbon is also bonded to a sulfur atom, which is part of a phosphorus-containing group. The phosphorus atom is double-bonded to a sulfur atom and single-bonded to two methoxy groups (OCH₃).</p>
140.9822	140.9781	$C_2H_6O_3PS$	 <p>The structure shows a phosphorus atom double-bonded to an oxygen atom and single-bonded to two methoxy groups (OCH₃). The phosphorus atom is also single-bonded to a sulfur atom with a negative charge (S⁻).</p>

Figure 19 **Representative LC-MS Analysis of Malathion Monocarboxylic Acid confirmed at 48.0 min in Plasma (P2)**

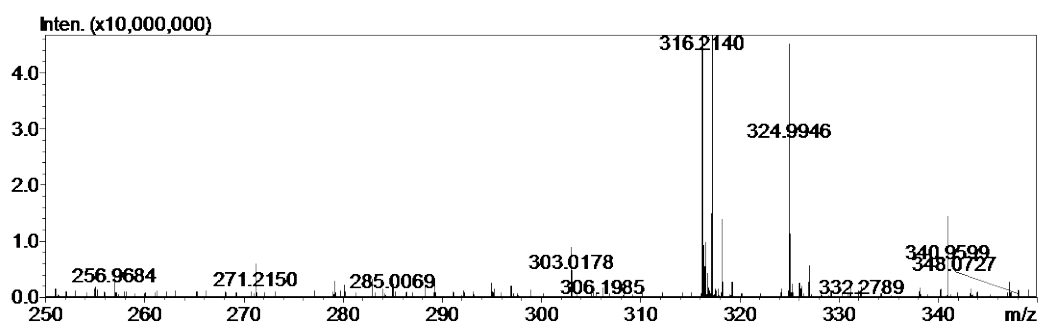
A. Extracted Ion Chromatogram for m/z 324.9940 ($[M+Na]^+$)

MS File: CHE018133



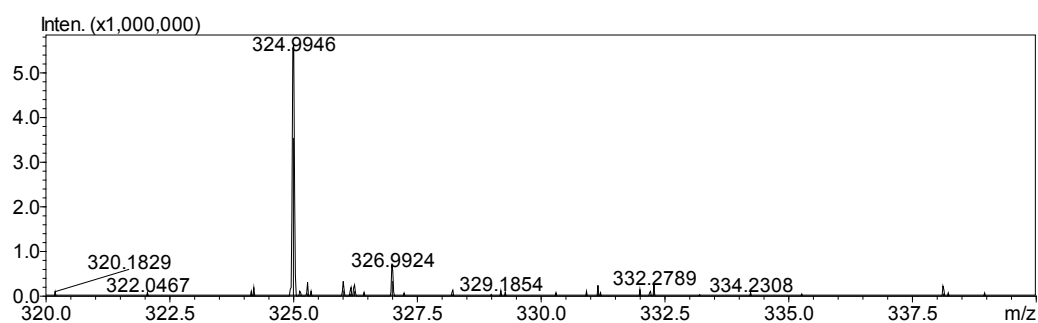
B. Expanded Full Scan Mass Spectrum

Event#: 1 MS(E+) Ret. Time : 48.148 -> 48.237 - 47.758 -> 48.082 Scan# : 8895 -> 8911 - 8823 -> 8883



**Figure 19 Representative LC-MS Analysis of Malathion Monocarboxylic Acid
(continued) confirmed at 48.0 min in Plasma (P2)****C. Expanded Full Scan Mass Spectrum**

Event#: 1 MS(E+) Ret. Time : 48.148 -> 48.237 - 47.758 -> 48.082 Scan# : 8895 -> 8911 - 8823 -> 8883

**D. MS/MS Product Ion Spectrum**

Event#: 2 MS/MS(E+) Ret. Time : 48.205 Scan# : 8906 Precursor : 324.9840 Cutoff : 89

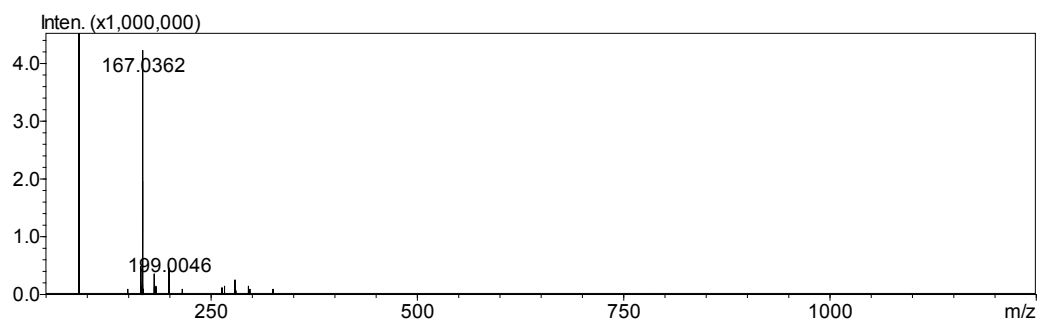


Figure 19 **Representative LC-MS Analysis of Malathion Monocarboxylic Acid**
(continued) **confirmed at 48.0 min in Plasma (P2)****E. Postulated Structures of Observed Ions**

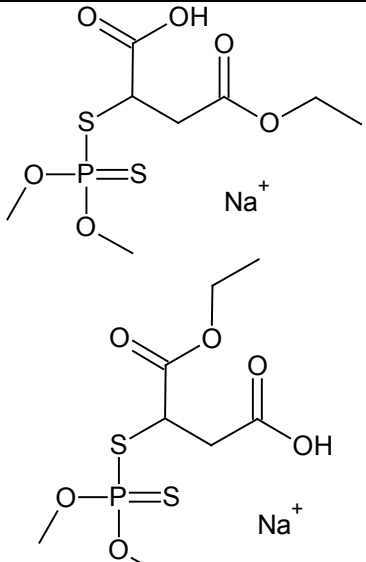
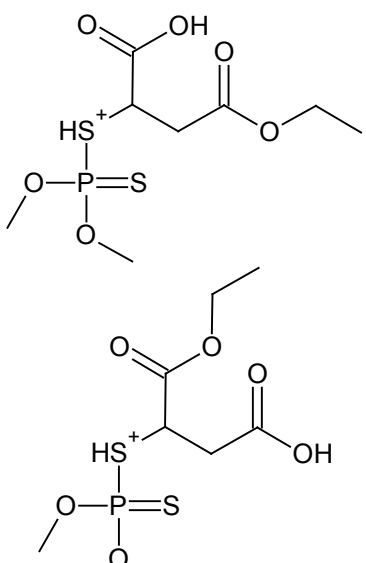
Observed Mass	Calculated Mass	Chemical Formula	Postulated Structure
324.9946	324.9940	$C_8H_{15}NaO_6PS_2$	
303.0178 (in-source)	303.0120	$C_8H_{16}O_6PS_2$	

Figure 19 **Representative LC-MS Analysis of Malathion Monocarboxylic Acid**
(continued) **confirmed at 48.0 min in Plasma (P2)****E. Postulated Structures of Observed Ions (Continued)**

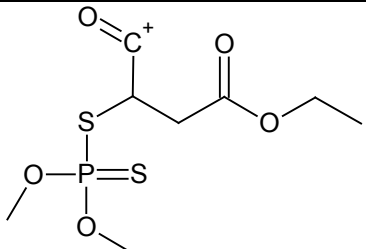
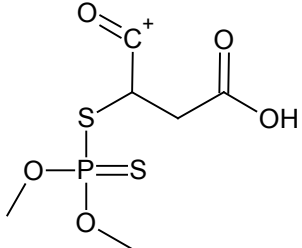
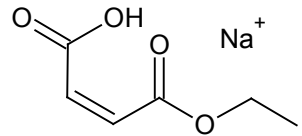
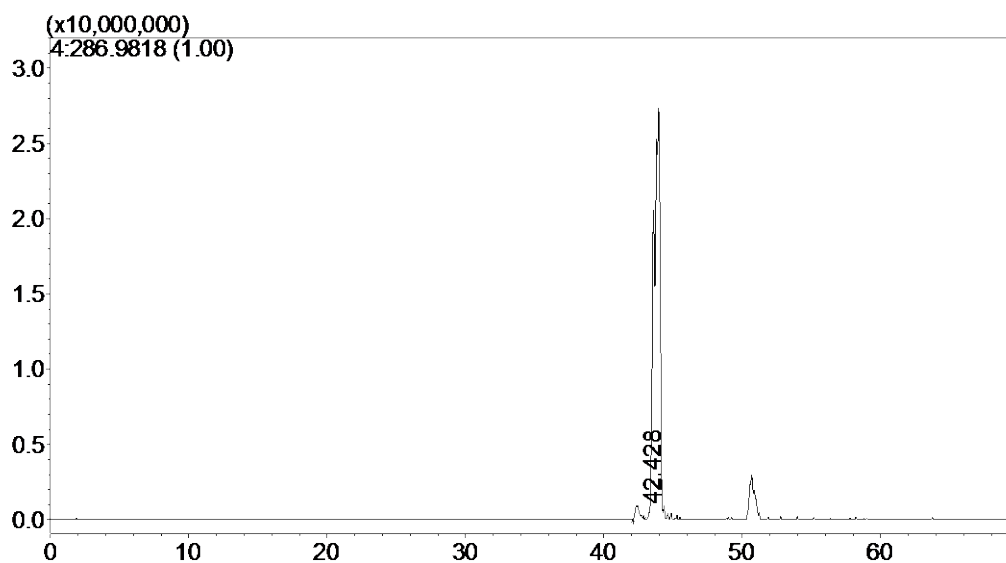
Observed Mass	Calculated Mass	Chemical Formula	Postulated Structure
285.0069 (in-source)	285.0015	$C_8H_{14}O_5PS_2$	
256.9684 (in-source)	256.9702	$C_6H_{10}O_5PS_2$	
167.0362	167.0315	$C_6H_8NaO_4$	

Figure 20 **Representative LC-MS Analysis of Desmethyl Malathion**
Monocarboxylic Acid confirmed at 42.0 min in Plasma (P2)

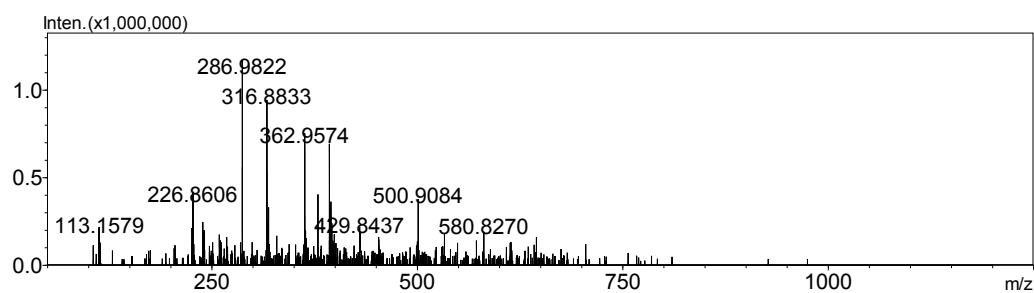
A. Extracted Ion Chromatogram for m/z 286.9818 ($[M-H]^-$)

MS File: CHE018133



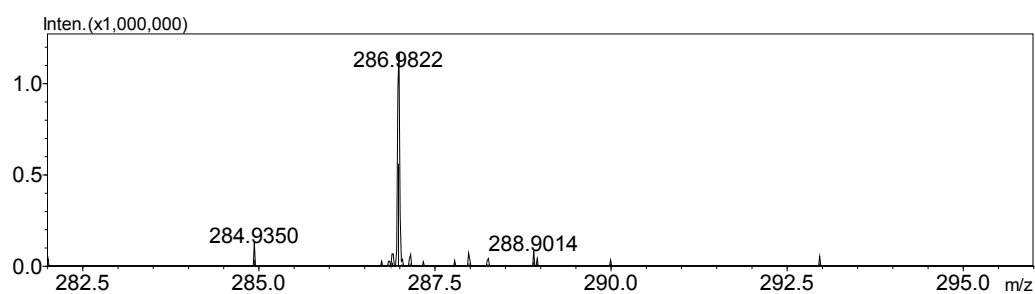
B. Full Scan Mass Spectrum (42.0 min)

Event#: 4 MS(E-) Ret. Time : 42.137 -> 42.383 - 41.610 -> 41.817 Scan# : 7792 -> 7835 - 7694 -> 7734



**Figure 20 Representative LC-MS Analysis of Desmethyl Malathion
(continued) Monocarboxylic Acid confirmed at 42.0 min in Plasma (P2)****C. Expanded Full Scan Mass Spectrum (42.0 min)**

Event#: 4 MS(E-) Ret. Time : 42.137 -> 42.383 - 41.610 -> 41.817 Scan# : 7792 -> 7835 - 7694 -> 7734

**D. MS/MS Product Ion Spectrum (42.0 min)**

Event#: 5 MS/MS(E-) Ret. Time : 42.210 -> 42.267 Scan# : 7805 -> 7815 Precursor : 286.9840 Cutoff : 79

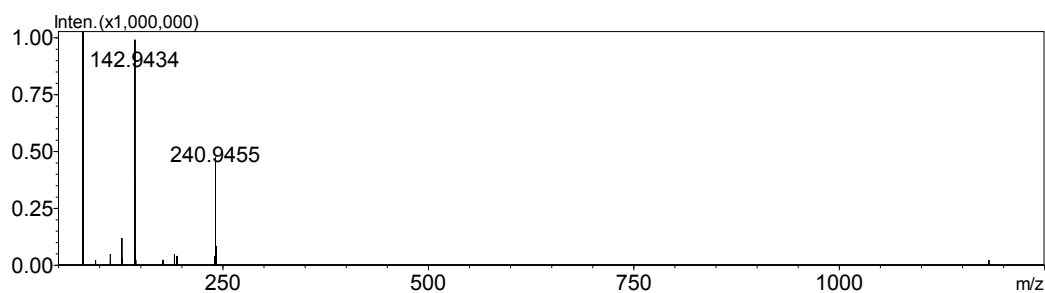


Figure 20 **Representative LC-MS Analysis of Desmethyl Malathion**
(continued) **Monocarboxylic Acid confirmed at 42.0 min in Plasma (P2)**

E. Postulated Structures of Observed Ions

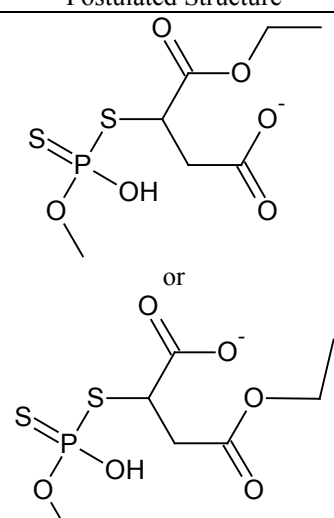
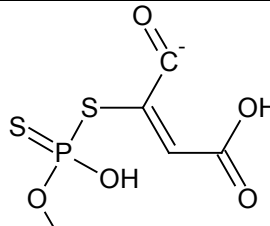
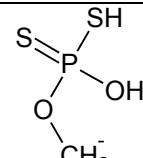
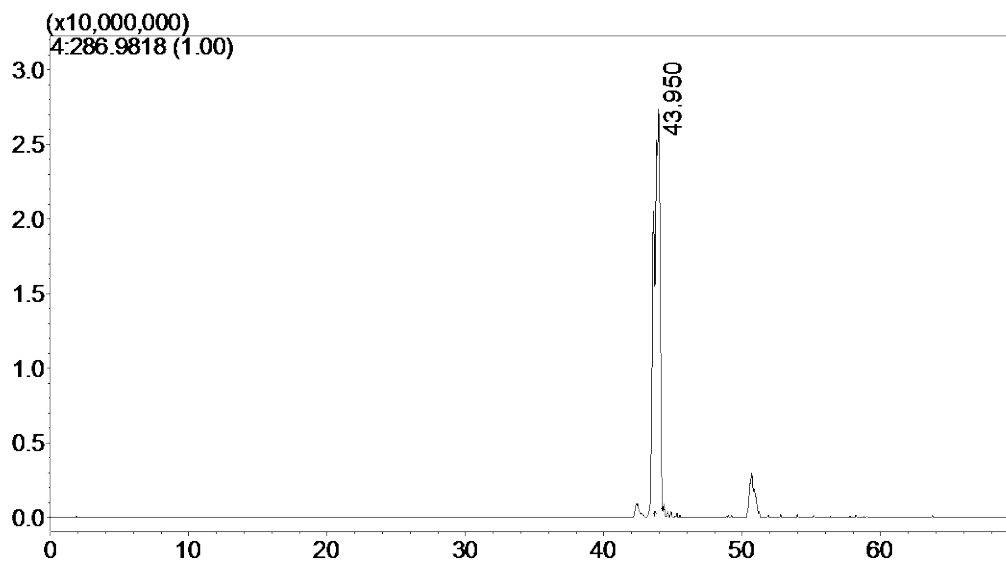
Observed Mass	Calculated Mass	Chemical Formula	Postulated Structure
286.9822	286.9818	$C_7H_{12}O_6PS_2$	
240.9455	240.9400	$C_5H_6O_5PS_2$	
142.9434	142.9400	$CH_4O_2PS_2$	

Figure 21 **Representative LC-MS Analysis of Desmethyl Malathion**
Monocarboxylic Acid confirmed at 43.3 min in Plasma (P2)

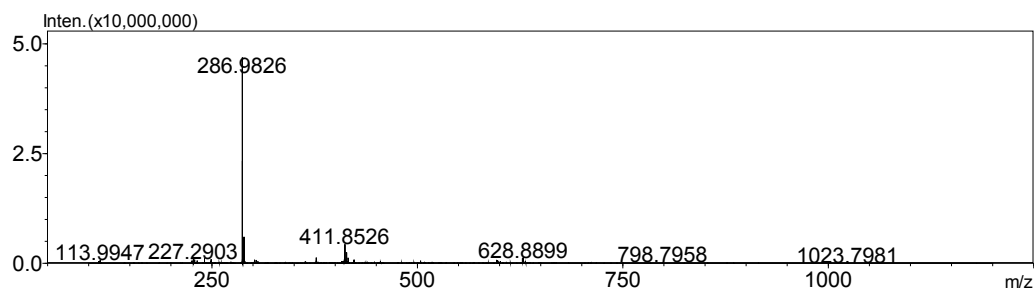
A. Extracted Ion Chromatogram for m/z 286.9818 ($[M-H]^-$)

MS File: CHE018133



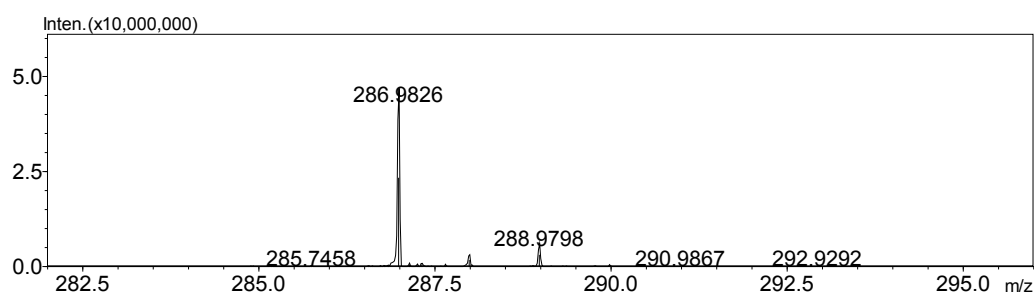
B. Full Scan Mass Spectrum (43.3 min)

Event#: 4 MS(E-) Ret. Time : 43.887 -> 44.123 - 44.607 -> 45.088 Scan# : 8113 -> 8156 - 8245 -> 8334

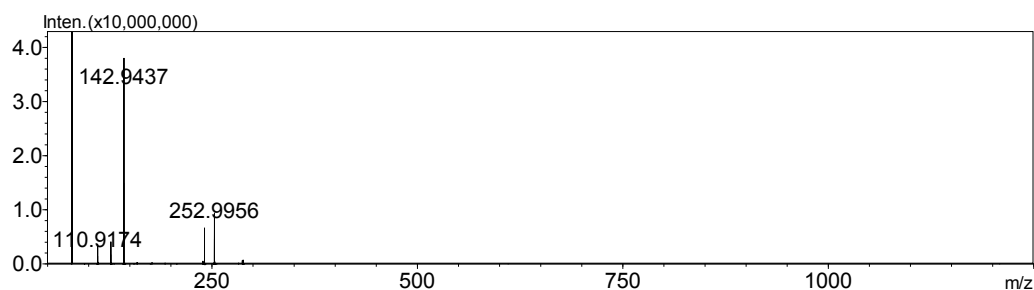


**Figure 21 Representative LC-MS Analysis of Desmethyl Malathion
(continued) Monocarboxylic Acid confirmed at 43.3 min in Plasma (P2)****C. Expanded Full Scan Mass Spectrum (43.3 min)**

Event#: 4 MS(E-) Ret. Time : 43.887 -> 44.123 - 44.607 -> 45.088 Scan# : 8113 -> 8156 - 8245 -> 8334

**D. MS/MS Product Ion Spectrum (43.3 min)**

Event#: 5 MS/MS(E-) Ret. Time : 43.997 -> 44.060 Scan# : 8133 -> 8145 Precursor : 286.9825 Cutoff : 79

**E. Expanded MS/MS Product Ion Spectrum (43.3 min)**

Event#: 5 MS/MS(E-) Ret. Time : 43.997 -> 44.060 Scan# : 8133 -> 8145 Precursor : 286.9825 Cutoff : 79

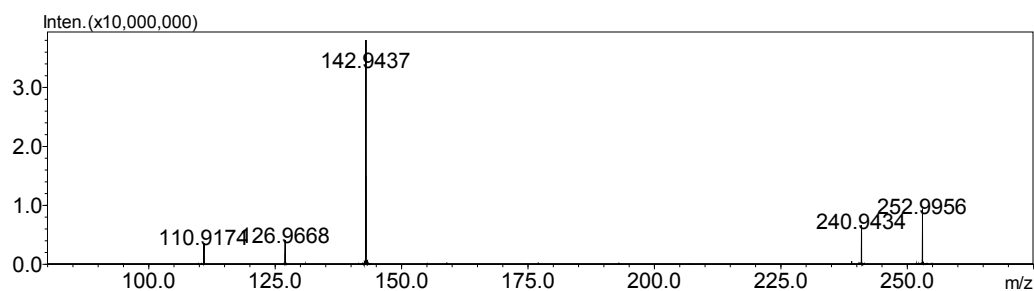


Figure 21 **Representative LC-MS Analysis of Desmethyl Malathion**
(continued) **Monocarboxylic Acid confirmed at 43.3 min in Plasma (P2)**

F. Postulated Structures of Observed Ions

Observed Mass	Calculated Mass	Chemical Formula	Postulated Structure
286.9826	286.9818	$C_7H_{12}O_6PS_2$	
252.9956	252.9941	$C_7H_{10}O_6PS$	
240.9434	240.9400	$C_5H_6O_5PS_2$	
142.9437	142.9400	$CH_4O_2PS_2$	

Figure 21 **Representative LC-MS Analysis of Desmethyl Malathion**
(continued) **Monocarboxylic Acid confirmed at 43.3 min in Plasma (P2)**

F. Postulated Structures of Observed Ions (continued)

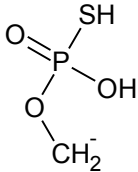
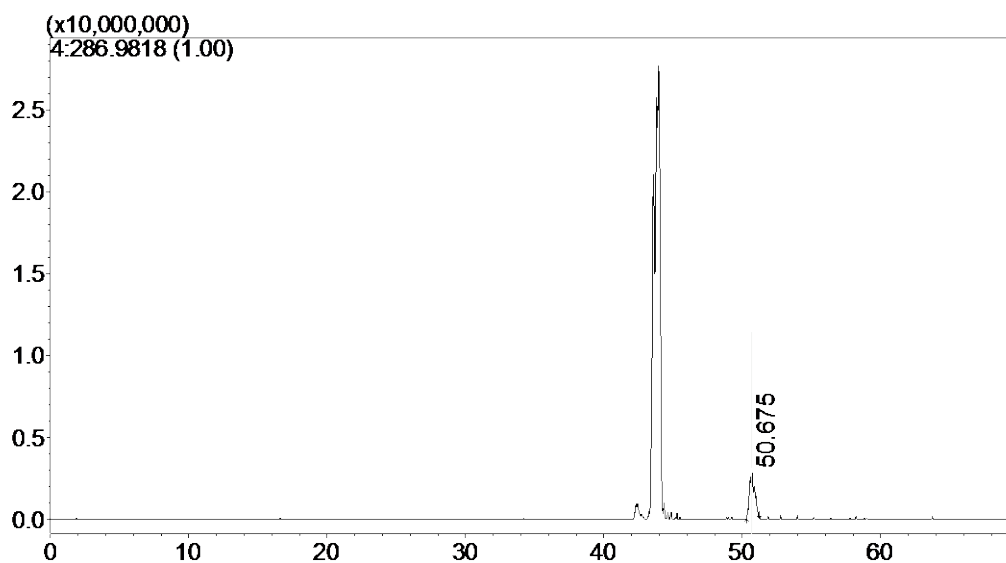
Observed Mass	Calculated Mass	Chemical Formula	Postulated Structure
126.9668	126.9624	CH ₄ O ₃ PS	

Figure 22 **Representative LC-MS Analysis of Desmethyl Malathion**
Monocarboxylic Acid confirmed at 50.5 min in Plasma (P2)

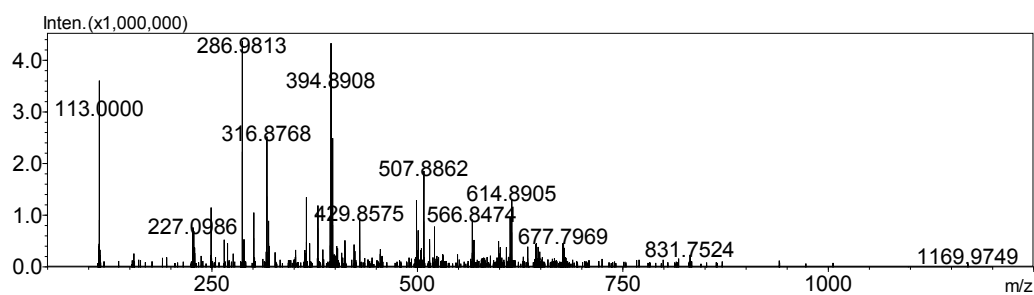
A. Extracted Ion Chromatogram for m/z 286.9818 ($[M-H]^-$)

MS File: CHE018133



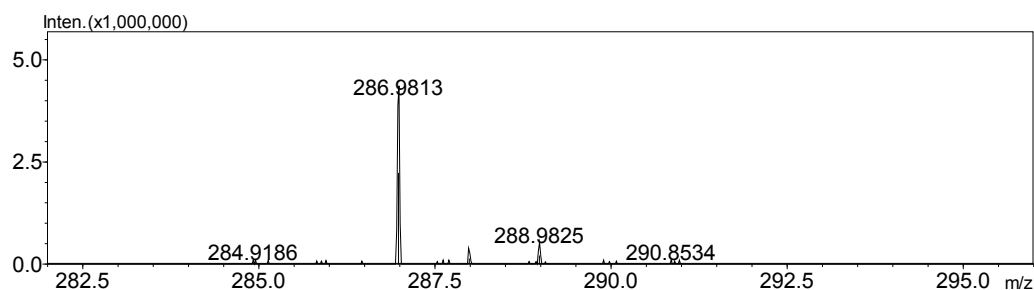
B. Full Scan Mass Spectrum (50.5 min)

Event#: 4 MS(E-) Ret. Time : 50.443 -> 50.905 - 49.762 -> 49.992 Scan# : 9325 -> 9411 - 9198 -> 9242

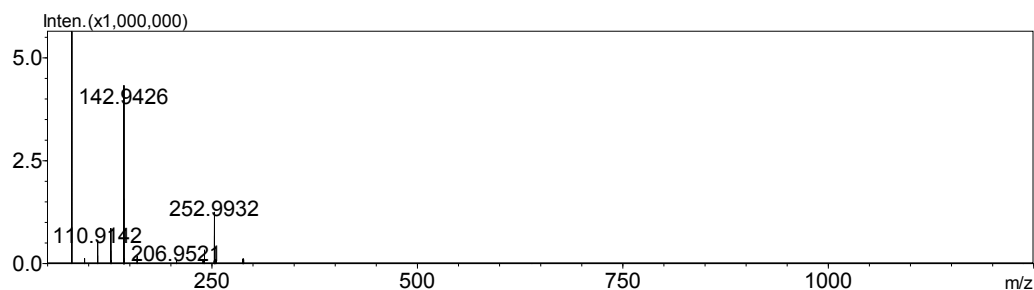


**Figure 22 Representative LC-MS Analysis of Desmethyl Malathion
(continued) Monocarboxylic Acid confirmed at 50.5 min in Plasma (P2)****C. Expanded Full Scan Mass Spectrum (50.5 min)**

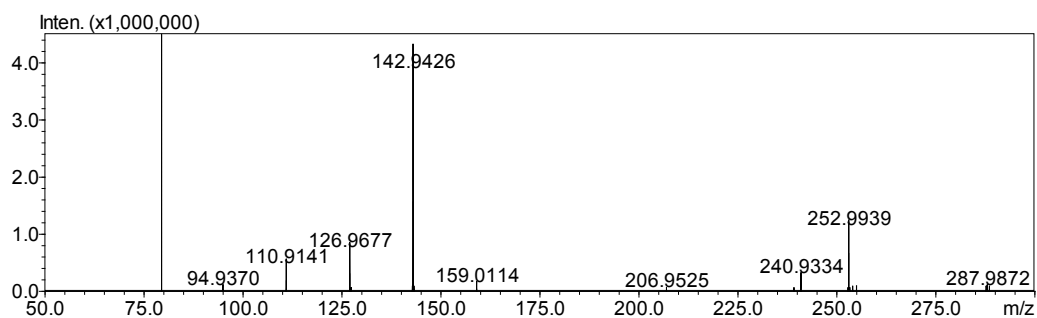
Event#: 4 MS(E-) Ret. Time : 50.443 -> 50.905 - 49.762 -> 49.992 Scan# : 9325 -> 9411 - 9198 -> 9242

**D. MS/MS Product Ion Spectrum (50.5 min)**

Event#: 5 MS/MS(E-) Ret. Time : 50.475 -> 50.475 Scan# : 9332 -> 9332 Precursor : 286.9661 Cutoff : 79

**E. Expanded MS/MS Product Ion Spectrum (50.5 min)**

Event#: 5 MS/MS(E-) Ret. Time : 50.475 -> 50.475 Scan# : 9332 -> 9332 Precursor : 286.9661 Cutoff : 79



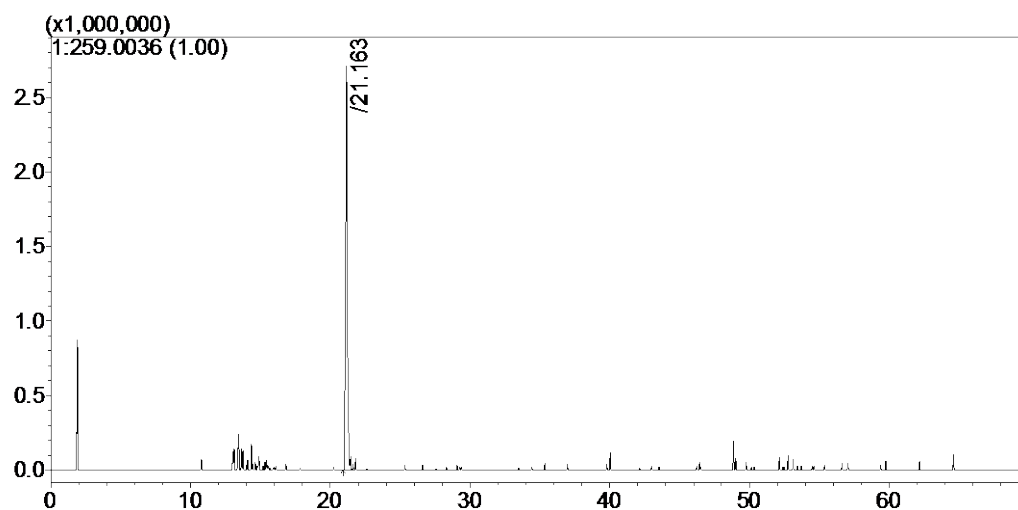
**Figure 22 Representative LC-MS Analysis of Desmethyl Malathion
(continued) Monocarboxylic Acid confirmed at 50.5 min in Plasma (P2)****F. Postulated Structures of Observed Ions**

Observed Mass	Calculated Mass	Chemical Formula	Postulated Structure
286.9813	286.9818	$C_7H_{12}O_6PS_2$	
252.9932	252.9941	$C_7H_{10}O_6PS$	
142.9426	142.9400	$CH_4O_2PS_2$	
126.9677	126.9624	CH_4O_3PS	

Figure 23 **Representative LC-MS Analysis of Metabolite 1 Identified at 21.0 min in Plasma (P2)**

A. Extracted Ion Chromatogram for m/z 259.0036 ($[M+Na]^+$)

MS File: CHE018133



B. Full Scan Mass Spectrum

Event#: 1 MS(E+) Ret. Time : 21.053 -> 21.138 - 20.668 -> 21.003 Scan# : 3890 -> 3905 - 3819 -> 3880

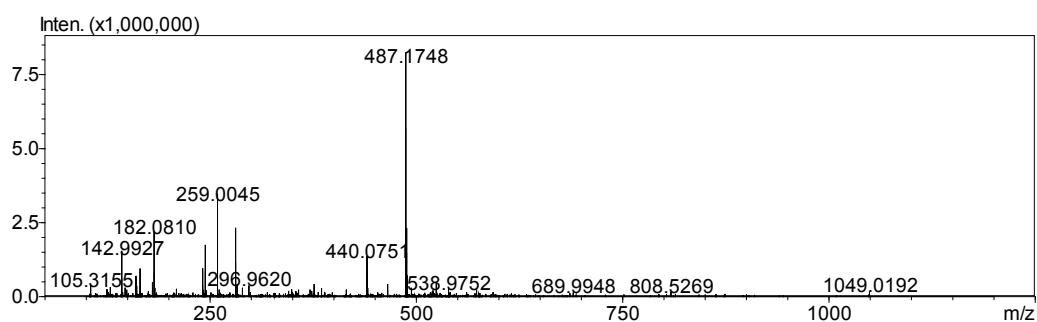
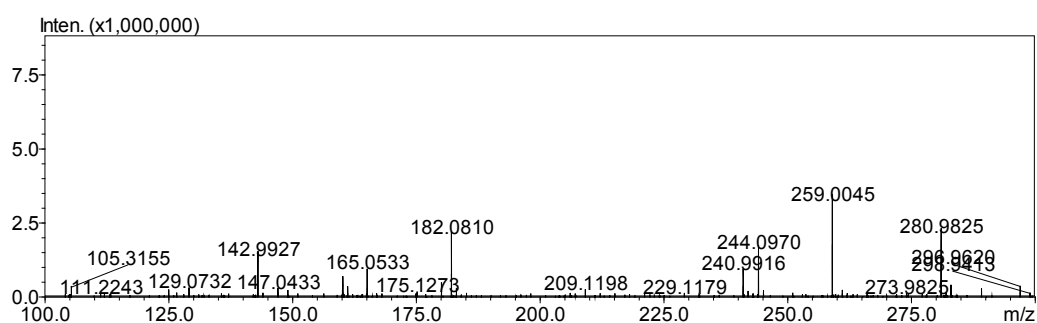


Figure 23 **Representative LC-MS Analysis of Metabolite 1 Identified at 21.0 min**
(continued) **in Plasma (P2)**

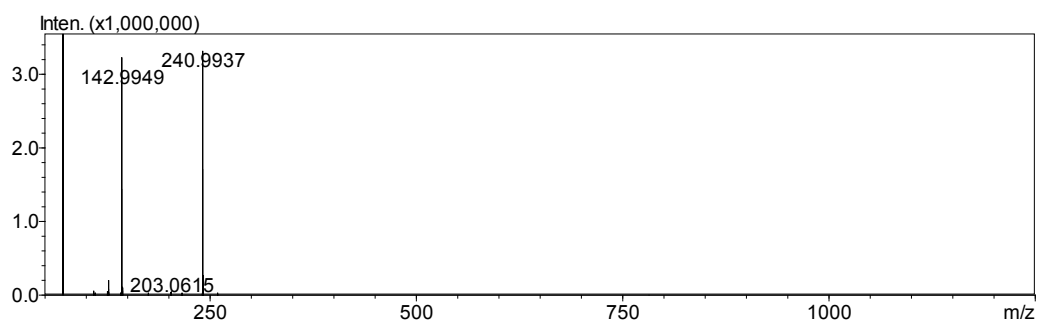
C. Expanded Full Scan Mass Spectrum

Event#: 1 MS(E+) Ret. Time : 21.053 -> 21.138 - 20.668 -> 21.003 Scan# : 3890 -> 3905 - 3819 -> 3880

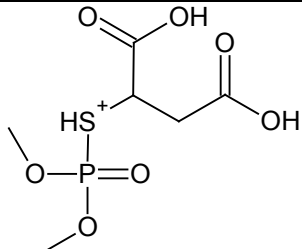
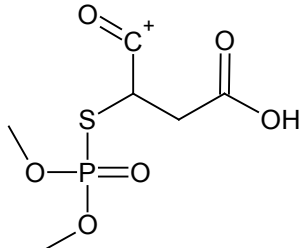
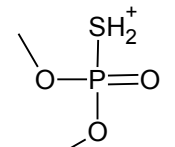


D. MS/MS Product Ion Spectrum

Event#: 2 MS/MS(E+) Ret. Time : 21.053 -> 21.110 Scan# : 3891 -> 3901 Precursor : 259.0063 Cutoff : 71



**Figure 23 Representative LC-MS Analysis of Metabolite 1 Identified at 21.0 min
(continued) in Plasma (P2)****E. Postulated Structures of Observed Ions**

Observed Mass	Calculated Mass	Chemical Formula	Postulated Structure
259.0045	259.0036	$C_6H_{12}O_7PS$	
240.9937	240.9930	$C_6H_{10}O_6PS$	
142.9949	142.9926	$C_2H_8O_3PS$	

11 TABLES**Table 1 Mean Concentrations of Total Radioactivity in Whole Blood Following a Single Oral Administration of [¹⁴C]-Malathion to Male Rats at Target Dose Levels of 40, 800 and 1200 mg/kg**

Nominal Time after dosing (hours)	µg equiv/g (n=4 with standard deviation (SD))					
	40 mg/kg		800 mg/kg		1200 mg/kg	
	Mean	SD	Mean	SD	Mean	SD
0.25	6.6	1.5	71	25	88	11
0.50	9.8	2.6	75	28	146	29
1	15.7	6.6	66	24	126	21
2	12.4	4.9	68	39	76	13
4	5.4	1.3	74	31	78	28
8	2.6	0.3	71	19	96	36
12	2.3	0.8	41	14	57	14
24	0.3	0.0	24	24	29	7
30	°0.2	°0.0	11	7	17	3
48	°0.2	°0.0	5	2	9	1
72	°0.2	°0.0	3	1	6	1
96	°0.1	°0.0	°2	°0	5	1

°=Mean includes results calculated from data less than 30 d.p.m. above background

Table 2 **Mean Concentrations of Total Radioactivity in Whole Blood Following a Single Intravenous Administration of [¹⁴C]-Malathion to Male Rats at a Target Dose Level of 4 mg/kg**

Nominal Time after dosing (hours)	µg equiv/g (n=4 with standard deviation (SD))	
	Mean	SD
5 min	9.35	2.32
0.25	5.26	1.93
0.50	2.95	1.19
1	1.25	0.78
2	0.46	0.37
4	0.13	0.05
8	0.07	0.02
12	0.07	0.02
24	0.03	0.01
30	°0.02	°0.01
48	°0.01	°0.00
72	°0.01	°0.00
96	°0.01	°0.00

°=Mean includes results calculated from data less than 30 d.p.m. above background

Table 3 **Mean Concentrations of Total Radioactivity in Plasma Following a Single Oral Administration of [¹⁴C]-Malathion to Male Rats at Target Dose Levels of 40, 800 and 1200 mg/kg**

Nominal Time after dosing (hours)	µg equiv/g (n=4 with standard deviation (SD))					
	40 mg/kg		800 mg/kg		1200 mg/kg	
	Mean	SD	Mean	SD	Mean	SD
0.25	13.3	4.1	119	31	173	9
0.50	19.5	7.6	152	31	249	34
1	27.3	10.7	119	32	206	38
2	22.7	9.7	127	88	144	49
4	14.7	9.0	150	65	145	56
8	4.6	0.8	121	39	167	65
12	5.3	4.1	68	26	88	21
24	0.5	0.1	37	36	47	11
30	0.3	0.0	15	13	26	4
48	0.2	0.0	5	1	11	1
72	°0.1	°0.0	4	1	6	1
96	°0.1	°0.0	2	0	4	1

°=Mean includes results calculated from data less than 30 d.p.m. above background

Table 4 **Mean Concentrations of Total Radioactivity in Plasma Following a Single Intravenous Administration of [¹⁴C]-Malathion to Male Rats at a Target Dose Level of 4 mg/kg**

Nominal Time after dosing (hours)	µg equiv/g (n=4 with standard deviation (SD))	
	Mean	SD
5 min	17.56	3.72
0.25	9.76	3.04
0.50	5.72	2.34
1	2.26	1.55
2	0.75	0.66
4	0.20	0.11
8	0.11	0.05
12	0.10	0.02
24	0.04	0.01
30	0.03	0.01
48	0.02	0.00
72	°0.01	°0.00
96	°0.01	°0.00

°=Mean includes results calculated from data less than 30 d.p.m. above background

Table 5 **Mean Blood to Plasma Ratios of Radioactivity Following a Single Oral Administration of [¹⁴C]-Malathion to Male Rats**

Nominal Time after dosing (hours)	Blood to Plasma Ratio (n=4)		
	40 mg/kg	800 mg/kg	1200 mg/kg
0.25	0.51	0.59	0.51
0.50	0.53	0.48	0.58
1	0.57	0.55	0.62
2	0.55	0.57	0.56
4	0.44	0.50	0.54
8	0.57	0.60	0.57
12	0.52	0.62	0.65
24	0.61	0.63	0.62
30	NQ	0.74	0.65
48	NQ	0.85	0.83
72	NQ	0.84	1.05
96	NQ	NQ	1.22

NQ – Not quantifiable

Table 6 **Mean Blood to Plasma Ratios of Radioactivity Following a Single Intravenous Administration of [¹⁴C]-Malathion to Male Rats**

Nominal Time after dosing (hours)	Blood to Plasma Ratio (n=4)
	4 mg/kg
5 min	0.53
0.25	0.54
0.50	0.53
1	0.57
2	0.66
4	0.69
8	0.68
12	0.71
24	0.79
30	NQ
48	NQ
72	NQ
96	NQ

NQ – Not quantifiable

Table 7 Pharmacokinetic Parameters of Total Radioactivity in Whole Blood and Plasma Following Single Oral Administration of [¹⁴C]-Malathion at a Target Dose Level of 40 mg/kg (Phase 1)

Matrix	Animal number	T _{max} (h)	C _{max} (µg equiv/mL) ^a	C _{max} /D (µg equiv/mL) ^a / (mg/kg)	AUC(0-t) (µg equiv.h/mL) ^a	AUC(0-t)/D (µg equiv.h/mL) ^a / (mg/kg)	AUC(0-inf) (µg equiv.h/mL) ^a	AUC(0-inf)/D (µg equiv.h/mL) ^a / (mg/kg)	T _{1/2} (h)	Absolute Bioavailability (F) (%)
Whole Blood	001M	1	7.20	0.169	67.6	1.59	68.8	1.62	4.38	102
	002M	1	22.9	0.538	93.0	2.18	95.1	2.23	4.68	
	003M	1	18.2	0.427	93.3	2.19	94.8	2.23	5.38	
	004M	1	14.6	0.343	77.6	1.82	80.6	1.89	6.96	
	n	4	4	4	4	4	4	4	4	
	Mean*	1	15.7	0.369	82.9	1.95	84.8	1.99	5.35	
	SD	-	6.62	0.155	12.6	0.295	12.6	0.297	1.15	
Plasma	001M	1	13.0	0.305	163	3.82	166	3.91	25.0	117
	002M	1	36.6	0.859	175	4.11	178	4.17	9.44	
	003M	1	34.2	0.803	187	4.38	191	4.49	30.8	
	004M	4	27.5	0.646	201	4.71	NR	NR	NR	
	n	4	4	4	4	4	3	3	3	
	Mean*	1	27.8	0.653	181	4.26	178	4.19	21.7	
	SD	-	10.6	0.249	16.2	0.380	12.3	289	11.0	

^a Units of volume are 'mL' for plasma and 'g' for whole blood. Units reported in mL assuming 1 mL = 1 g.

*Median reported for T_{max}.

NR = the coefficient of determination of the elimination phase was <0.800.

Table 8 Pharmacokinetic Parameters of Total Radioactivity in Whole Blood and Plasma Following Single Oral Administration of [¹⁴C]-Malathion at a Target Dose Level of 800 mg/kg (Phase 2)

Matrix	Animal number	T _{max} (h)	C _{max} (µg equiv/mL) ^a	C _{max} /D (µg equiv/mL) ^a /(mg/kg)	AUC(0-t) (µg equiv.h/mL) ^a	AUC(0-t)/D (µg equiv.h/mL) ^a /(mg/kg)	AUC(0-inf) (µg equiv.h/mL) ^a	AUC(0-inf)/D (µg equiv.h/mL) ^a /(mg/kg)	T _{1/2} (h)	Absolute Bioavailability (F) (%)
Whole Blood	005M	2	126	0.161	1220	1.56	NR	NR	NR	121
	006M	1	74.0	0.0943	1170	1.49	1260	1.61	32.4	
	007M	0.5	114	0.145	2370	3.02	2480	3.16	24.8	
	008M	8	95.0	0.121	1680	2.14	1810	2.30	29.8	
	n	4	4	4	4	4	3	3	3	
	Mean*	1.5	102	0.130	1610	2.05	1850	2.35	29.0	
	SD	-	22.8	0.029	557	0.709	610	0.777	3.83	
Plasma	005M	2	258	0.329	2040	2.59	2070	2.64	24.0	98
	006M	0.5	133	0.169	1950	2.48	2050	2.61	36.3	
	007M	4	225	0.287	3880	4.95	3960	5.04	26.6	
	008M	8	168	0.214	2830	3.60	2930	3.74	36.3	
	n	4	4	4	4	4	4	4	4	
	Mean*	3	196	0.250	2670	3.41	2750	3.51	30.8	
	SD	-	56.1	0.0715	898	1.14	903	1.15	6.45	

^a Units of volume are 'mL' for plasma and 'g' for whole blood. Units reported in mL assuming 1 mL = 1 g.

*Median reported for T_{max}.

NR = Not Reported

Table 9 Pharmacokinetic Parameters of Total Radioactivity in Whole Blood and Plasma Following Single Oral Administration of [¹⁴C]-Malathion at a Target Dose Level of 1200 mg/kg (Phase 3)

Matrix	Animal number	Tmax (h)	Cmax (µg equiv/mL) ^a	Cmax/D (µg equiv/mL) ^a / (mg/kg)	AUC(0-t) (µg equiv.h/mL) ^a	AUC(0-t)/D (µg equiv.h/mL) ^a / (mg/kg)	AUC(0-inf) (µg equiv.h/mL) ^a	AUC(0-inf)/D (µg equiv.h/mL) ^a / (mg/kg)	T _{1/2} (h)	Absolute Bioavailability (F) (%)
Whole Blood	009M	0.5	172	0.160	2400	2.23	2680	2.49	38.2	118
	010M	0.5	155	0.144	2130	1.98	2380	2.21	34.7	
	011M	4	116	0.108	2500	2.32	2770	2.57	37.3	
	012M	0.5	150	0.139	1790	1.66	2020	1.88	40.6	
	n	4	4	4	4	4	4	4	4	
	Mean*	0.5	148	0.138	2210	2.05	2460	2.29	37.7	
	SD	-	23.5	0.0218	320	0.297	338	0.314	2.46	
Plasma	009M	8	254	0.236	3790	3.52	3880	3.61	21.5	94
	010M	0.5	292	0.271	3230	3.00	3370	3.13	24.0	
	011M	4	214	0.199	3950	3.67	4150	3.85	27.9	
	012M	0.5	244	0.227	2850	2.65	3020	2.81	29.2	
	n	4	4	4	4	4	4	4	4	
	Mean*	2.25	251	0.233	3450	3.21	3610	3.35	25.7	
	SD	-	32.2	0.0299	504	0.468	505	0.469	3.54	

^a Units of volume are 'mL' for plasma and 'g' for whole blood. Units reported in mL assuming 1 mL = 1 g.

*Median reported for Tmax.

Table 10 Pharmacokinetic Parameters of Total Radioactivity in Whole Blood and Plasma Following Single Intravenous Administration of [¹⁴C]-Malathion at a Target Dose Level of 4 mg/kg (Phase 4)

Matrix	Animal number	C0(μg equiv/mL) ^a	AUC(0-t) (μg equiv.h/mL) ^a	AUC(0-t)/D (μg equiv.h/mL) ^a /(mg/kg)	AUC(0-inf) (μg equiv.h/mL) ^a	AUC(0-inf)/D (μg equiv.h/mL) ^a /(mg/kg)	T _{1/2} (h)	CL (mL/h/kg)	Vd (mL/kg)
Whole Blood	013M	13.2	7.08	1.85	7.53	1.97	10.3	509	7590
	014M	15.5	10.7	2.80	11.3	2.94	12.0	341	5900
	015M	9.94	3.89	1.02	4.19	1.09	10.5	912	13900
	016M	11.5	6.36	1.66	6.84	1.79	11.2	559	9030
	n	4	4	4	4	4	4	4	4
	Mean	12.5	7.02	1.83	7.45	1.95	11.0	580	9090
	SD	2.38	2.83	0.739	2.91	0.760	0.751	240	3420
Plasma	013M	24.5	12.7	3.32	13.2	3.45	18.0	291	7560
	014M	28.3	19.8	5.18	20.3	5.29	29.1	190	7980
	015M	19.2	7.68	2.00	8.35	2.18	46.4	463	31000
	016M	22.7	12.6	3.30	13.0	3.41	14.6	295	6210
	n	4	4	4	4	4	4	4	4
	Mean	23.7	13.2	3.45	13.7	3.58	27.0	310	13200
	SD	3.82	5.00	1.31	4.91	1.28	14.3	113	11900

^a Units of volume are 'mL' for plasma and 'g' for whole blood. Units reported in mL assuming 1 mL = 1 g.

Table 11 Quantification of Metabolites in Plasma and Red Blood Cells

Assignment	<i>m/z</i>	Approx. RT (min)	Concentration (µg equiv/g)			
			P1 ^B	P2 ^B	R1 ^B	R2 ^B
Unassigned	NA	2.3	1.365	2.099	< 1.092	< 1.543
Unassigned	NA	5.5	1.191	< 1.626	< 1.092	ND
Unassigned	NA	10.3	< 1.130	ND	ND	ND
Unassigned	NA	18.0	< 1.130	ND	ND	ND
Metabolite 1 (<i>m/z</i> 259)	259 [M+H] ⁺	21.0	1.795	2.230	< 1.092	< 1.543
Unassigned	NA	31.5	ND	ND	< 1.092	ND
Unassigned	NA	32.8	2.811	2.350	< 1.092	< 1.543
Unassigned	NA	33.5	ND	ND	< 1.092	ND
Unassigned	NA	35.3	< 1.130	ND	< 1.092	< 1.543
Desmethyl Malathion Monocarboxylic Acid 1	287 [M-H] ⁻	36.5	< 1.130 ^A	< 1.626 ^A	2.013 ^{A, C}	< 1.543 ^A
Unassigned	NA	37.0	ND	ND	< 1.092	< 1.543
Malathion Dicarboxylic Acid	273 [M-H] ⁻	39.8	121.264	117.342	47.368	33.262
Unassigned	NA	40.5	ND	ND	ND	< 1.543
Desmethyl Malathion Monocarboxylic Acid 1	287 [M-H] ⁻	42.0	3.607	2.290	1.275 ^A	< 1.543 ^A
Desmethyl Malathion Monocarboxylic Acid 2	287 [M-H] ⁻	43.3	17.438	14.191	4.581	3.403
Malathion Monocarboxylic Acid	325 [M+Na] ⁺	48.0	12.270	5.436	10.018	3.878
Desmethyl Malathion Monocarboxylic Acid 2	287 [M-H] ⁻	50.5	3.627	2.258	< 1.092	< 1.543
Unassigned	NA	54.3	ND	ND	ND	< 1.543

ND = not detected by radio-LC-MS

NA = not assigned

A) Assignment by comparison of retention time with Reference Standard, not detected by LC-MS

B) LOQ for P1S.7E = 1.130, P2S.5E = 1.626, R1S.5E = 1.092 and R2S.5E = 1.543 µg equiv/g

C) Radio-peak not baseline resolved

Table 12 **Cumulative Concentrations for Desmethyl Malathion Monocarboxylic Acid 1 and 2**

Assignment	<i>m/z</i>	Approx. RT (min)	Concentration (µg equiv/g)			
			P1	P2	R1	R2
Desmethyl Malathion Monocarboxylic Acid 1	287 [M-H] ⁻	42.0	3.607- 4.737	2.290- 3.916	3.288 ^A	< 3.086
Desmethyl Malathion Monocarboxylic Acid 2	287 [M-H] ⁻	43.3	21.065	16.449	4.581- 5.673	3.403- 4.946

A) Radio-peak for desmethyl Malathion monocarboxylic acid 1 at 36.0 min not baseline resolved

Table 13 **Identified Metabolites**

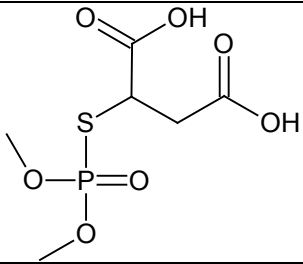
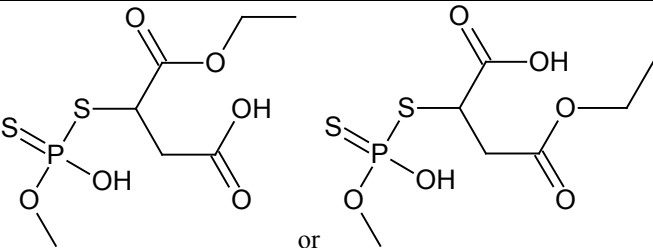
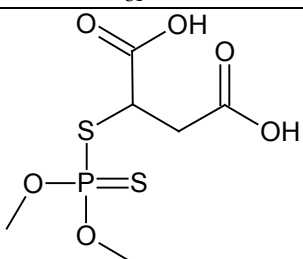
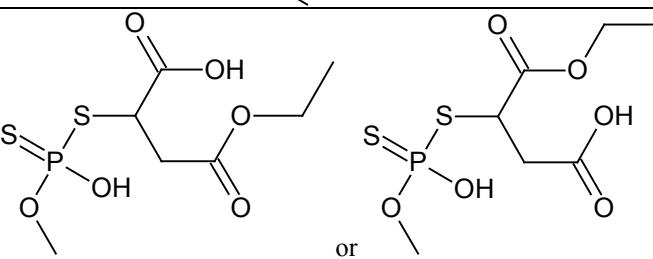
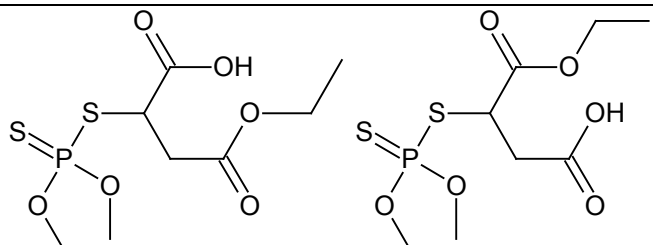
Retention time (min)	Nominal m/z	Compound	Structure Proposal
21.0	259 $[M+H]^+$	Metabolite 1 (m/z 259)	
36.5 and 42.0	287 $[M-H]^-$	Desmethyl Malathion Monocarboxylic Acid 1	
39.8	273 $[M-H]^-$	Malathion Dicarboxylic Acid	
43.3 and 50.5	287 $[M-H]^-$	Desmethyl Malathion Monocarboxylic Acid 2	
48.0	325 $[M+Na]^+$	Malathion Monocarboxylic Acid	

Table 14 **Qualitative Table of Identified Metabolites by LC-MS**

Identified Metabolite	Nominal m/z	Approx. RT (min)	Samples			
			P1	P2	R1	R2
Metabolite 1 (m/z 259)	259 [M+H] ⁺	21.0	Y	Y	Y	Y
Desmethyl Malathion Monocarboxylic Acid 1	287 [M-H] ⁻	36.5	N	N	N	N
Malathion Dicarboxylic Acid	273 [M-H] ⁻	39.8	Y	Y	Y	Y
Desmethyl Malathion Monocarboxylic Acid 1	287 [M-H] ⁻	42.0	Y	Y	N	N
Desmethyl Malathion Monocarboxylic Acid 2	287 [M-H] ⁻	43.3	Y	Y	Y	Y
Malathion Monocarboxylic Acid	325 [M+Na] ⁺	48.0	Y	Y	Y	Y
Desmethyl Malathion Monocarboxylic Acid 2	287 [M-H] ⁻	50.5	Y	Y	Y	Y

Y = detected by LC-MS analysis

N = not detected

Table 15 Summary of Reference Standards Analysed

Component Analysed	<i>m/z</i> [M+H] ⁺	<i>m/z</i> [M-H] ⁻	Approximate Retention Time (min)	Detected (Y/N)
Fumaric acid	117.0182	115.0037	5.5	N
Maleic acid	117.0182	115.0037	17.9	N
Mercaptosuccinic acid	151.0060	148.9914	6.7	N
Diethylmethylthiosuccinate	221.0842	219.0697	47.7	N
Diethyl maleate	173.0808	171.0663	37.5	N
Malaoxon	315.0662	313.0516	43.8	N
Isomalathion	331.0433	329.0288	46.7	N
Malathion dicarboxylic acid	274.9807	272.9662	39.4	Y
Diethyl fumarate	173.0808	171.0663	45.7	N
Monoethyl maleic acid	145.0495	143.0350	12.4	N
Diethylmethyl-malathion	317.0277	315.0131	50.4	<LOQ ^A
Desmethyl malathion dicarboxylic acid	260.9651	258.9505	40.6, 43.5	N
Desmethyl malathion monocarboxylic acid	288.9964	286.9818	36.3, 42.1, 43.5, 50.6	Y
Malathion monocarboxylic acid	303.0120	300.9975	47.5	Y
O,O-dimethyl-thiophosphoric acid	142.9926	140.9781	15.4	N
Diethyl mercaptosuccinate	207.0686	205.0540	46.1	N
Desmethyl malaoxon dicarboxylic acid	244.9879	242.9734	32.8	N
O,O-dimethyl dithiophosphoric acid	158.9698	156.9552	28.7	N
Tetra ethyl dithiosuccinate	411.1142	409.0996	58.0	N
Monoethyl fumarate	145.0495	143.0350	14.1	N
Malathion	331.0433	329.0288	55.8	N

Y=Detected by radio-LC/MS

N=Not detected by radio-LC/MS

LOQ for analysis of plasma and red blood cells by radio-HPLC = 1.092 µg/g – 1.626 µg/g

^ADetected in P1S.7E and R1S.5E by MS only, not detected by radio-HPLC therefore present <LOQ (<1.13 µg/g)

12 APPENDICES**Appendix 1 Certificate of Good Laboratory Practice****THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM****GOOD LABORATORY PRACTICE****STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC****TEST FACILITY**

CHARLES RIVER LABORATORIES EDINBURGH LIMITED
ELPHINSTONE RESEARCH CENTRE
TRANENT
EH33 2NE
UNITED KINGDOM

Including satellite facilities at:

CHARLES RIVER
TRADE TERMINAL DIETZENBACH
MAX-PLANK-STR 6
53128, DIETZENBACH
GERMANY

TEST TYPE(S)

Analytical/Clinical Chemistry
Environmental Fate
Environmental Toxicity
Ecosystems
Physical/Chemical Testing
Residue Studies
Toxicology

Residue Studies

DATE OF INSPECTION: 26/07/2016 – 28/07/2016

DATE OF ISSUE: 10/11/2016

An Inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above named test facility as part of the UK Good Laboratory Practice Compliance Monitoring Programme.

This statement confirms that, on the date of issue, the UK Good Laboratory Practice Monitoring Authority were satisfied that the above named test facility was operating in compliance with the OECD Principles of Good Laboratory Practice.

This statement constitutes a Good Laboratory Practice Instrument (as defined in the UK Good Laboratory Practice Regulations 1999).

Issued by
Dr Andrew J Gray
Head, UK GLP Monitoring Authority



Medicines & Healthcare products
Regulatory Agency



Appendix 2 Copy of Study Plan and Amendment 4



FINAL PROTOCOL

Test Facility Study No. 172891

Sponsor Reference No. 2016MET-FYF2662

**[¹⁴C]-Malathion: The Pharmacokinetics of [¹⁴C]-Malathion in the Rat
Following Single Oral and Intravenous Administration**

SPONSOR:
Cheminova A/S
P.O. Box 9
DK-7620 Lemvig
Denmark

TEST FACILITY:
Charles River Laboratories Edinburgh Ltd
Elphinstone Research Centre
Tranent, East Lothian, EH33 2NE
UK

15 July 2016

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**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****TABLE OF CONTENTS**

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**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****1. OBJECTIVE(S)**

This study has been designed to examine the pharmacokinetics of total radioactivity following single oral and intravenous administration of [¹⁴C]-Malathion to male rats. Pharmacokinetic parameters will be investigated in blood and plasma using Phoenix pharmacokinetic software.

The nature of the radioactivity present in selected plasma samples will also be examined.

2. PROPOSED STUDY SCHEDULE

Experimental Start Date:	August 2016
Completion of Total Radioactivity Analysis:	November 2016
Experimental Completion Date:	December 2016
Draft Report:	January 2017

3. GUIDELINES FOR STUDY DESIGN

OECD guideline reference 417 (2010): Toxicokinetics.

United States Environmental Protection Agency, Health Effects Test Guidelines (August 1998) OPPTS 870.7485 (Metabolism and Pharmacokinetics).

Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market.

Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.

Japanese Ministry of Agriculture, Forestry and Fisheries, Test Data for Registration of Agricultural Chemicals, 12 Nohsan No 8147, Agricultural Production Bureau, November 24, 2000.

4. REGULATORY COMPLIANCE

The study will be performed in accordance with the OECD Principles of Good Laboratory Practice as incorporated into the United Kingdom Statutory Instrument for GLP and as accepted by Regulatory Authorities throughout the European Union, United States of America (FDA and EPA) and Japan (MHLW, MAFF and METI) and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

All routine activities performed during the conduct of this study are detailed in Charles River Standard Operating Procedures.

5. QUALITY ASSURANCE**5.1. Test Facility**

The Test Facility Quality Assurance Unit (QAU) will monitor the study to assure the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with Good Laboratory Practice regulations. The QAU will review the protocol, conduct inspections at intervals adequate to assure the integrity of the study, and audit the Final

Appendix 2 Copy of Study Plan and Amendment 4 (continued)

Report to assure that it accurately describes the methods and standard operating procedures and that the reported results accurately reflect the raw data of the study.

6. SPONSOR

Sponsor Representative

Mette Jensen
Tel: +45 9690 9775
Fax: +45 9690 9691
E-mail: mette.jensen@fmc.com

7. RESPONSIBLE PERSONNEL

Study Director

Mhairi Libberton, BSc
Charles River Laboratories Edinburgh Ltd
Elphinstone Research Centre
Tranent, East Lothian, EH33 2NE
UK
Tel: +44 (0) 1875 618292
Fax: +44 (0) 1875 614555
E-mail: mhairi.libberton@crl.com

Management Contact

Stephen Madden, BSc PhD CBiol FRSB
Charles River Laboratories Edinburgh Ltd
Elphinstone Research Centre
Tranent, East Lothian, EH33 2NE
UK
Tel: +44 (0) 1875 618316
Fax: +44 (0) 1875 614555
E-mail: stephen.madden@crl.com

Principal Analyst (Responsible for Purity Assessment)

Gary Wicksted, BSc
Charles River Laboratories Edinburgh Ltd
Elphinstone Research Centre
Tranent, East Lothian, EH33 2NE
UK
Tel: +44 (0) 1875 888764
Fax: +44 (0) 1875 614555
E-mail: gary.wicksted@crl.com

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****Principal Analyst (Responsible for Biotransformation Analysis)**

Martha A Green, BSc PhD
Charles River Laboratories Edinburgh Ltd
Elphinstone Research Centre
Tranent, East Lothian, EH33 2NE
UK
Tel: +44 (0) 1875 886267
Fax: +44 (0) 1875 614555
E-mail: martha.green@crl.com

8. TEST ITEMS AND DOSE VEHICLES**8.1. Test Item**

Identification: [¹⁴C]-Malathion
Batch (Lot) Number: 8357RDB001-1
Specific Activity: To be confirmed in the study data
Radiochemical Purity: To be confirmed in the study data
Purity: To be confirmed in the study data, dose calculations will not be corrected for purity.
Storage Conditions: To be confirmed in the study data

Identification: Malathion Technical
Batch (Lot) Number: P2168-IbW-05
Expiration Date: 06 April 2019
Physical Description: Pale yellow liquid
Molecular Weight: 330.36
Purity: 95%; dose calculations will be corrected for purity.
Storage Conditions: Kept in a freezer set to maintain -20°C

8.2. Dose Vehicles

Corn oil. Intravenous dose vehicle to be decided following trial assessment – refer to Section 10.2.

8.3. Reference Standards

Any available reference standards will be supplied by the Sponsor. These will be supplied and stored as outlined for the unlabelled material in Section 8.1 (unless otherwise instructed by the Sponsor) except that no reserve sample will be retained.

8.4. Test Item Characterisation

The Sponsor will provide to the Test Facility documentation of the identity, strength, purity, composition, and stability for the test item. A Certificate of Analysis or equivalent documentation will be provided for inclusion in the Final Report.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

The Sponsor has appropriate documentation on file concerning the method of synthesis, fabrication or derivation of the test item, and this information is available to the appropriate regulatory agencies should it be requested.

8.5. Analysis of Test Item

The stability of the bulk test item will not be determined during the course of this study. Information to support the stability of each lot of the bulk test item will be provided by the Sponsor.

8.6. Reserve Samples

For each batch (lot) of unlabelled test item, a reserve sample (approximately 20 mg or appropriate) will be collected and maintained under the appropriate storage conditions by the Test Facility.

8.7. Test Item Inventory and Disposition

Records of the receipt, distribution, and storage of test items will be maintained. After finalisation of the study report, the Sponsor will be contacted with regard to the disposal, shipment, or continued storage of the test item. The fees for either outcome will be the subject of a contract extension once the costs are known.

8.8. Other Materials

Other materials will be obtained by Charles River. Chemicals will be of analytical grade where available.

9. SAFETY

Safety instructions for this study are provided on the Sponsor supplied safety data sheet. An internal COSHH safety sheet will be prepared at Charles River.

10. DOSE FORMULATION AND ANALYSIS**10.1. Radiochemical Purity and Stability**

Charles River will confirm the radiochemical purity of the radiolabelled test item prior to animal dosing using a preliminary HPLC method supplied by the Sponsor. This method may be optimised to improve the retention time of the test item if necessary and will be compatible with LC-MS whenever possible.

10.2. Trial Dose Formulations

Prior to preparation of the animal dose, trial oral formulations will be performed to assess the suitability of the dose formulation procedures and to assess the stability of the radiochemical over the anticipated formulation and administration periods. The trial dose preparation will mimic the procedures required for the main animal study but will be prepared using the minimum practical quantities of test item. The trial oral dose formulations will be prepared at 8 and 160 mg/mL. An additional trial oral formulation will also be prepared at the Phase 3 nominal dose concentration (to be detailed by formal protocol amendment) if the concentration is >160 mg/mL. Radiochemical purity will be assessed at the following timepoints:

24 and 48 h after preparation

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

Prior to the preparation of the trial intravenous dose solution, preliminary experiments will be carried out to assess the solubility of unlabelled Malathion in an appropriate vehicle suitable for intravenous dosing. A trial intravenous dose formulation will then be performed to assess the suitability of the dose formulation procedures and to assess the stability of the radiochemical in the formulation over the anticipated formulation and administration period. The trial IV dose formulation will be prepared at 0.8 mg/mL. Radiochemical purity will be assessed at the following timepoints:

3 and 24 h after preparation

10.3. Summary of Radiochemical Purity Measurements

A summary of the radiochemical purity measurements to be performed is detailed below:

Sample	Time
Stock radiochemical	On receipt/as deemed necessary
Trial oral formulations	Stability at 24 and 48 h
Trial IV formulation	Stability at 3 and 24 h
Dose Preparations	Predose and post dose

10.4. Dose Levels, Dose Regimen and Routes of Administration

Phase No.	Dose Route	Nominal Dose	Nominal Radioactive Dose	Nominal Dose Concentration	Nominal Dose Volume
1	Oral	40 mg/kg	5 MBq/kg	8 mg/mL	5 mL/kg
2	Oral	800 mg/kg	10 MBq/kg	160 mg/mL	
3	Oral	TBC		TBC	
4	IV	4 mg/kg	5 MBq/kg	0.8 mg/mL	
5	Oral	TBC	10 MBq/kg	TBC	
6	IV	4 mg/kg	5 MBq/kg	0.8 mg/mL	

10.5. Dose Formulations

The oral dose formulations will be prepared as a suspension in corn oil.

The intravenous dose formulation will be prepared as a non-irritant solution in a dose vehicle to be selected following trial stability work (section 10.2). The dose vehicle selection will be documented in the raw data.

The dose formulations will be stored in a refrigerator set to maintain a temperature of 4°C until prior to administration, with the exception of the trial intravenous formulation which will be stored at ambient until 3 h post preparation, then stored in a refrigerator set to maintain a temperature of 4°C.

The purity of the dose formulations will be determined by HPLC prior to and following dose administration.

10.6. Dose Determination

The actual dose received by each animal will be determined with reference to the dose concentration, the weight of dose administered and the specific activity of [¹⁴C]-Malathion in the formulated dose. Where necessary, these calculations will take into account any undosed residue.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****11. TEST SYSTEM**

Species: Rat
Strain: Sprague-Dawley
Condition: Purpose-bred, naive
Source: Charles River UK Limited, Margate, Kent, UK
Number of Males Ordered: Twenty
Target Age at the Initiation of Dosing: Approximately 8-10 weeks at dosing
Target Weight at the Initiation of Dosing: Appropriate for age and strain of animal.
The actual age, weight, and number of animals received will be listed in the Final Report.

11.1. Justification of Test System and Number of Animals

The Sprague Dawley rat has been chosen as the species for this study as this is a species which has been used in the toxicological evaluation of the test item.

The study will be conducted using male rats only since there is no sex difference apparent from existing ADME and toxicity data available for Malathion.

The number of rats chosen for this study is the smallest number considered necessary to provide reliable data and is compliant with regulatory requirements.

11.2. Animal Identification

Each cage will be given a cage card which identifies each animal within the cage by study number, animal number and sex. Animals assigned to the study will be uniquely identified by tail marking and will be accurately weighed predose.

11.3. Environmental Acclimation

The animals will be allowed to acclimatise to the Charles River rodent accommodation for a period of a minimum of 5 days before the commencement of dosing.

11.4. Selection and Assignment to Study

The animals will be carefully observed during the acclimatisation period to ensure that they are in good health and suitable for inclusion in the study.

12. HUSBANDRY**12.1. Housing**

During pre-trial, animals will be multiply housed (where possible) by sex in solid floored polycarbonate and stainless steel cages with bedding and/or nesting material.

Bedding material will be sterilised white wood shavings which will be provided with a certificate of analysis for significant contaminants. An analytical certificate for each batch of bedding used will be retained at Charles River, Edinburgh.

During on-study periods, rats will be multiply housed (where possible) by sex in polycarbonate and stainless steel cages with raised wire mesh floors.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****12.2. Environmental Conditions**

The targeted conditions for animal room environment will be as follows:

Temperature:	19 - 23°C
Humidity:	40 -70%
Ventilation:	A minimum of 10 air changes per hour
Light Cycle:	12 hours light and 12 hours dark (except when interrupted by study procedures/activities).

There will be automatic control of temperature which will be continuously monitored and recorded. Humidity will be continuously monitored and recorded. Deviations from target temperature and humidity ranges will be presented in the study report.

There will be automatic control of light cycle.

12.3. Food

SDS Rat and Mouse (modified) No. 1 Diet SQC Expanded will be provided *ad libitum* throughout the study, except during designated procedures. The same diet in meal form may be provided to individual animals as warranted by clinical signs (e.g. broken/damaged incisors or other health changes).

The feed is analysed by the supplier for nutritional components and environmental contaminants. Results of the analysis are provided by the supplier and will be retained at Charles River, Edinburgh.

It is considered that there are no known contaminants in the feed that would interfere with the objectives of the study.

12.4. Water

The animals will have access to water *ad libitum* from the public supply from water bottles which will be changed as necessary throughout the course of the study.

The water used by Charles River Edinburgh is analysed at regular intervals for dissolved materials, heavy metals, pesticide residues, pH, nitrates and nitrites. Microbiological screening is also conducted. An analytical certificate for each analysis will be retained at Charles River, Edinburgh.

The water used is considered not to contain any additional substances, in sufficient concentration, to have any influence on the outcome of the study.

12.5. Animal Enrichment

Animals will be socially housed for psychological/environmental enrichment and will be provided with items such as a device for hiding and an object for chewing, except when interrupted by study procedures/activities.

Objects for chewing and devices for hiding will be provided with a certificate of analysis for significant contaminants. An analytical certificate for each batch of chewing objects and hiding devices used will be retained at Charles River, Edinburgh.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

Other items may be included to enrich the cage environment. Details will be given in the study report.

12.6. Veterinary Care

Veterinary care will be available throughout the course of the study and animals will be examined by the veterinary staff as warranted by clinical signs or other changes. All veterinary examinations and recommended therapeutic treatments, if any, will be documented in the study records.

In the event that animals show signs of illness or distress, the responsible veterinarian may make initial recommendations about treatment of the animal(s) and/or alteration of study procedures, which must be approved by the Study Director. All such actions will be properly documented in the study records and, when appropriate, by protocol amendment. Treatment of the animal(s) for minor injuries or ailments may be approved without prior consultation with the Sponsor representative when such treatment does not impact fulfilment of the study objectives. If the condition of the animal(s) warrants significant therapeutic intervention or alterations in study procedures, the Sponsor representative will be contacted, when possible, to discuss appropriate action. If the condition of the animal(s) is such that emergency measures must be taken, the Study Director and/or attending veterinarian will attempt to consult with the Sponsor representative prior to responding to the medical crisis, but the Study Director and/or veterinarian has authority to act immediately at his/her discretion to alleviate suffering. The Sponsor representative will be fully informed of any such events.

13. EXPERIMENTAL DESIGN

Phase No.	Test Item	Dose Route	Dose Level* (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals	Animal Numbers
						Main Study Males	
1	¹⁴ C]-Malathion	Oral	40	5	8	4	001M-004M
2		Oral	800		160	4	005M-008M
3		Oral	TBC		TBC	4	009M-012M
4		IV	4		0.8	4	013M-016M
5		Oral	TBC		TBC	2	017M-018M
6		IV	4		0.8	2	019M-020M

* Each rat in Phase 2, 3 and 5 will receive a target radiochemical dose of 10 MBq/kg. Each rat in Phase 1, 4 and 6 will receive a target radiochemical dose of 5 MBq/kg

TBC = To be confirmed, dose levels will be selected following discussion of Phase 2 results with the Sponsor and detailed by a formal protocol amendment.

The study will be conducted in 6 phases. In Phases 1 and 2, the blood and plasma kinetics of total radioactivity will be investigated in groups of 4 male rats following a single oral administration of [¹⁴C]-Malathion at 40 mg/kg or 800 mg/kg.

In Phase 3, the blood and plasma kinetics of total radioactivity will be investigated in a group of 4 male rats following a single oral administration of [¹⁴C]-Malathion at a dose level to be decided following a review of Phase 2 results, in agreement with the Sponsor.

In Phase 4, the blood and plasma kinetics of total radioactivity will be investigated in a group of 4 male rats following a single intravenous administration of [¹⁴C]-Malathion at 4 mg/kg.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

In Phase 5, a group of 2 male rats will receive a single oral administration of [¹⁴C]-Malathion at a dose level to be decided, in agreement with the Sponsor. The nature of the radioactivity present in the plasma samples will be examined.

In Phase 6, a group of 2 male rats will receive a single intravenous administration of [¹⁴C]-Malathion at 4 mg/kg. The nature of the radioactivity present in the plasma samples will be examined.

For Phases 1-4, the blood and plasma pharmacokinetic parameters will be investigated for total radioactivity animal data using Phoenix pharmacokinetic software (see Section 16).

13.1. Administration of Test Item

For oral administration, the dose will be given by gastric gavage. Intravenous administration will be *via* a tail vein as a slow bolus over a period of *ca* 30 sec.

13.2. Justification of Route and Dosage Levels

The oral route is used as this is a possible route of exposure and has been used in the toxicological evaluation of the test item. The intravenous route is used as reference route for pharmacokinetic evaluation.

It is a requirement of the Home Office Licence that the dose levels used in toxicokinetic studies will not result in extreme toxicity. The dose levels selected for oral administration will normally be dose levels used previously in the toxicological evaluation of the test item and should exhibit no overt toxic effects. Similarly, the dose level for intravenous administration will be a no-effect dose level used previously. Where no tolerability information is available for the intravenous route of administration, the dose level used will normally be at least a factor of 10 lower than the oral dose level.

Prior to administration, the Sponsor will provide evidence (based on previous studies undertaken with the test item) to support the conclusion that the dose levels selected for use in the study are well tolerated.

14. EXPERIMENTAL PROCEDURE**14.1. Phase 1: Blood and Plasma Kinetics Following Oral Administration at 40 mg/kg**

Four male rats will each receive a single oral administration of [¹⁴C]-Malathion and will be multiply housed (where possible) in polycarbonate and stainless steel cages with raised wire mesh floors.

Blood samples (*ca* 0.2 mL) will be collected into heparinised tubes by venepuncture of a tail or jugular vein, at the following target times (actual times will be recorded):

0.25, 0.5, 1, 2, 4, 8, 12, 24, 30, 48, 72 and 96 h post dose

Total radioactivity will be determined in each blood and plasma sample collected.

14.2. Phase 2: Blood and Plasma Kinetics Following Oral Administration at 800 mg/kg

Four male rats will each receive a single oral administration of [¹⁴C]-Malathion. The animals will be housed and samples collected and analysed as described in Section 14.1 above.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****14.3. Phase 3: Blood and Plasma Kinetics Following Oral Administration (dose level to be detailed by protocol amendment)**

Four male rats will each receive a single oral administration of [¹⁴C]-Malathion. The animals will be housed and samples collected and analysed as described in Section 14.1.

14.4. Phase 4: Blood and Plasma Kinetics Following Intravenous Administration at 4 mg/kg

Four male rats will each receive a single intravenous administration of [¹⁴C]-Malathion and will be multiply housed (where possible) in polycarbonate and stainless steel cages with raised wire mesh floors.

Blood samples (ca 0.2 mL) will be collected into heparinised tubes by venepuncture of a jugular vein, at the following target times (actual times will be recorded):

5 minutes, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 30, 48, 72 and 96 h post dose

Total radioactivity will be determined in each blood and plasma sample collected.

14.5. Phase 5: Collection of Plasma for Chromatographic Analysis Following Oral Administration (dose level to be detailed by protocol amendment)

Two male rats will receive a single oral administration of [¹⁴C]-Malathion and housed as in Section 14.1 above. Rats will be humanely killed (CO₂ narcosis) at 1 timepoint post dose (time to be detailed by a formal protocol amendment following discussion with the Sponsor).

A sample of whole blood (ca 3-10 mL) will be collected from the heart into heparinised tubes. Plasma will be separated from each sample by centrifugation. Blood cells will be discarded.

Total radioactivity will be determined in each plasma sample collected.

14.6. Phase 6: Collection of Plasma for Chromatographic Analysis Following Intravenous Administration at 4 mg/kg

Two male rats will receive a single oral administration of [¹⁴C]-Malathion and housed as in Section 14.1 above. Rats will be humanely killed (CO₂ narcosis) at 1 timepoint post dose (time to be detailed by a formal protocol amendment following discussion with the Sponsor).

A sample of whole blood (ca 3-10 mL) will be collected from the heart into heparinised tubes. Plasma will be separated from each sample by centrifugation. Blood cells will be discarded.

Total radioactivity will be determined in each plasma sample collected.

14.7. Sample Storage

Biological samples not analysed immediately including those collected for chromatographic analysis will be stored frozen (ca -20°C) until taken for analysis. After analysis, samples will be returned to storage at ca -20°C.

Samples of dose determinations and dose residues (where relevant) will be stored at room temperature prior to and following analysis. These samples will be discarded at the Study Director's discretion following acceptance of the study results.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****14.8. Sample Processing**

Volumes or weights of all samples will be measured where appropriate.

Duplicate portions of each blood sample will be combusted using a Perkin Elmer Model 307 Sample Oxidiser. The resultant $^{14}\text{CO}_2$ generated will be collected in a suitable absorbent scintillation system. The efficiency of oxidation will be determined by combustion of quality control standards.

Duplicate portions of liquid samples will be diluted with water or compatible solvent (if necessary) and dissolved in scintillation fluid.

14.9. Total Radioactivity Analysis

All samples prepared in scintillation fluid will be subjected to liquid scintillation counting for 5 min, together with representative blank samples, using a Liquid Scintillation Analyser with automatic quench correction by an external standard method. Prior to analysis, samples will be allowed to stabilise with regard to light and temperature.

Representative blank sample values will be subtracted from sample count rates to give net d.p.m. per sample.

A limit of reliable measurement of 30 d.p.m. above background has been instituted in these laboratories. If results arise from data below the limit of reliable measurement, the fact will be noted in the Results section of the report.

Radioactivity in samples analysed by HPLC will be quantified by either on-line radiodetection with peak integration or by fraction collection with liquid scintillation counting.

15. METABOLITE CHARACTERISATION**15.1. Sample Pooling**

Plasma samples following oral (Phase 5 only) and intravenous (Phase 6 only) administration will be pooled to generate a single plasma sample per dose route for metabolite profiling. Volumes pooled will be detailed in the study data. Pools will be prepared as detailed in the table below:

Matrix	Group	Dose Route	Dose Level	Animals
Plasma	5	Oral	TBC	017M-018M
	6	IV	4 mg/kg	019M-020M

TBC = To be confirmed, dose level will be selected following discussion of Phase 2 results with the Sponsor and detailed by a formal protocol amendment.

15.1.1. Extraction of Samples

Samples of plasma will be extracted prior to analysis. Extraction methods may be supplied by the Sponsor or developed at Charles River.

Methods will be compatible with mass spectrometry. Sample preparation procedures will be conducted with the aim of achieving $\geq 85\%$ recovery of radioactivity. Recovery will be determined at all appropriate stages (e.g. extraction and concentration). Investigation of any

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

unextracted radiolabelled material will be conducted at the discretion of the Study Director. The stability and extractability of the test item may be investigated by extraction and analysis of pre-dose or control samples fortified with [^{14}C]-Malathion. Should this be required details will be included in a protocol amendment.

15.1.2. Chromatography

Final sample extracts will be analysed by HPLC with concurrent radiodetection and mass spectrometry. The parent molecule and any available reference standards will also be analysed to aid identification of metabolites.

Each resolved metabolite will be expressed as a concentration in plasma.

Following initial sample analysis, further development of the chromatographic method may be necessary to provide suitable resolution of metabolites present. This will be discussed with the Sponsor and will be the subject of a contract extension if required.

A representative column recovery will be established for each matrix. The radioactivity collected in the post column eluent will be quantified against the actual radioactivity injected.

15.2. Additional Investigations

The presence of conjugated metabolites may be investigated by treating selected extracts with non-specific β -glucuronidase containing residual sulphatase activity (from *Helix pomatia*). The resulting hydrolysates will be analysed using the same chromatographic methods as used for quantification and identification of unconjugated metabolites.

Further work to facilitate metabolite characterisation and or/identification involving *e.g.* additional extraction procedures, sample clean-up and/or metabolite isolation may be required.

Additional investigations will be conducted following discussion with the Sponsor and the intended methods will be documented in the study files and approved by the Study Director prior to commencement. Additional investigations may be the subject of a contract extension.

16. DATA ANALYSIS AND PRESENTATION**16.1. Pharmacokinetic Evaluation**

Pharmacokinetic parameters will be estimated using Phoenix pharmacokinetic software. A non-compartmental approach consistent with the extravascular and intravenous bolus routes of administration will be used for parameter estimation. All parameters will be generated from individual total radioactivity in plasma and whole blood from Phases 1-4, whenever practical.

Parameters to be Estimated

Parameter	Description of Parameter
T_{max}	The time after dosing at which the maximum concentration was observed.
C_0	The theoretical concentration at time zero after intravenous bolus dosing.
C_{max}	The maximum observed concentration measured after dosing.
C_{max}/D	The C_{max} divided by the dose administered.
$AUC(0-t)$	The area under the concentration versus time curve from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed, using the linear or linear/log trapezoidal method.
$AUC(0-t)/D$	The $AUC(0-t)$ divided by the dose administered.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

When data permits, the slope of the terminal elimination phase of each concentration versus time curve will be determined by log-linear regression, and the following additional parameters will also be estimated.

Additional Parameters to be Estimated

Parameter	Description of Parameter
$T_{1/2}$	The apparent terminal elimination half life.
$AUC_{(0-\infty)}$	The area under the concentration versus time curve from time zero to infinity.
$AUC_{(0-\infty)}/D$	The $AUC_{(0-\infty)}$ divided by the dose administered.
CL	The apparent clearance rate of total radioactivity from the analysed test matrix, following intravenous dosing only
Vd	The apparent volume of distribution of total radioactivity in the test system.
F	Absolute bioavailability, calculated as the ratio of the dose-normalised AUC (oral) to dose normalised AUC (intravenous)

Descriptive statistics (means and standard deviations) for appropriate grouping and sorting variables will be generated using Phoenix. For T_{max} , median values will be reported.

17. TERMINAL PROCEDURES**17.1. Unscheduled Deaths**

Animals that are euthanized for humane reasons, pre-treatment or post treatment will undergo exsanguination from the abdominal aorta following carbon dioxide asphyxiation. If necessary, the animal will be refrigerated to minimize autolysis.

A complete gross pathology examination of the carcass will be conducted on all animals found dead or euthanized for humane reasons following dosing; all other animal carcasses will be discarded without examination. No tissues will be retained for any animal.

18. COMPUTERISED SYSTEMS

The following critical computerised systems may be used in the study. The actual critical computerised systems used will be specified in the Final Report.

System Name	Description of Data Collected and/or Analysed
Atlas and/or Laura	Chromatography data
DEBRA® Laboratory Information System	Total radioactivity and sample weight data
Phoenix	Pharmacokinetics
Mass Lynx and/or LC-MS Solutions	Mass Spectrometry data

19. STATISTICAL ANALYSIS

Statistical analyses shall be limited to derivation of means, standard deviations and coefficients of variation where appropriate.

20. AMENDMENTS AND DEVIATIONS

Changes to the approved protocol shall be made in the form of an amendment, which will be signed and dated by the Study Director. Every reasonable effort will be made to discuss any necessary protocol changes in advance with the Sponsor.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

All protocol and SOP deviations will be documented in the study records. Deviations from the protocol and/or SOP related to the phase(s) of the study conducted at a Test Site shall be documented, acknowledged by the PI/IS, and reported to the Study Director for authorisation/acknowledgement. The Study Director will notify the Sponsor of deviations that may result in a significant impact on the study as soon as possible.

21. RETENTION OF RECORDS, SAMPLES AND SPECIMENS

All study-specific raw data, electronic data, documentation, protocol, protocol amendments and interim (if applicable) reports from this study will be transferred to the Charles River archive by no later than the date of final report issue. Two years after issue of the final report, the Sponsor will be contacted to determine the disposition of these materials. The original signed copy of the Final Report will be archived indefinitely at the Test Facility.

Electronic data generated by the Test Facility will be archived and the software and hardware required to produce it in a readable form will be maintained and available.

All records, retained samples and specimens and reports generated from phases or segments performed by Test Facility-designated subcontractors will be returned to the Test Facility for archiving.

Records to be maintained will include, but will not be limited to, documentation and data for the following:

- Protocol, protocol amendments, and deviations
- Study schedule
- Study-related correspondence
- Test system receipt, health, and husbandry
- Test item receipt, identification, preparation, and analysis
- In-life measurements and observations
- Statistical analysis results

Biological samples generated during the course of this study will be held deep frozen until issue of the final report. Charles River will contact the Sponsor to discuss the fate of the samples (disposal, return or retain at Charles River) on issue of the final report. Samples will be disposed of unless Charles River receives written instruction regarding shipment of the samples to the Sponsor or continued storage at Charles River. Shipment of samples or long term retention will incur additional costs.

22. REPORTING

A comprehensive Draft Report will be prepared following completion of the study and will be finalised following consultation with the Sponsor. The report will include all information necessary to provide a complete and accurate description of the experimental methods and results and any circumstances that may have affected the quality or integrity of the study.

The Sponsor will receive an electronic version of the Draft and Final Report provided in Adobe Acrobat PDF format (hyperlinked and searchable at final) along with a Microsoft Word version of the text. The PDF document will be created from native electronic files to the extent possible, including text and tables generated by the Test Facility. Report

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

components not available in native electronic files and/or original signature pages will be scanned and converted to PDF image files for incorporation. An original copy of the report with the Test Facility's handwritten signatures will be retained.

Reports should be finalised within 6 months of issue of the audited Draft Report. If the Sponsor has not provided comments to the report within 6 months of draft issue, the report will be finalised by the Test Facility unless other arrangements are made by the Sponsor.

23. ANIMAL WELFARE

The UK Home Office controls scientific procedures on animals in the UK and does so by the issue of licences under the Animals (Scientific Procedures) Act 1986. The regulations conform to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, Council of Europe) and achieve the standard of care required by the US Department of Health and Human Services' Guide for the Care and Use of Laboratory Animals.

The Home Office licence governing this study strictly specifies the limits of severity of effects on the animals. From the available information, the procedures described in the protocol are not anticipated to cause any effects which exceed the severity limit of the procedure. Any animal which shows unacceptable reactions may be euthanised or other actions taken as required by the Home Office to alleviate distress.

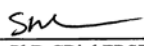
23.1. Home Office Project Licence No.

The animal work in this study will be conducted under the UK Home Office Project Licence No. PPL 70/8628, Metabolism of Chemicals, Protocol Reference Number 1.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

24. TEST FACILITY APPROVAL

The signature below acknowledges Test Facility Management's responsibility to the study as defined by the relevant GLP regulations.



Stephen Madden, BSc PhD CBiol FRSB
Test Facility Management

Date: 15 July 2016

The signature below indicates that the Study Director approves the study protocol.



Mhairi Libberton, BSc
Study Director

Date: 15 July 2016

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

25. SPONSOR APPROVAL

The signature below confirms the approval of the protocol by the Sponsor Representative.



Mette Jensen
Sponsor Representative

Date: July 25, 2016

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**



PROTOCOL AMENDMENT NO. 4

Test Facility Study No. 172891

Sponsor Reference No. 2016MET-FYF2662

**[¹⁴C]-Malathion: The Pharmacokinetics of [¹⁴C]-Malathion in the Rat
Following Single Oral and Intravenous Administration**

SPONSOR:
Cheminova A/S
P.O. Box 9
DK-7620 Lemvig
Denmark

TEST FACILITY:
Charles River Laboratories Edinburgh Ltd
Elphinstone Research Centre
Tranent, East Lothian, EH33 2NE
UK

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****SUMMARY OF CHANGES AND JUSTIFICATIONS**

Note: When applicable, additions are indicated in bold underlined text and deletions are indicated in bold strikethrough text in the affected sections of the document.

Item or Section(s)	Justification
Amendment 1	Date: 14 September 2016
8. Test Items and Dose Vehicles 8.1. Test Item	To update the batch number of the radiolabelled test item to be consistent with the batch number on the Certificate of Analysis.
10. Dose Formulation and Analysis 10.4. Dose Levels, Dose Regimen and Routes of Administration	To include the details of the dose level selected for Phase 3, following a review of the results and in agreement with the Sponsor.
13. Experimental Design	To include the details of the dose level selected for Phase 3, following a review of the results and in agreement with the Sponsor.
14. Experimental Procedure 14.3. Phase 3: Blood and Plasma Kinetics Following Oral Administration (dose level to be detailed by protocol amendment)	To update the section title to include the details of the dose level selected for Phase 3, following a review of the results and in agreement with the Sponsor.
Amendment 2	Date: 18 October 2016
1. Objectives	To detail that the nature of radioactivity in red blood cells will now also be investigated, as requested by the Sponsor.
6. Sponsor	Addition of Sponsor address and details of Sponsor study monitor, previously omitted in error.
10. Dose Formulation and Analysis 10.2 Trial Dose Formulations	To detail the selected Phase 3 dose concentration
10. Dose Formulation and Analysis 10.4 Dose Levels, Dose Regimen and Routes of Administration	To detail the selected dose route, level and concentration for Phases 5 and 6, in agreement with the Sponsor.
13. Experimental Design	To detail the selected dose route, level and concentration for Phases 5 and 6, in agreement with the Sponsor. Also, to detail that the nature of radioactivity in red blood cells in Phase 5 and 6 will now also be investigated, as requested by the Sponsor.
14. Experimental Procedure 14.5 Phase 5: Collection of Plasma for Chromatographic Analysis Following Oral Administration (dose level to be detailed by protocol amendment)	To detail the selected dose level for Phase 5, in agreement with the Sponsor. Also, to detail that whole blood and red blood cells samples will now also be retained and analysed. Section title also updated to state red blood cells will be collected for chromatographic analysis.
14. Experimental Procedure 14.6 Phase 6: Collection of Plasma for Chromatographic Analysis Following Intravenous Administration at 4 mg/kg	To detail the updated dose route and level for Phase 6, in agreement with the Sponsor. Also, to detail that whole blood and red blood cells samples will now also be retained and analysed. Section title also updated to state red blood cells will be collected for chromatographic analysis.
15. Metabolite Characterisation 15.1 Sample pooling	To detail the selected dose route and level for Phases 5 and 6 and that red blood cells sample pools will be prepared, as requested by the Sponsor.
15. Metabolite Characterisation 15.1 Sample pooling 15.1.1 Extraction of samples	To detail the inclusion of red blood cells samples for metabolite characterisation, as requested by the Sponsor.
15. Metabolite Characterisation 15.1 Sample pooling 15.1.2 Chromatography	To detail the inclusion of red blood cells samples for metabolite characterisation, as requested by the Sponsor.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

Item or Section(s)	Justification
Amendment 3	Date: 19 October 2016
14. Experimental Procedure 14.5 Phase 5: Collection of Plasma and Red Blood Cells for Chromatographic Analysis Following Oral Administration at 800 mg/kg	To detail the selected sacrifice timepoint, in agreement with the Sponsor.
14. Experimental Procedure 14.6 Phase 6: Collection of Plasma and Red Blood Cells for Chromatographic Analysis Following Oral Administration at 1200 mg/kg	To detail the selected sacrifice timepoint, in agreement with the Sponsor.
Amendment 4	Date: 11 January 2017
2. Proposed Study Schedule	To amend the Draft Report date in line with new study timelines, in agreement with the Sponsor.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****TABLE OF CONTENTS**

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**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****1. OBJECTIVE(S)**

This study has been designed to examine the pharmacokinetics of total radioactivity following single oral and intravenous administration of [¹⁴C]-Malathion to male rats. Pharmacokinetic parameters will be investigated in blood and plasma using Phoenix pharmacokinetic software.

The nature of the radioactivity present in selected plasma and red blood cells samples will also be examined.

2. PROPOSED STUDY SCHEDULE

Experimental Start Date:	August 2016
Completion of Total Radioactivity Analysis:	November 2016
Experimental Completion Date:	December 2016
Draft Report:	January <u>February</u> 2017

3. GUIDELINES FOR STUDY DESIGN

OECD guideline reference 417 (2010): Toxicokinetics.

United States Environmental Protection Agency, Health Effects Test Guidelines (August 1998) OPPTS 870.7485 (Metabolism and Pharmacokinetics).

Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market.

Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.

Japanese Ministry of Agriculture, Forestry and Fisheries, Test Data for Registration of Agricultural Chemicals, 12 Nohsan No 8147, Agricultural Production Bureau, November 24, 2000.

4. REGULATORY COMPLIANCE

The study will be performed in accordance with the OECD Principles of Good Laboratory Practice as incorporated into the United Kingdom Statutory Instrument for GLP and as accepted by Regulatory Authorities throughout the European Union, United States of America (FDA and EPA) and Japan (MHLW, MAFF and METI) and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

All routine activities performed during the conduct of this study are detailed in Charles River Standard Operating Procedures.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****5. QUALITY ASSURANCE****5.1. Test Facility**

The Test Facility Quality Assurance Unit (QAU) will monitor the study to assure the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with Good Laboratory Practice regulations. The QAU will review the protocol, conduct inspections at intervals adequate to assure the integrity of the study, and audit the Final Report to assure that it accurately describes the methods and standard operating procedures and that the reported results accurately reflect the raw data of the study.

6. SPONSOR**Sponsor Representative**

Mette Jensen
Cheminova A/S
P.O. Box 9
DK-7620 Lemvig
Denmark
Tel: +45 9690 9775
Fax: +45 9690 9691
E-mail: mette.jensen@finc.com

Sponsor Study Monitor

Alex Gledhill
JSC International Limited
The Exchange
Station Parade
Harrogate, North Yorkshire, HG1 1TS
UK
Tel: +44 (0)1423 700241
Fax: +44(0)1423 520297
E-mail: alex.gledhill@jsc.co.uk

7. RESPONSIBLE PERSONNEL**Study Director**

Mhairi Libberton, BSc
Charles River Laboratories Edinburgh Ltd
Elphinstone Research Centre
Tranent, East Lothian, EH33 2NE
UK
Tel: +44 (0) 1875 618292
Fax: +44 (0) 1875 614555
E-mail: mhairi.libberton@crl.com

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****Management Contact**

Stephen Madden, BSc PhD CBiol FRSB
Charles River Laboratories Edinburgh Ltd
Elphinstone Research Centre
Tranent, East Lothian, EH33 2NE
UK
Tel: +44 (0) 1875 618316
Fax: +44 (0) 1875 614555
E-mail: stephen.madden@crl.com

Principal Analyst (Responsible for Purity Assessment)

Gary Wicksted, BSc
Charles River Laboratories Edinburgh Ltd
Elphinstone Research Centre
Tranent, East Lothian, EH33 2NE
UK
Tel: +44 (0) 1875 888764
Fax: +44 (0) 1875 614555
E-mail: gary.wicksted@crl.com

Principal Analyst (Responsible for Biotransformation Analysis)

Martha A Green, BSc PhD
Charles River Laboratories Edinburgh Ltd
Elphinstone Research Centre
Tranent, East Lothian, EH33 2NE
UK
Tel: +44 (0) 1875 886267
Fax: +44 (0) 1875 614555
E-mail: martha.green@crl.com

8. TEST ITEMS AND DOSE VEHICLES**8.1. Test Item**

Identification: [¹⁴C]-Malathion
Batch (Lot) Number: 9587CEO001-1
Specific Activity: To be confirmed in the study data
Radiochemical Purity: To be confirmed in the study data
Purity: To be confirmed in the study data, dose calculations will not be corrected for purity.
Storage Conditions: To be confirmed in the study data

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

Identification: Malathion Technical
Batch (Lot) Number: P2168-IbW-05
Expiration Date: 06 April 2019
Physical Description: Pale yellow liquid
Molecular Weight: 330.36
Purity: 95%; dose calculations will be corrected for purity.
Storage Conditions: Kept in a freezer set to maintain -20°C

8.2. Dose Vehicles

Corn oil. Intravenous dose vehicle to be decided following trial assessment – refer to Section 10.2.

8.3. Reference Standards

Any available reference standards will be supplied by the Sponsor. These will be supplied and stored as outlined for the unlabelled material in Section 8.1 (unless otherwise instructed by the Sponsor) except that no reserve sample will be retained.

8.4. Test Item Characterisation

The Sponsor will provide to the Test Facility documentation of the identity, strength, purity, composition, and stability for the test item. A Certificate of Analysis or equivalent documentation will be provided for inclusion in the Final Report.

The Sponsor has appropriate documentation on file concerning the method of synthesis, fabrication or derivation of the test item, and this information is available to the appropriate regulatory agencies should it be requested.

8.5. Analysis of Test Item

The stability of the bulk test item will not be determined during the course of this study. Information to support the stability of each lot of the bulk test item will be provided by the Sponsor.

8.6. Reserve Samples

For each batch (lot) of unlabelled test item, a reserve sample (approximately 20 mg or appropriate) will be collected and maintained under the appropriate storage conditions by the Test Facility.

8.7. Test Item Inventory and Disposition

Records of the receipt, distribution, and storage of test items will be maintained. After finalisation of the study report, the Sponsor will be contacted with regard to the disposal, shipment, or continued storage of the test item. The fees for either outcome will be the subject of a contract extension once the costs are known.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****8.8. Other Materials**

Other materials will be obtained by Charles River. Chemicals will be of analytical grade where available.

9. SAFETY

Safety instructions for this study are provided on the Sponsor supplied safety data sheet. An internal COSHH safety sheet will be prepared at Charles River.

10. DOSE FORMULATION AND ANALYSIS**10.1. Radiochemical Purity and Stability**

Charles River will confirm the radiochemical purity of the radiolabelled test item prior to animal dosing using a preliminary HPLC method supplied by the Sponsor. This method may be optimised to improve the retention time of the test item if necessary and will be compatible with LC-MS whenever possible.

10.2. Trial Dose Formulations

Prior to preparation of the animal dose, trial oral formulations will be performed to assess the suitability of the dose formulation procedures and to assess the stability of the radiochemical over the anticipated formulation and administration periods. The trial dose preparation will mimic the procedures required for the main animal study but will be prepared using the minimum practical quantities of test item. The trial oral dose formulations will be prepared at 8 and 160 mg/mL. An additional trial oral formulation will also be prepared at the Phase 3 nominal dose concentration (240 mg/mL) if the concentration is >160 mg/mL. Radiochemical purity will be assessed at the following timepoints:

24 and 48 h after preparation

Prior to the preparation of the trial intravenous dose solution, preliminary experiments will be carried out to assess the solubility of unlabelled Malathion in an appropriate vehicle suitable for intravenous dosing. A trial intravenous dose formulation will then be performed to assess the suitability of the dose formulation procedures and to assess the stability of the radiochemical in the formulation over the anticipated formulation and administration period. The trial IV dose formulation will be prepared at 0.8 mg/mL. Radiochemical purity will be assessed at the following timepoints:

3 and 24 h after preparation

10.3. Summary of Radiochemical Purity Measurements

A summary of the radiochemical purity measurements to be performed is detailed below:

Sample	Time
Stock radiochemical	On receipt/as deemed necessary
Trial oral formulations	Stability at 24 and 48 h
Trial IV formulation	Stability at 3 and 24 h
Dose Preparations	Predose and post dose

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****10.4. Dose Levels, Dose Regimen and Routes of Administration**

Phase No.	Dose Route	Nominal Dose	Nominal Radioactive Dose	Nominal Dose Concentration	Nominal Dose Volume
1	Oral	40 mg/kg	5 MBq/kg	8 mg/mL	5 mL/kg
2	Oral	800 mg/kg	10 MBq/kg	160 mg/mL	
3	Oral	1200 mg/kg		240 mg/mL	
4	IV	4 mg/kg	5 MBq/kg	0.8 mg/mL	
5	Oral	800 mg/kg	10 MBq/kg	160 mg/mL	
6	Oral	1200 mg/kg	10 MBq/kg	240 mg/mL	

10.5. Dose Formulations

The oral dose formulations will be prepared as a suspension in corn oil.

The intravenous dose formulation will be prepared as a non-irritant solution in a dose vehicle to be selected following trial stability work (section 10.2). The dose vehicle selection will be documented in the raw data.

The dose formulations will be stored in a refrigerator set to maintain a temperature of 4°C until prior to administration, with the exception of the trial intravenous formulation which will be stored at ambient until 3 h post preparation, then stored in a refrigerator set to maintain a temperature of 4°C.

The purity of the dose formulations will be determined by HPLC prior to and following dose administration.

10.6. Dose Determination

The actual dose received by each animal will be determined with reference to the dose concentration, the weight of dose administered and the specific activity of [¹⁴C]-Malathion in the formulated dose. Where necessary, these calculations will take into account any undosed residue.

11. TEST SYSTEM

Species: Rat
 Strain: Sprague-Dawley
 Condition: Purpose-bred, naive
 Source: Charles River UK Limited, Margate, Kent, UK
 Number of Males Ordered: Twenty
 Target Age at the Initiation of Dosing: Approximately 8-10 weeks at dosing
 Target Weight at the Initiation of Dosing: Appropriate for age and strain of animal.

The actual age, weight, and number of animals received will be listed in the Final Report.

11.1. Justification of Test System and Number of Animals

The Sprague Dawley rat has been chosen as the species for this study as this is a species which has been used in the toxicological evaluation of the test item.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

The study will be conducted using male rats only since there is no sex difference apparent from existing ADME and toxicity data available for Malathion.

The number of rats chosen for this study is the smallest number considered necessary to provide reliable data and is compliant with regulatory requirements.

11.2. Animal Identification

Each cage will be given a cage card which identifies each animal within the cage by study number, animal number and sex. Animals assigned to the study will be uniquely identified by tail marking and will be accurately weighed predose.

11.3. Environmental Acclimation

The animals will be allowed to acclimatise to the Charles River rodent accommodation for a period of a minimum of 5 days before the commencement of dosing.

11.4. Selection and Assignment to Study

The animals will be carefully observed during the acclimatisation period to ensure that they are in good health and suitable for inclusion in the study.

12. HUSBANDRY**12.1. Housing**

During pre-trial, animals will be multiply housed (where possible) by sex in solid floored polycarbonate and stainless steel cages with bedding and/or nesting material.

Bedding material will be sterilised white wood shavings which will be provided with a certificate of analysis for significant contaminants. An analytical certificate for each batch of bedding used will be retained at Charles River, Edinburgh.

During on-study periods, rats will be multiply housed (where possible) by sex in polycarbonate and stainless steel cages with raised wire mesh floors.

12.2. Environmental Conditions

The targeted conditions for animal room environment will be as follows:

Temperature:	19 - 23°C
Humidity:	40 -70%
Ventilation:	A minimum of 10 air changes per hour
Light Cycle:	12 hours light and 12 hours dark (except when interrupted by study procedures/activities).

There will be automatic control of temperature which will be continuously monitored and recorded. Humidity will be continuously monitored and recorded. Deviations from target temperature and humidity ranges will be presented in the study report.

There will be automatic control of light cycle.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****12.3. Food**

SDS Rat and Mouse (modified) No. 1 Diet SQC Expanded will be provided *ad libitum* throughout the study, except during designated procedures. The same diet in meal form may be provided to individual animals as warranted by clinical signs (*e.g.* broken/damaged incisors or other health changes).

The feed is analysed by the supplier for nutritional components and environmental contaminants. Results of the analysis are provided by the supplier and will be retained at Charles River, Edinburgh.

It is considered that there are no known contaminants in the feed that would interfere with the objectives of the study.

12.4. Water

The animals will have access to water *ad libitum* from the public supply from water bottles which will be changed as necessary throughout the course of the study.

The water used by Charles River Edinburgh is analysed at regular intervals for dissolved materials, heavy metals, pesticide residues, pH, nitrates and nitrites. Microbiological screening is also conducted. An analytical certificate for each analysis will be retained at Charles River, Edinburgh.

The water used is considered not to contain any additional substances, in sufficient concentration, to have any influence on the outcome of the study.

12.5. Animal Enrichment

Animals will be socially housed for psychological/environmental enrichment and will be provided with items such as a device for hiding and an object for chewing, except when interrupted by study procedures/activities.

Objects for chewing and devices for hiding will be provided with a certificate of analysis for significant contaminants. An analytical certificate for each batch of chewing objects and hiding devices used will be retained at Charles River, Edinburgh.

Other items may be included to enrich the cage environment. Details will be given in the study report.

12.6. Veterinary Care

Veterinary care will be available throughout the course of the study and animals will be examined by the veterinary staff as warranted by clinical signs or other changes. All veterinary examinations and recommended therapeutic treatments, if any, will be documented in the study records.

In the event that animals show signs of illness or distress, the responsible veterinarian may make initial recommendations about treatment of the animal(s) and/or alteration of study procedures, which must be approved by the Study Director. All such actions will be properly documented in the study records and, when appropriate, by protocol amendment. Treatment of the animal(s) for minor injuries or ailments may be approved without prior consultation with the Sponsor representative when such treatment does not impact fulfilment of the study

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

objectives. If the condition of the animal(s) warrants significant therapeutic intervention or alterations in study procedures, the Sponsor representative will be contacted, when possible, to discuss appropriate action. If the condition of the animal(s) is such that emergency measures must be taken, the Study Director and/or attending veterinarian will attempt to consult with the Sponsor representative prior to responding to the medical crisis, but the Study Director and/or veterinarian has authority to act immediately at his/her discretion to alleviate suffering. The Sponsor representative will be fully informed of any such events.

13. EXPERIMENTAL DESIGN

Phase No.	Test Item	Dose Route	Dose Level ^a (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals	Animal Numbers
						Main Study	
1	[¹⁴ C]-Malathion	Oral	40	5	8	Males	001M-004M
2		Oral	800		160	4	005M-008M
3		Oral	1200		240	4	009M-012M
4		IV	4		0.8	4	013M-016M
5		Oral	800		160	2	017M-018M
6		Oral	1200		240	2	019M-020M

^a Each rat in Phase 2, 3, 5 and 6 will receive a target radiochemical dose of 10 MBq/kg. Each rat in Phase 1 and 4 will receive a target radiochemical dose of 5 MBq/kg

The study will be conducted in 6 phases. In Phases 1 and 2, the blood and plasma kinetics of total radioactivity will be investigated in groups of 4 male rats following a single oral administration of [¹⁴C]-Malathion at 40 mg/kg or 800 mg/kg.

In Phase 3, the blood and plasma kinetics of total radioactivity will be investigated in a group of 4 male rats following a single oral administration of [¹⁴C]-Malathion at 1200 mg/kg.

In Phase 4, the blood and plasma kinetics of total radioactivity will be investigated in a group of 4 male rats following a single intravenous administration of [¹⁴C]-Malathion at 4 mg/kg.

In Phase 5, a group of 2 male rats will receive a single oral administration of [¹⁴C]-Malathion at 800 mg/kg. The nature of the radioactivity present in the plasma and red blood cells samples will be examined.

In Phase 6, a group of 2 male rats will receive a single oral administration of [¹⁴C]-Malathion at 1200 mg/kg. The nature of the radioactivity present in the plasma and red blood cells samples will be examined.

For Phases 1-4, the blood and plasma pharmacokinetic parameters will be investigated for total radioactivity animal data using Phoenix pharmacokinetic software (see Section 16).

13.1. Administration of Test Item

For oral administration, the dose will be given by gastric gavage. Intravenous administration will be *via* a tail vein as a slow bolus over a period of *ca* 30 sec.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****13.2. Justification of Route and Dosage Levels**

The oral route is used as this is a possible route of exposure and has been used in the toxicological evaluation of the test item. The intravenous route is used as reference route for pharmacokinetic evaluation.

It is a requirement of the Home Office Licence that the dose levels used in toxicokinetic studies will not result in extreme toxicity. The dose levels selected for oral administration will normally be dose levels used previously in the toxicological evaluation of the test item and should exhibit no overt toxic effects. Similarly, the dose level for intravenous administration will be a no-effect dose level used previously. Where no tolerability information is available for the intravenous route of administration, the dose level used will normally be at least a factor of 10 lower than the oral dose level.

Prior to administration, the Sponsor will provide evidence (based on previous studies undertaken with the test item) to support the conclusion that the dose levels selected for use in the study are well tolerated.

14. EXPERIMENTAL PROCEDURE**14.1. Phase 1: Blood and Plasma Kinetics Following Oral Administration at 40 mg/kg**

Four male rats will each receive a single oral administration of [¹⁴C]-Malathion and will be multiply housed (where possible) in polycarbonate and stainless steel cages with raised wire mesh floors.

Blood samples (ca 0.2 mL) will be collected into heparinised tubes by venepuncture of a tail or jugular vein, at the following target times (actual times will be recorded):

0.25, 0.5, 1, 2, 4, 8, 12, 24, 30, 48, 72 and 96 h post dose

Total radioactivity will be determined in each blood and plasma sample collected.

14.2. Phase 2: Blood and Plasma Kinetics Following Oral Administration at 800 mg/kg

Four male rats will each receive a single oral administration of [¹⁴C]-Malathion. The animals will be housed and samples collected and analysed as described in Section 14.1 above.

14.3. Phase 3: Blood and Plasma Kinetics Following Oral Administration at 1200 mg/kg

Four male rats will each receive a single oral administration of [¹⁴C]-Malathion. The animals will be housed and samples collected and analysed as described in Section 14.1.

14.4. Phase 4: Blood and Plasma Kinetics Following Intravenous Administration at 4 mg/kg

Four male rats will each receive a single intravenous administration of [¹⁴C]-Malathion and will be multiply housed (where possible) in polycarbonate and stainless steel cages with raised wire mesh floors.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

Blood samples (*ca* 0.2 mL) will be collected into heparinised tubes by venepuncture of a jugular vein, at the following target times (actual times will be recorded):

5 minutes, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 30, 48, 72 and 96 h post dose

Total radioactivity will be determined in each blood and plasma sample collected.

14.5. Phase 5: Collection of Plasma and Red Blood Cells for Chromatographic Analysis Following Oral Administration at 800 mg/kg

Two male rats will receive a single oral administration of [¹⁴C]-Malathion and housed as in Section 14.1 above. Rats will be humanely killed (CO₂ narcosis) at 1.5 h post dose.

A sample of whole blood (*ca* 3-10 mL) will be collected from the heart into heparinised tubes. A sample of whole blood will be retained for radioanalysis (*ca* 0.5 mL). Plasma will be separated from the remaining sample by centrifugation. Blood cells will be retained.

Total radioactivity will be determined in each sample collected.

14.6. Phase 6: Collection of Plasma and Red Blood Cells for Chromatographic Analysis Following Oral Administration at 1200 mg/kg

Two male rats will receive a single oral administration of [¹⁴C]-Malathion and housed as in Section 14.1 above. Rats will be humanely killed (CO₂ narcosis) at 1.5 h post dose.

A sample of whole blood (*ca* 3-10 mL) will be collected from the heart into heparinised tubes. A sample of whole blood will be retained for radioanalysis (*ca* 0.5 mL). Plasma will be separated from the remaining sample by centrifugation. Blood cells will be retained.

Total radioactivity will be determined in each sample collected.

14.7. Sample Storage

Biological samples not analysed immediately including those collected for chromatographic analysis will be stored frozen (*ca* -20°C) until taken for analysis. After analysis, samples will be returned to storage at *ca* -20°C.

Samples of dose determinations and dose residues (where relevant) will be stored at room temperature prior to and following analysis. These samples will be discarded at the Study Director's discretion following acceptance of the study results.

14.8. Sample Processing

Volumes or weights of all samples will be measured where appropriate.

Duplicate portions of each blood sample will be combusted using a Perkin Elmer Model 307 Sample Oxidiser. The resultant ¹⁴CO₂ generated will be collected in a suitable absorbent scintillation system. The efficiency of oxidation will be determined by combustion of quality control standards.

Duplicate portions of liquid samples will be diluted with water or compatible solvent (if necessary) and dissolved in scintillation fluid.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****14.9. Total Radioactivity Analysis**

All samples prepared in scintillation fluid will be subjected to liquid scintillation counting for 5 min, together with representative blank samples, using a Liquid Scintillation Analyser with automatic quench correction by an external standard method. Prior to analysis, samples will be allowed to stabilise with regard to light and temperature.

Representative blank sample values will be subtracted from sample count rates to give net d.p.m. per sample.

A limit of reliable measurement of 30 d.p.m. above background has been instituted in these laboratories. If results arise from data below the limit of reliable measurement, the fact will be noted in the Results section of the report.

Radioactivity in samples analysed by HPLC will be quantified by either on-line radiodetection with peak integration or by fraction collection with liquid scintillation counting.

15. METABOLITE CHARACTERISATION**15.1. Sample Pooling**

Plasma and red blood cells samples following oral (Phase 5 and 6 only) administration will be pooled to generate a single plasma or red blood cells sample per dose level for metabolite profiling. Volumes or weights pooled will be detailed in the study data. Pools will be prepared as detailed in the table below:

Matrix	Group	Dose Route	Dose Level	Animals
Plasma	5	Oral	800 mg/kg	017M-018M
Red blood cells				017M-018M
Plasma	6	Oral	1200 mg/kg	019M-020M
Red blood cells				019M-020M

15.1.1. Extraction of Samples

Samples of plasma and red blood cells will be extracted prior to analysis. Extraction methods may be supplied by the Sponsor or developed at Charles River.

Methods will be compatible with mass spectrometry. Sample preparation procedures will be conducted with the aim of achieving $\geq 85\%$ recovery of radioactivity. Recovery will be determined at all appropriate stages (e.g. extraction and concentration). Investigation of any unextracted radiolabelled material will be conducted at the discretion of the Study Director. The stability and extractability of the test item may be investigated by extraction and analysis of pre-dose or control samples fortified with [^{14}C]-Malathion. Should this be required details will be included in a protocol amendment.

15.1.2. Chromatography

Final sample extracts will be analysed by HPLC with concurrent radiodetection and mass spectrometry. The parent molecule and any available reference standards will also be analysed to aid identification of metabolites.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

Each resolved metabolite will be expressed as a concentration in plasma and red blood cells.

Following initial sample analysis, further development of the chromatographic method may be necessary to provide suitable resolution of metabolites present. This will be discussed with the Sponsor and will be the subject of a contract extension if required.

A representative column recovery will be established for each matrix. The radioactivity collected in the post column eluent will be quantified against the actual radioactivity injected.

15.2. Additional Investigations

The presence of conjugated metabolites may be investigated by treating selected extracts with non-specific β -glucuronidase containing residual sulphatase activity (from *Helix pomatia*). The resulting hydrolysates will be analysed using the same chromatographic methods as used for quantification and identification of unconjugated metabolites.

Further work to facilitate metabolite characterisation and/or identification involving *e.g.* additional extraction procedures, sample clean-up and/or metabolite isolation may be required.

Additional investigations will be conducted following discussion with the Sponsor and the intended methods will be documented in the study files and approved by the Study Director prior to commencement. Additional investigations may be the subject of a contract extension.

16. DATA ANALYSIS AND PRESENTATION**16.1. Pharmacokinetic Evaluation**

Pharmacokinetic parameters will be estimated using Phoenix pharmacokinetic software. A non-compartmental approach consistent with the extravascular and intravenous bolus routes of administration will be used for parameter estimation. All parameters will be generated from individual total radioactivity in plasma and whole blood from Phases 1-4, whenever practical.

Parameters to be Estimated

Parameter	Description of Parameter
T_{max}	The time after dosing at which the maximum concentration was observed.
C_0	The theoretical concentration at time zero after intravenous bolus dosing.
C_{max}	The maximum observed concentration measured after dosing.
C_{max}/D	The C_{max} divided by the dose administered.
$AUC(0-t)$	The area under the concentration versus time curve from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed, using the linear or linear/log trapezoidal method.
$AUC(0-t)/D$	The $AUC(0-t)$ divided by the dose administered.

When data permits, the slope of the terminal elimination phase of each concentration versus time curve will be determined by log-linear regression, and the following additional parameters will also be estimated.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

Additional Parameters to be Estimated

Parameter	Description of Parameter
$T_{1/2}$	The apparent terminal elimination half life.
$AUC_{(0-\infty)}$	The area under the concentration versus time curve from time zero to infinity.
$AUC_{(0-\infty)}/D$	The $AUC_{(0-\infty)}$ divided by the dose administered.
CL	The apparent clearance rate of total radioactivity from the analysed test matrix, following intravenous dosing only
Vd	The apparent volume of distribution of total radioactivity in the test system.
F	Absolute bioavailability, calculated as the ratio of the dose-normalised AUC (oral) to dose normalised AUC (intravenous)

Descriptive statistics (means and standard deviations) for appropriate grouping and sorting variables will be generated using Phoenix. For T_{max} , median values will be reported.

17. TERMINAL PROCEDURES**17.1. Unscheduled Deaths**

Animals that are euthanized for humane reasons, pre-treatment or post treatment will undergo exsanguination from the abdominal aorta following carbon dioxide asphyxiation. If necessary, the animal will be refrigerated to minimize autolysis.

A complete gross pathology examination of the carcass will be conducted on all animals found dead or euthanized for humane reasons following dosing; all other animal carcasses will be discarded without examination. No tissues will be retained for any animal.

18. COMPUTERISED SYSTEMS

The following critical computerised systems may be used in the study. The actual critical computerised systems used will be specified in the Final Report.

System Name	Description of Data Collected and/or Analysed
Atlas and/or Laura	Chromatography data
DEBRA® Laboratory Information System	Total radioactivity and sample weight data
Phoenix	Pharmacokinetics
Mass Lynx and/or LC-MS Solutions	Mass Spectrometry data

19. STATISTICAL ANALYSIS

Statistical analyses shall be limited to derivation of means, standard deviations and coefficients of variation where appropriate.

20. AMENDMENTS AND DEVIATIONS

Changes to the approved protocol shall be made in the form of an amendment, which will be signed and dated by the Study Director. Every reasonable effort will be made to discuss any necessary protocol changes in advance with the Sponsor.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

All protocol and SOP deviations will be documented in the study records. Deviations from the protocol and/or SOP related to the phase(s) of the study conducted at a Test Site shall be documented, acknowledged by the PI/IS, and reported to the Study Director for authorisation/acknowledgement. The Study Director will notify the Sponsor of deviations that may result in a significant impact on the study as soon as possible.

21. RETENTION OF RECORDS, SAMPLES AND SPECIMENS

All study-specific raw data, electronic data, documentation, protocol, protocol amendments and interim (if applicable) reports from this study will be transferred to the Charles River archive by no later than the date of final report issue. Two years after issue of the final report, the Sponsor will be contacted to determine the disposition of these materials. The original signed copy of the Final Report will be archived indefinitely at the Test Facility.

Electronic data generated by the Test Facility will be archived and the software and hardware required to produce it in a readable form will be maintained and available.

All records, retained samples and specimens and reports generated from phases or segments performed by Test Facility-designated subcontractors will be returned to the Test Facility for archiving.

Records to be maintained will include, but will not be limited to, documentation and data for the following:

- Protocol, protocol amendments, and deviations
- Study schedule
- Study-related correspondence
- Test system receipt, health, and husbandry
- Test item receipt, identification, preparation, and analysis
- In-life measurements and observations
- Statistical analysis results

Biological samples generated during the course of this study will be held deep frozen until issue of the final report. Charles River will contact the Sponsor to discuss the fate of the samples (disposal, return or retain at Charles River) on issue of the final report. Samples will be disposed of unless Charles River receives written instruction regarding shipment of the samples to the Sponsor or continued storage at Charles River. Shipment of samples or long term retention will incur additional costs.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)****22. REPORTING**

A comprehensive Draft Report will be prepared following completion of the study and will be finalised following consultation with the Sponsor. The report will include all information necessary to provide a complete and accurate description of the experimental methods and results and any circumstances that may have affected the quality or integrity of the study.

The Sponsor will receive an electronic version of the Draft and Final Report provided in Adobe Acrobat PDF format (hyperlinked and searchable at final) along with a Microsoft Word version of the text. The PDF document will be created from native electronic files to the extent possible, including text and tables generated by the Test Facility. Report components not available in native electronic files and/or original signature pages will be scanned and converted to PDF image files for incorporation. An original copy of the report with the Test Facility's handwritten signatures will be retained.

Reports should be finalised within 6 months of issue of the audited Draft Report. If the Sponsor has not provided comments to the report within 6 months of draft issue, the report will be finalised by the Test Facility unless other arrangements are made by the Sponsor.

23. ANIMAL WELFARE

The UK Home Office controls scientific procedures on animals in the UK and does so by the issue of licences under the Animals (Scientific Procedures) Act 1986. The regulations conform to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, Council of Europe) and achieve the standard of care required by the US Department of Health and Human Services' Guide for the Care and Use of Laboratory Animals.

The Home Office licence governing this study strictly specifies the limits of severity of effects on the animals. From the available information, the procedures described in the protocol are not anticipated to cause any effects which exceed the severity limit of the procedure. Any animal which shows unacceptable reactions may be euthanised or other actions taken as required by the Home Office to alleviate distress.

23.1. Home Office Project Licence No.

The animal work in this study will be conducted under the UK Home Office Project Licence No. PPL 70/8628, Metabolism of Chemicals, Protocol Reference Number 1.

**Appendix 2 Copy of Study Plan and Amendment 4
(continued)**

24. AMENDMENT APPROVAL:



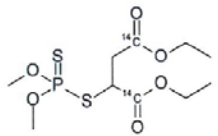
Mhairi Libberton, BSc
Study Director
Date: 11 Jan 2017



Mette Jensen
Sponsor Representative
Date: 12 Jan, 2017



Appendix 3 Certificate of Analysis for [^{14}C]-Malathion

	CERTIFICATE OF ANALYSIS		
	Analysis reference SEL/9587/2	Document revision 1	Page 1 of 1

 <p>^{14}C label shared between each carbonyl</p>	Common name	[carbonyl- ^{14}C]Malathion
	Chemical name	2-(Dimethoxythiophosphorylsulfanyl)-[1(4)-carbonyl- ^{14}C]succinic acid diethyl ester
	Batch ID	9587CEO001-1
	Molecular formula	$\text{C}_{11}\text{H}_{19}\text{O}_6\text{P}_2\text{S}_2$
	Storage conditions	Glass bottle, below -15 °C
	Date of manufacture	23 June 2016

Analyses were performed in accordance with internal Quality Program procedures by the Analytical Support Group and the Radiochemistry Department of Selcia Ltd. Comparison was with a reference sample (batch SZBF028XV) where appropriate.

Test	Method	Result
Appearance & physical form	Visual inspection	Colourless solution in ethanol
Structure, identity & residual solvents	^1H and ^{31}P NMR	Compatible with proposed structure & comparable with supplied reference Total detected residual solvents 1.1 % w/w
Structure & identity	LC/MS	Compatible with proposed structure & comparable with supplied reference
Radiochemical purity	HPLC with radio detection	99.2 area%
Chemical purity & identity	HPLC with UV detection (220 nm)	98.6 area% Retention time comparable with supplied reference sample
Specific activity & labelled molecular weight	Gravimetric analysis by LSC	58.12 mCi/mmol 2150 MBq/mmol 174.9 $\mu\text{Ci}/\text{mg}$ 6.47 MBq/mg 332.22 g/mol @ 58.12 mCi/mmol
Specific concentration	Volumetric analysis by LSC	1.009 mCi/ml 37.32 MBq/ml

Issuer:	Date:	Reviewer:	Date:
 S. J. Knight, Ph.D., MRSC Analytical Project Manager, Analytical Support	5 July 2016	 P. Morgan, Ph.D., MRQA Quality Assurance	5 July 2016

Caution: Radioactive material for research use only. Not suitable for human use.
Expiry date not determined. In the absence of stability data a purity check is recommended before use

Appendix 4 Certificate of Analysis for Malathion Technical



Test substance certified:

Test substance:	Malathion Technical
CHA Code No.:	CHA 300
Batch No.:	P2168-lbW-05
Origin of test substance:	<input checked="" type="checkbox"/> Laboratory <input type="checkbox"/> Pilot plant <input type="checkbox"/> Commercial

Analysis:

Content of Malathion:	95.0% w/w
Identified by:	¹ H-NMR, ¹³ C-NMR, ³¹ P-NMR Spectroscopy, Mass Spectrometry (GC/MS) and IR Spectroscopy
Quantified by:	GC (Method VAM 001-02)
Date of analysis:	April 06, 2016

Information of the test substance:

Appearance:	Pale yellowish liquid
Storage:	Store frozen (nominally -20°C)
Tap density:	Not determined
Expiry date:	April 06, 2019

Information of analyte(s):

Common name:	Malathion
CAS name:	Butanedioic acid, 2-[(dimethoxyphosphinothioyl)thio]-, 1,4-diethyl ester
CAS No.:	121-75-5
Molecular formula:	C ₁₀ H ₁₉ O ₆ P ₂ S ₂
Molecular mass:	330.36 g/mol
Structure formula:	

Statement of GLP Compliance

The identification and quantification were performed at Cheminova A/S and conducted according to FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices.

Date: April 15, 2016

Barbara Hinz

**Appendix 4 Certificate of Analysis for Malathion Technical
(continued)**Cheminova A/S
P.O. Box 9
DK-7520 Løngby
DenmarkPhone (+45) 99 90 99
Fax (+45) 99 00 99 91
www.cheminova.com
CVR-No. DK 12 76 00 43

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Certificate of AnalysisTEM 010-13
AmendmentTest substance: Malathion technical
Batch No.: P2168-lbW-05

Content of impurities (cont.):

Cheminova name	CAS name	Content [% w/w]	Method of analysis	Date of analysis
ME	2-Butenedioic acid, 1,4-diethylester	< 0.030	VAM 060-01	April 05, 2016
Mixed ester of Malathion	Mixture of Butanedioic acid, [[dimethoxyphosphinothioyl]thio], 1-ethyl-4-methyl ester and Butanedioic acid, [[dimethoxyphosphinothioyl] thio], 1-methyl-4-ethyl ester	0.43 ± 0.00071	VAM 060-01	April 05, 2016
MeCOOPS-triester	Phosphorothioic acid, O,O,O- trimethyl ester	0.47 ± 0.00	VAM 130-01	April 04, 2016
MeCOSPS-triester	Phosphorodithioic acid, O, O, S- trimethyl ester	1.5 ± 0.014	VAM 130-01	April 04, 2016
Water	Water	0.16 ± 0.0085	VAM 022-03	March 30, 2016

Statement of GLP Compliance

The identification and quantification were performed at Cheminova A/S and conducted according to FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices.

Date

April 15, 2016

Barbara Hinz

Appendix 5 Certificate of Analysis for Diethyl Mercaptosuccinate

Cheminova A/S
P.O. Box 9
DK-7650 Lemvig
DenmarkPhone (+45) 96 96 96 96
Fax (+45) 96 96 96 91
www.cheminova.com
CVR-No. DK 12 76 00 43

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Certificate of Analysis

REF 016-02

Test substance certified:

Test substance:	Diethyl Mercaptosuccinate
Batch No.:	849-BSa-31B
Origin of test substance:	<input checked="" type="checkbox"/> Laboratory <input type="checkbox"/> Pilot plant <input type="checkbox"/> Commercial

Analysis:

Content:	99.6 % w/w
Identified by:	¹ H-NMR, ¹³ C-NMR, IR and UV Spectroscopy and Mass Spectrometry (GC/MS)
Determination of purity by:	High Performance Liquid Chromatography and Gas Chromatography
Date of analysis:	August 27, 2015

Information of the test substance:

Appearance:	Colourless liquid
Storage:	Store frozen (nominally -20°C)
Expiry date:	August 27, 2018

Information of analyte(s):

Common name:	Diethyl Mercaptosuccinate, ME+H ₂ S
CAS name:	Butanedioic acid, 2-mercapto-, 1,4-diethyl ester
CAS No.:	23060-14-2
Molecular formula:	C ₈ H ₁₄ O ₄ S
Molecular mass:	206.26 g/mol
Structure formula:	

Statement of GLP Compliance

The identification and determination of purity were performed at Cheminova A/S and conducted according to FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices.

Date

September 16, 2015

Barbara Hinz

Appendix 6 Certificate of Analysis for O,O-Dimethyldithiophosphoric Acid



Chemnova A/S
P.O. Box 9
DK-7850 Lemvig
Denmark

Phone (+45) 96 90 98 90
Fax (+45) 96 90 98 91
www.chemnova.com
CVR-No. DK12 76 00 43

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Certificate of Analysis

REF 057-01

Test substance certified:

Test substance:	Analytical standard of MP-1-K-salt		
Batch No.:	291-BSe-62A		
Origin of test substance:	<input checked="" type="checkbox"/> Laboratory	<input type="checkbox"/> Pilot plant	<input type="checkbox"/> Commercial

Analysis:

Content of MP-1-K-salt:	98.7 % w/w
Identified by:	¹ H-NMR and ³¹ P-NMR Spectroscopy, IR Spectroscopy and UV Spectroscopy
Determination of purity by:	Quantitative ³¹ P-NMR
Date of analysis:	April 11, 2013

Information of the test substance:

Appearance:	White solid
Storage:	Ambient temperature
Expiry date:	April 11, 2018

Information of analyte(s):

Common name:	MP-1-K-salt
CAS name:	Phosphorodithioic acid, O,O-dimethyl ester, potassium salt
CAS No.:	16001-68-6
Molecular formula:	C ₂ H ₆ KO ₂ PS ₂
Molecular mass:	196.27 g/mol
Structure formula:	

Statement of GLP Compliance

The identification and determination of purity were performed at Chemnova A/S and conducted according to FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices.

Date

May 23, 2013

Appendix 7 Certificate of Analysis for Desmethyl Malaoxon Dicarboxylic Acid,
Trisodium SaltCheminova A/S
P.O. Box 9
DK-7620 Lønmø
DenmarkPhone (+45) 98 80 96 90
Fax (+45) 98 90 96 91
www.cheminova.com
CVR-No. DK12760043

Page 1 of 1

Certificate of Analysis

TEM 220-01

Test substance certified:

Test substance:	Desmethyl malaoxon dicarboxylic acid, trisodium salt
CHA Code No.:	MoxDCA
Batch No.:	P1334-CSO-15-filtered
Origin of test substance:	<input checked="" type="checkbox"/> Laboratory <input type="checkbox"/> Pilot plant <input type="checkbox"/> Commercial

Analysis:

Content of	23.9% w/w (Aqueous solution)
Identified by:	¹ H-NMR Spectroscopy, ¹³ C-NMR Spectroscopy, and ³¹ P-NMR Spectroscopy
Quantified by:	³¹ P-NMR Spectroscopy
Date of analysis:	May 26, 2016.

Information of the test substance:

Appearance:	Clear, light-purple liquid (Aqueous solution)
Storage:	Store frozen (nominally -20° C).
Density:	Not determined.
Expiry date:	May 26, 2017.

Information of analyte(s):

Common name:	Desmethyl malaoxon dicarboxylic acid, trisodium salt (MoxDCA)
Systematic name:	Sodium, 2-((methoxyoxodiphosphoryl)thio)succinate
CAS No.:	-
Molecular formula:	C ₇ H ₆ Na ₃ O ₇ PS
Molecular mass:	310.10 g/mol
Structural formula:	

Statement of GLP Compliance

The identification and quantification were performed at Cheminova A/S and conducted according to FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices.

Date

June 7, 2016

Signature

(M.H.)

Appendix 8 Certificate of Analysis for Tetra Ethyl Dithiosuccinate

Cheminova A/S
Thyborønvej 78
DK-7673 Harboøre
Denmark

tel: +45 9690 9690
fax: +45 9690 9691
info@cheminova.com
www.cheminova.com
SE No. DK 12 76 00 43



KSJ/15.11.2016

CERTIFICATE OF ANALYSIS TETRA ETHYL DITHIOSUCCINATE

We, CHEMINOVA A/S, DK-7620 Lemvig, Denmark do hereby certify that we have analyzed a representative sample of the following product:

Tetra ethyl dithiosuccinate:

Batch No.: 195-ABB-79-1
CAS No.: 2090-25-7
Expiry date: November 16, 2017
Storage conditions: In deep freezer

and certify that the purity is 95.5% w/w.

This is an electronically generated certificate and is valid without a signature.



Appendix 9 Certificate of Analysis for Monoethyl Fumarate



Cheminova A/S
Thyborøenvej 78
DK-7673 Harboøre
Denmark
+45 9690 9690
info@cheminova.com
www.cheminova.com
SE No. DK 12 76 00 43

KSJ/15.11.2016

CERTIFICATE OF ANALYSIS

Monoethyl fumarate

We, CHEMINOVA A/S, DK-7620 Lemvig, Denmark do hereby certify
that we have analyzed a representative sample of the following formulation:

Monoethyl fumarate:

Batch No.: 43-IA-167
CAS No.: 2459-05-4
Expiry date.: November 16, 2017

and certify that the content of active ingredient is: 92 % w/w

This is an electronically generated certificate and is valid without a signature.

Appendix 10 Certificate of Analysis for Mercaptosuccinic Acid

11/4/2016

Certificate Of Analysis

Certificate of Analysis

SIGMA-ALDRICH

Product Name	Mercaptosuccinic acid, 97%
Product Number	M6182
Product Brand	ALDRICH
CAS Number	70-49-5
Molecular Formula	HOOCCH(SH)CH ₂ COOH
Molecular Weight	150.15

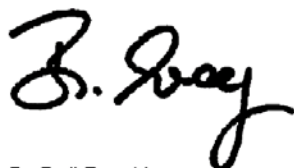
TEST

SPECIFICATION

LOT STBF9504V RESULTS

PDF

[Click here:](#) Certificate of Analysis and
Specifications only available in PDF
format



Dr. Beril Eray, Manager
Quality Control
Steinheim, Germany

Appendix 11 Certificate of Analysis for Fumaric Acid

11/4/2016

Certificate Of Analysis

Certificate of Analysis

SIGMA-ALDRICH

Product Name	Fumaric acid, ≥99.0% (T)
Product Number	47910
Product Brand	SIAL
CAS Number	<u>110-17-8</u>
Molecular Formula	HOOCCH=CHCOOH
Molecular Weight	116.07

TEST

SPECIFICATION

LOT BCBP4470V RESULTS

PDF

[Click here:](#) Certificate of Analysis and
Specifications only available in PDF
format



Dr. Claudia Geitner
Manager Quality Control
Buchs, Switzerland

Appendix 12 Certificate of Analysis for Succinic Acid

SIGMA-ALDRICH3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sigmaaldrich.com Outside USA: eurtechserv@sigmaaldrich.com**Certificate of Analysis**

Product Name: SUCCINIC ACID
purum p.a., >= 99.0 % T
Product Number: 14080
Batch Number: BCBR9019V
Brand: Sigma-Aldrich
CAS Number: 110-15-6
Formula: HOOCCH₂CH₂COOH
Formula Weight: 118.09
Quality Release Date: 04 APR 2016

TEST	SPECIFICATION	RESULT
APPEARANCE (COLOR)	COLORLESS OR WHITE	WHITE
APPEARANCE (FORM)	POWDER OR CRYSTALS	FINE CRYSTALS
TITRATION (T) NaOH 1M	99.0 - 101.0 %	99.7 %
MELTING POINT	185 - 188 C	187 C
PROTON NMR SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS
METAL TRACE ANALYSIS (ICP)	CORRESPONDS TO REQUIREMENTS	PASSED
CALCIUM (ICP)	≤ 50 MG/KG	< 50 MG/KG
CADMIUM (ICP)	≤ 50 MG/KG	< 50 MG/KG
COBALT (ICP)	≤ 50 MG/KG	< 50 MG/KG
COPPER (ICP)	≤ 50 MG/KG	< 50 MG/KG
IRON (ICP)	≤ 50 MG/KG	< 50 MG/KG
POTASSIUM (ICP)	≤ 100 MG/KG	< 100 MG/KG
SODIUM (ICP)	≤ 100 MG/KG	< 100 MG/KG
NICKEL (ICP)	≤ 50 MG/KG	< 50 MG/KG
LEAD (ICP)	≤ 50 MG/KG	< 50 MG/KG
ZINC (ICP)	≤ 50 MG/KG	< 50 MG/KG
TOTAL SULFUR AS SO ₄ (ICP)	≤ 100 MG/KG	< 30 MG/KG
CHLORIDE (CL)	≤ 50 MG/KG	< 10 MG/KG



Dr. Claudia Galtner
Manager Quality Control
Buchs, Switzerland

Sigma-Aldrich warrants that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Appendix 13 Certificate of Analysis for Maleic Acid

SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA

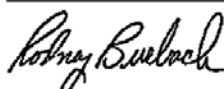
Website: www.sigmaaldrich.com

Email USA: techserv@sial.com

Outside USA: eurtechserv@sial.com

Certificate of AnalysisProduct Name:
Maleic acid – ReagentPlus®, ≥99.0% (HPLC)Product Number: M0375
Batch Number: SLBL3881V
Brand: SIAL
CAS Number: 110-16-7
MDL Number: MFCD00063177
Formula: C4H4O4
Formula Weight: 116.07 g/mol
Quality Release Date: 15 OCT 2014
Recommended Retest Date: OCT 2022

Test	Specification	Result
Appearance (Color)	White	White
Appearance (Form)	Powder	Powder
Solubility (Color)	Colorless	Colorless
Solubility (Turbidity)	Clear	Clear
333 mg/mL, H ₂ O		
Water	≤ 2.0 %	0.1 %
Residue on Ignition	≤ 0.1 %	0.0 %
Proton NMR Spectrum	Conforms to Structure	Conforms
Purity (HPLC)	≥ 99.0 %A	99.1 %A

Rodney Burbach, Manager
Analytical Services
St. Louis, Missouri US

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Appendix 14 Certificate of Analysis for Monoethyl Maleate, Potassium Salt

Cheminova A/S
P.O. Box 9
DK-7620 Lemvig
DenmarkPhone (+45) 96 90 95 90
Fax (+45) 96 90 95 91
www.cheminova.com
CVR-No. DK12769043

Page 1 of 1

Certificate of Analysis

REF 012-02

Test substance certified:

Test substance:	Analytical standard of 2-Butenedioic acid (Z)-monoethylester, potassium salt		
Batch No.:	91-FVL-122-2		
Origin of test substance:	<input checked="" type="checkbox"/> Laboratory	<input type="checkbox"/> Pilot plant	<input type="checkbox"/> Commercial

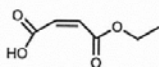
Analysis:

Content:	94.3 % w/w
Identified by:	¹ H-NMR and ¹³ C-NMR Spectroscopy and UV Spectroscopy
Determination of purity by:	High Performance Liquid Chromatography and Water determination (Karl Fischer)
Date of analysis:	November 05, 2014

Information of the test substance:

Appearance:	White solid
Storage:	Store frozen (nominally - 20°C)
Expiry date:	November 05, 2019

Information of analyte(s):

Common name:	Monoethyl maleate, potassium salt
CAS name:	2-Butenedioic acid (2Z)-, monoethyl ester, potassium salt
CAS No.:	50848-96-9
Molecular formula:	C ₈ H ₈ O ₄ · K
Molecular mass:	183.22 g/mol
Structure formula:	

Statement of GLP Compliance

The identification and determination of purity were performed at Cheminova A/S and conducted according to FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices.

Date

November 6, 2014Barbara E. H.

Appendix 15 Certificate of Analysis for Malathion Monocarboxylic Acid



Page 1 of 1

Certificate of Analysis

REF 119-08

Test substance certified:

Test substance:	Analytical standard of Malathion Monocarboxylic Acid ($\alpha + \beta$ mixture)
Batch No.:	1017-BSa-56B
Origin of test substance:	<input checked="" type="checkbox"/> Laboratory <input type="checkbox"/> Pilot plant <input type="checkbox"/> Commercial

Analysis:

Content:	89.4 % w/w
Identified by:	¹ H-NMR Spectroscopy, ¹³ C-NMR Spectroscopy, ³¹ P-NMR Spectroscopy, Mass Spectrometry (GC/MS), IR Spectroscopy and UV Spectroscopy
Determination of purity by:	Quantitative ³¹ P-NMR
Date of analysis:	January 31, 2014

Information of the test substance:

Appearance:	Light yellowish liquid.
Storage:	Store frozen (nominally - 20°C)
Expiry date:	January 31, 2017

Information of analyte(s):

Common name:	-
CAS name:	Butanedioic acid, 2-[(dimethoxyphosphinothioyl)thio]-, monoethyl ester
CAS No.:	35884-78-5
Molecular formula:	C ₉ H ₁₅ O ₆ PS ₂
Molecular mass:	302.30 g/mol
Structure formula:	

Statement of GLP Compliance

The identification and quantification were performed at Cheminova A/S and conducted according to FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices.

310

February 10, 2014

Appendix 16 Certificate of Analysis for Malathion Dicarboxylic Acid



Page 1 of 1

Certificate of Analysis

REF 120-02

Test substance certified:

Test substance:	Analytical standard of Malathion Dicarboxylic Acid		
Batch No.:	621-BSe-61A		
Origin of test substance:	<input checked="" type="checkbox"/> Laboratory	<input type="checkbox"/> Pilot plant	<input type="checkbox"/> Commercial

Analysis:

Content:	98.8 % w/w
Identified by:	¹ H-NMR Spectroscopy, ¹³ C-NMR Spectroscopy, ³¹ P-NMR Spectroscopy, Mass Spectrometry, IR Spectroscopy and UV Spectroscopy
Determination of purity by:	Quantitative ³¹ P-NMR
Date of analysis:	February 11, 2016

Information of the test substance:

Appearance:	White solid.
Storage:	Store frozen (nominally - 20°C)
Expiry date:	February 11, 2026

Information of analyte(s):

Common name:	Butanedioic acid, [(dimethoxyphosphinothioyl)thio]-
CAS name:	1190-28-9
CAS No.:	
Molecular formula:	C ₆ H ₁₁ O ₆ P ₂ S ₂
Molecular mass:	274.25 g/mol
Structure formula:	

Statement of GLP Compliance

The identification and quantification were performed at Cheminova A/S and conducted according to FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices.

Date

February 19, 2016

**Appendix 17 Certificate of Analysis for Desmethyl Malathion Monocarboxylic Acid,
Potassium Salt**Cheminova A/S
P.O. Box 8
DK-7850 Lemvig
DenmarkPhone (+45) 96 90 96 90
Fax (+45) 96 90 96 91
www.cheminova.com
CVR-No. DK12 76 00 43

Page 1 of 2

Certificate of Analysis

REF 046-02

Test substance certified:

Test substance:	Analytical standard of Desmethyl malathion Monocarboxylic Acid, Potassium salt		
Batch No.:	676-BSe-16A		
Origin of test substance:	<input checked="" type="checkbox"/> Laboratory	<input type="checkbox"/> Pilot plant	<input type="checkbox"/> Commercial

Analysis:

Content of Desmethyl malathion Monocarboxylic Acid, Potassium salt:	73.9 % w/w
Identified by:	¹ H-NMR and ¹³ C-NMR Spectroscopy, IR Spectroscopy and UV spectroscopy
Determination of purity by:	Quantitative ³¹ P-NMR
Date of analysis:	February 02, 2016

Information of the test substance:

Appearance:	White solid
Storage:	Store frozen (nominally -20°C)
Expiry date:	February 02, 2018

Statement of GLP Compliance

The identification and determination of purity were performed at Cheminova A/S and conducted according to
FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory
Practices.

Date

February 11, 2016

**Appendix 17 Certificate of Analysis for Desmethyl Malathion Monocarboxylic Acid,
(continued) Potassium Salt**Cheminova A/S
P.O. Box 9
DK-7820 Lenebø
DenmarkPhone (+45) 96 96 96 90
Fax (+45) 96 96 96 91
www.cheminova.com
CVR-No. DK 12 78 60 43

Page 2 of 2

Certificate of Analysis

REF 046-02

Information of analyte(s):

Common name:	-
CAS name (free acid):	Butanediolo acid, 2-[(mercaptomethoxyphosphiny)thio]-, 1-ethyl ester
CAS No. (free acid):	Butanediolo acid, 2-[(mercaptomethoxyphosphiny)thio]-, 4-ethyl ester
CAS No. (free acid):	159776-73-5
CAS No. (free acid):	159776-74-6
Molecular formula:	$C_7H_{12}O_6PS_2K$
Molecular mass:	326.37 g/mol
Structure formula:	

Statement of GLP ComplianceThe identification and determination of purity were performed at Cheminova A/S and conducted according to
FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory
Practices.

Date

February 11, 2016

Appendix 18 Certificate of Analysis for Desmethyl Malathion Dicarboxylic Acid,
Dicyclohexylammonium SaltChemnova A/S
R.O. Box 9
DK-7620 Lemvig
DenmarkPhone (+45) 96 80 86 00
Fax (+45) 96 80 86 91
www.chemnova.com
CVR-No. DK 12 78 00 43

Page 1 of 1

Certificate of Analysis

REF 149-01

Test substance certified:

Test substance:	<i>Analytical standard of Characterization of (1RS,3RS)-Desmethyl-malathion-dicarboxylic acid, dicyclohexylammonium salt (CAS No. -), Batch No. 676-BSe-12A</i>		
Batch No.:	676-BSe-12A		
Origin of test substance:	<input checked="" type="checkbox"/> Laboratory	<input type="checkbox"/> Pilot plant	<input type="checkbox"/> Commercial

Analysis:

Content:	94.6 % w/w
Identified by:	¹ H-NMR Spectroscopy, ¹³ C-NMR Spectroscopy, ³¹ P-NMR Spectroscopy, Mass Spectrometry, IR Spectroscopy and UV Spectroscopy
Determination of purity by:	Quantitative ³¹ P-NMR Spectroscopy
Date of analysis:	August 21, 2013

Information of the test substance:

Appearance:	White solid.
Storage:	Store frozen (nominally - 20°C).
Expiry date:	August 21, 2023

Information of analyte(s):

Common name:	(1RS, 3RS)-Desmethyl-malathion-dicarboxylic acid, DCHA salt.
CAS name:	-
CAS No.:	-
Molecular formula:	C ₁₇ H ₂₅ NO ₆ PS ₂ .
Molecular mass:	441.54 g/mol
Structure formula:	

Statement of GLP Compliance

The identification and quantification were performed at Chemnova A/S and conducted according to FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practice.

Date

September 24, 2013

Appendix 19 Certificate of Analysis for S-(1,2-di(carbethoxy)ethyl)-O-methyl hydrogen phosphorodithioate Dicyclohexylammonium salt

Cheminova A/S
P.O. Box 9
DK-7620 Lørrig
DenmarkPhone (+45) 66 90 96 90
Fax (+45) 66 90 96 91
www.cheminova.com
CVR-No. DK 12 76 00 43

Page 1 of 1

Certificate of Analysis

REF 011-03

Test substance certified:

Test substance:	S-(1,2-di(carbethoxy)ethyl)-O-methyl hydrogen phosphorodithioate Dicyclohexylammonium salt		
Batch No.:	924-BSe-15A		
Origin of test substance:	<input checked="" type="checkbox"/> Laboratory	<input type="checkbox"/> Pilot plant	<input type="checkbox"/> Commercial

Analysis:

Content:	96.7 % w/w
Identified by:	¹ H-NMR, ¹³ C-NMR and ³¹ P-NMR Spectroscopy, UV Spectroscopy, IR Spectroscopy and Mass Spectrometry (LC/MS)
Determination of purity by:	Quantitative ³¹ P-NMR
Date of analysis:	April 4, 2016

Information of the test substance:

Appearance:	White solid
Storage:	Frozen (normally -20°C)
Expiry date:	April 4, 2019

Information of analyte(s):

Common name:	Desmethyl-malathion-Q-salt
CAS name:	-
CAS No.:	1116-04-7 (free acid)
Molecular formula:	C ₂₁ H ₄₀ O ₆ NPS ₂
Molecular mass:	497.65 g/mol
Structure formula:	

Statement of GLP Compliance

The identification and determination of purity were performed at Cheminova A/S and conducted according to FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices.

Date

May 17, 2016

Appendix 20 Certificate of Analysis for Malaoxon

Chemnova A/S
P.O. Box 9
DK-7620 Lemvig
DenmarkPhone (+45) 96 90 96 90
Fax (+45) 96 90 96 91
www.chemnova.com
CVR-Nr. DK 12 76 05 43

Page 1 of 1

Certificate of Analysis

TEM 045-01

Test substance certified:

Test substance:	Malaoxon
CHA Code No.:	-
Batch No.:	876-BSe-89A
Origin of test substance:	<input checked="" type="checkbox"/> Laboratory <input type="checkbox"/> Pilot plant <input type="checkbox"/> Commercial

Analysis:

Content of Malaoxon:	97.7 % w/w
Identified by:	¹ H-NMR Spectroscopy, ¹³ C-NMR Spectroscopy, ³¹ P-NMR Spectroscopy, Mass Spectrometry, IR Spectroscopy and UV Spectroscopy
Quantified by:	Quantitative ³¹ P NMR
Date of analysis:	February 02, 2016

Information of the test substance:

Appearance:	Colourless liquid
Storage:	Store frozen (nominally -20°C) in the dark
Density:	Not available
Expiry date:	February 02, 2021

Information of analyte(s):

Common name:	Malaoxon
CAS name:	Butanedioic acid, 2-[(dimethoxyphosphoryl)thio], 1,4-diethyl ester
CAS No.:	1634-78-2
Molecular formula:	C ₁₀ H ₁₈ O ₇ P ₂ S
Molecular mass:	314.29 g/mol
Structure formula:	

Statement of GLP Compliance

The identification and quantification were performed at Chemnova A/S and conducted according to FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices.

Date

February 02, 2016

Appendix 21 Certificate of Analysis for Isomalathion



Page 1 of 1

Certificate of Analysis

REF 080-05

Test substance certified:

Test substance:	Analytical standard of Isomalathion		
Batch No.:	924-8Se-58A		
Origin of test substance:	<input checked="" type="checkbox"/> Laboratory	<input type="checkbox"/> Pilot plant	<input type="checkbox"/> Commercial

Analysis:

Content:	97.2 % w/w
Identified by:	¹ H-NMR Spectroscopy, ¹³ C-NMR Spectroscopy, ³¹ P-NMR Spectroscopy, Mass Spectrometry, IR Spectroscopy and UV Spectroscopy
Determination of purity by:	³¹ P-NMR Spectroscopy and High Performance Liquid Chromatography
Date of analysis:	March 10, 2016

Information of the test substance:

Appearance:	Liquid, colourless
Storage:	Store frozen (nominally -20°C)
Expiry date:	March 10, 2018

Information of analyte(s):

Common name:	Isomalathion
CAS name:	Butanedioic acid, 2-[[methoxy(methylthio)phosphiny]thio]-, 1,4-diethyl ester
CAS No.:	3344-12-5
Molecular formula:	C ₁₂ H ₁₉ O ₆ P ₂ S ₂
Molecular mass:	330.36 g/mol
Structure formula:	

Statement of GLP Compliance

The identification and quantification were performed at Cheminova A/S and conducted according to FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices.

Date

April 20, 2016

(MLA)

Appendix 22 Certificate of Analysis for Diethyl Maleate

Chemnova A/S
P.O. Box 9
DK-7620 Lørrig
DenmarkPhone (+45) 06 90 90 90
Fax (+45) 06 90 90 91
www.chemnova.com
CVN-No. DK 12 78 50 43

Page 1 of 1

Certificate of Analysis

REF 018-01

Test substance certified:

Test substance:	<i>Analytical standard of Diethyl maleate</i>		
Batch No.:	021281 IA		
Origin of test substance:	<input checked="" type="checkbox"/> Laboratory	<input type="checkbox"/> Pilot plant	<input type="checkbox"/> Commercial

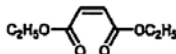
Analysis:

Content of Diethyl maleate:	97% w/w
Identified by:	¹ H-NMR and ¹³ C-NMR Spectroscopy, Mass Spectrometry, UV spectroscopy and IR spectroscopy
Determination of purity by:	High Pressure Liquid Chromatography and Gas chromatography
Date of analysis:	April 02, 2013

Information of the test substance:

Appearance:	Clear liquid
Storage:	< -20°C
Expiry date:	April 02, 2023

Information of analyte(s):

Common name:	Diethyl maleate
CAS name:	2-Butenedioic acid (2Z)-, 1,4-diethylester
CAS No.:	141-05-9
Molecular formula:	C ₈ H ₁₂ O ₄
Molecular mass:	172.18 g/mol
Structure formula:	

Statement of GLP Compliance

The identification and determination of purity were performed at Chemnova A/S and conducted according to FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practice.

Date

April 09, 2013



Appendix 23 Certificate of Analysis for Diethyl Fumarate

Chemnova A/S
P.O. Box 9
DK-7820 Lødvig
DenmarkPhone (+45) 98 90 96 80
Fax (+45) 98 90 96 91
www.chemnova.com
CVR-No. DK 12 78 00 43

Page 1 of 1

Certificate of Analysis

REF 014-02

Test substance certified:

Test substance:	<i>Analytical standard of Diethyl fumarate</i>		
Batch No.:	Aldrich S13344-424		
Origin of test substance:	<input type="checkbox"/> Laboratory	<input type="checkbox"/> Pilot plant	<input checked="" type="checkbox"/> Commercial

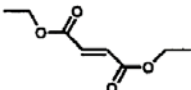
Analysis:

Content of Diethyl fumarate:	99.6 % w/w
Identified by:	¹ H-NMR and ¹³ C-NMR Spectroscopy, Mass Spectrometry, UV spectroscopy and IR spectroscopy
Determination of purity by:	High Pressure Liquid Chromatography and Gas chromatography
Date of analysis:	March 25, 2013

Information of the test substance:

Appearance:	Clear liquid
Storage:	< -20°C
Expiry date:	March 25, 2018

Information of analyte(s):

Common name:	Diethyl fumarate
CAS name:	2-Butenedioic acid (2E)-, 1,4-diethylester
CAS No.:	623-91-8
Molecular formula:	C ₈ H ₁₂ O ₄
Molecular mass:	172.18 g/mol
Structure formula:	

Statement of GLP Compliance

The identification and determination of purity were performed at Chemnova A/S and conducted according to FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices.

Date

March 26, 2013

Appendix 24 Certificate of Analysis for Diethylmethylthiosuccinate

Cheminova A/S
P.O. Box 9
DK-7620 Lemvig
DenmarkPhone (+45) 96 90 96 90
Fax (+45) 96 90 96 91
www.cheminova.com
CVR-No. DK 12 76 00 43

Page 1 of 1

Certificate of Analysis

REF 016-03

Test substance certified:

Test substance:	Analytical standard of Diethylmethylthiosuccinate
Batch No.:	D2014-BSe-MLT-07B
Origin of test substance:	<input checked="" type="checkbox"/> Laboratory <input type="checkbox"/> Pilot plant <input type="checkbox"/> Commercial

Analysis:

Content:	98.8 % w/w
Identified by:	¹ H-NMR Spectroscopy, ¹³ C-NMR Spectroscopy, Mass Spectrometry, IR Spectroscopy and UV Spectroscopy
Determination of purity by:	High Performance Liquid Chromatography and Gas Chromatography
Date of analysis:	July 05, 2016

Information of the test substance:

Appearance:	Liquid, colourless
Storage:	Store frozen (nominally -20° C)
Expiry date:	July 05, 2022

Information of analyte(s):

Common name:	Diethylmethylthiosuccinate.
CAS name:	Butanedioic acid, 2-(methylthio)-, 1,4-diethyl ester
CAS No.:	1642-46-2
Molecular formula:	C ₈ H ₁₆ O ₄ S
Molecular mass:	220.29 g/mol
Structure formula:	

Statement of GLP Compliance

The identification and quantification were performed at Cheminova A/S and conducted according to FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices.

Date	July 12, 2016
Signature	 (HSC)

Appendix 25 Certificate of Analysis for O,O-dimethyl-thiophosphoric acid,
Dicyclohexylammonium SaltCheminova A/S
P.O. Box 9
DK-7500 Lemvig
DenmarkPhone (+45) 96 90 96 90
Fax (+45) 96 90 96 91
www.cheminova.com
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Certificate of Analysis

REF 083-01

Test substance certified:

Test substance:	Analytical standard of O,O-dimethyl-thiophosphoric acid, dicyclohexylammonium salt.		
Batch No.:	267-OS-J-54B		
Origin of test substance:	<input checked="" type="checkbox"/> Laboratory	<input type="checkbox"/> Pilot plant	<input type="checkbox"/> Commercial

Analysis:

Content:	97.9 % w/w
Identified by:	¹ H-NMR Spectroscopy, ¹³ C-NMR Spectroscopy, ³¹ P-NMR Spectroscopy, IR Spectroscopy and UV Spectroscopy
Determination of purity by:	Quantitative ³¹ P-NMR
Date of analysis:	February 04, 2011

Information of the test substance:

Appearance:	White solid
Storage:	< - 20°C
Expiry date:	February 04, 2021

Information of analyte(s):

Common name:	-
CAS name:	Phosphorothioic acid, O,O-dimethyl ester (free acid)
CAS No.:	1112-38-8
Molecular formula:	C ₁₄ H ₂₆ NO ₃ PS
Molecular mass:	323.2 g/mol
Structure formula:	

Statement of GLP Compliance

The identification and determination of purity were performed at Cheminova A/S and conducted according to FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices.

Date

March 29, 2011

Appendix 26 Dosing Data**Phase 1 - Target Dose Level: 40 mg/kg (Oral)**

Animal ID	Animal Weight (g)	Dose Received			
		MBq	mg	mg/kg	MBq/kg
001M	373	1.82	15.7	42.0	4.87
002M	358	1.80	15.5	43.4	5.03
003M	365	1.81	15.6	42.7	4.95
004M	328	1.61	13.8	42.2	4.90

Phase 2 - Target Dose Level: 800 mg/kg (Oral)

Animal ID	Animal Weight (g)	Dose Received			
		MBq	mg	mg/kg	MBq/kg
005M	352	3.18	263	746	9.03
006M	350	3.40	281	804	9.73
007M	364	3.47	287	787	9.52
008M	332	3.21	266	801	9.69

Phase 3 - Target Dose Level: 1200 mg/kg (Oral)

Animal ID	Animal Weight (g)	Dose Received			
		MBq	mg	mg/kg	MBq/kg
009M	301	2.71	324	1074	8.99
010M	307	2.70	323	1053	8.81
011M	314	2.90	347	1105	9.24
012M	305	2.74	327	1071	8.96

Phase 4 - Target Dose Level: 4 mg/kg (Intravenous)

Animal ID	Animal Weight (g)	Dose Received			
		MBq	mg	mg/kg	MBq/kg
013M	331	1.45	1.25	3.76	4.37
014M	330	1.43	1.23	3.73	4.33
015M	333	1.49	1.28	3.86	4.47
016M	317	1.46	1.26	3.97	4.60

Appendix 26 Dosing Data**(continued)****Phase 5 - Target Dose Level: 800 mg/kg (Oral)**

Animal ID	Animal Weight (g)	Dose Received			
		MBq	mg	mg/kg	MBq/kg
017M	333	3.49	296	887	10.5
018M	334	3.48	295	882	10.4

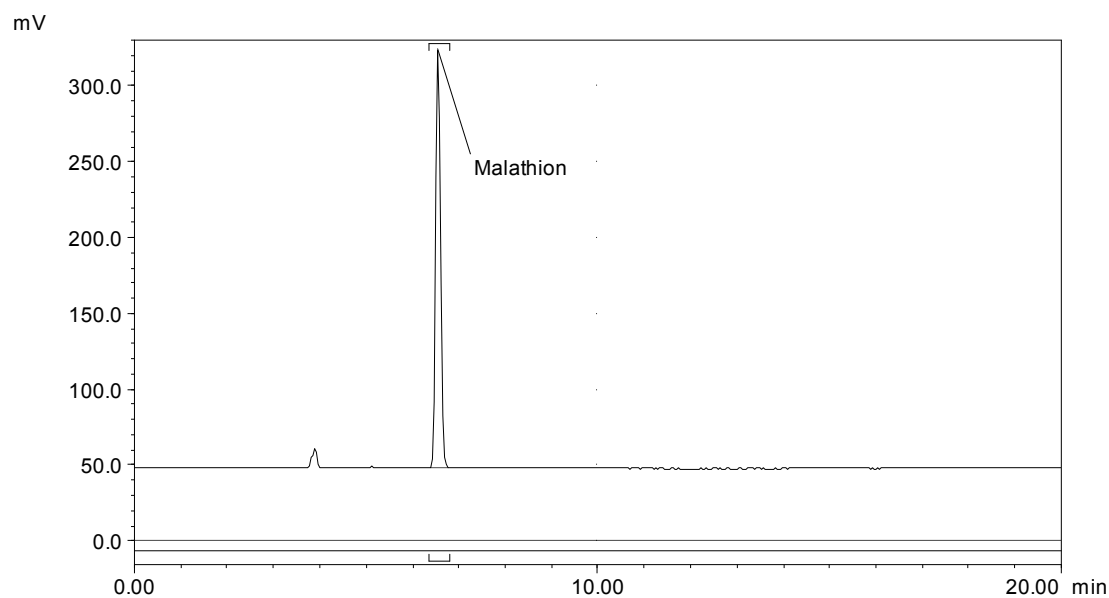
Phase 6 - Target Dose Level: 1200 mg/kg (Oral)

Animal ID	Animal Weight (g)	Dose Received			
		MBq	mg	mg/kg	MBq/kg
019M	316	3.21	391	1240	10.2
020M	314	3.19	389	1241	10.2

**Appendix 27 Representative UV Chromatogram for the Radiochemical Purity of
[¹⁴C]-Malathion**

Sample Name: UV Std

File Name: 172891_01Aug2016Run3Eval1

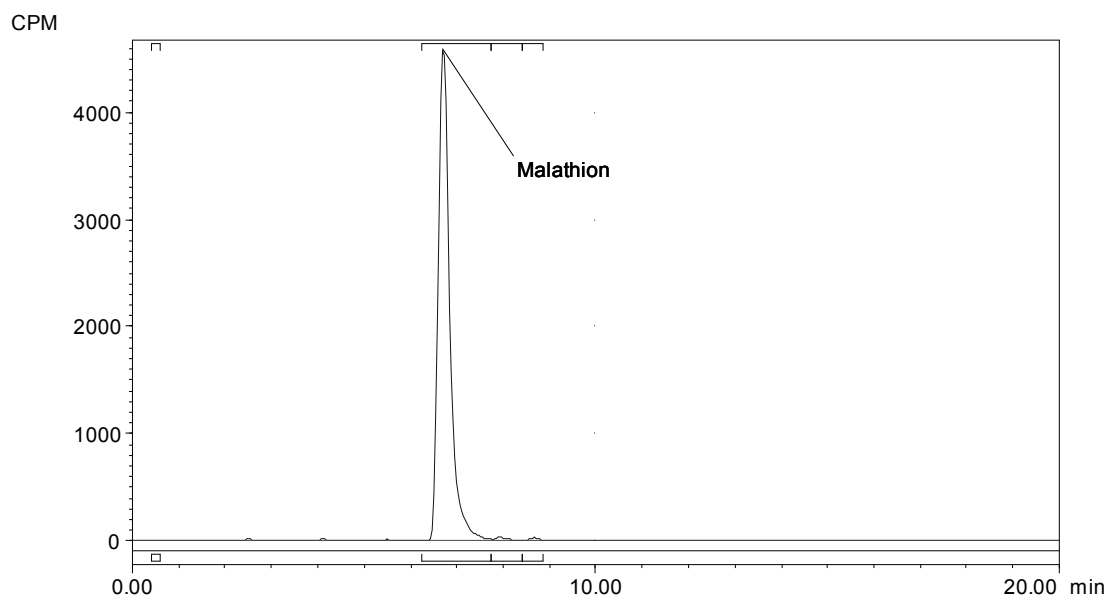


Peak Name	Retention Time (min)
Malathion	6.53

Appendix 28 Representative HPLC Radiochromatogram for the Radiochemical Purity of [¹⁴C]-Malathion

Sample Name: RCP 1

File Name: 172891_01Aug2016Run4Eval1

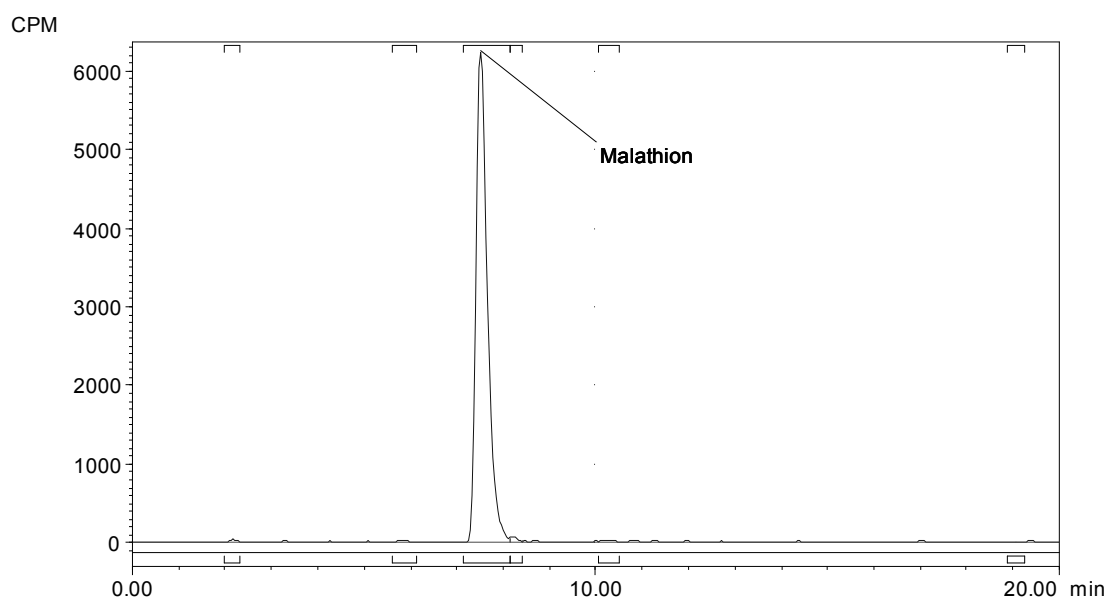


Peak Name	Retention Time (min)	%ROI
Malathion	6.70	98.9
-	7.87	0.7
-	8.75	0.5

Appendix 29 Representative HPLC Radiochromatogram for the Radiochemical Purity of [¹⁴C]-Malathion in the 800 mg/kg Oral Formulation Pre-dose Administration

Sample Name: Predose ph 2

File Name: 172891_24aug2016Run4Eval1

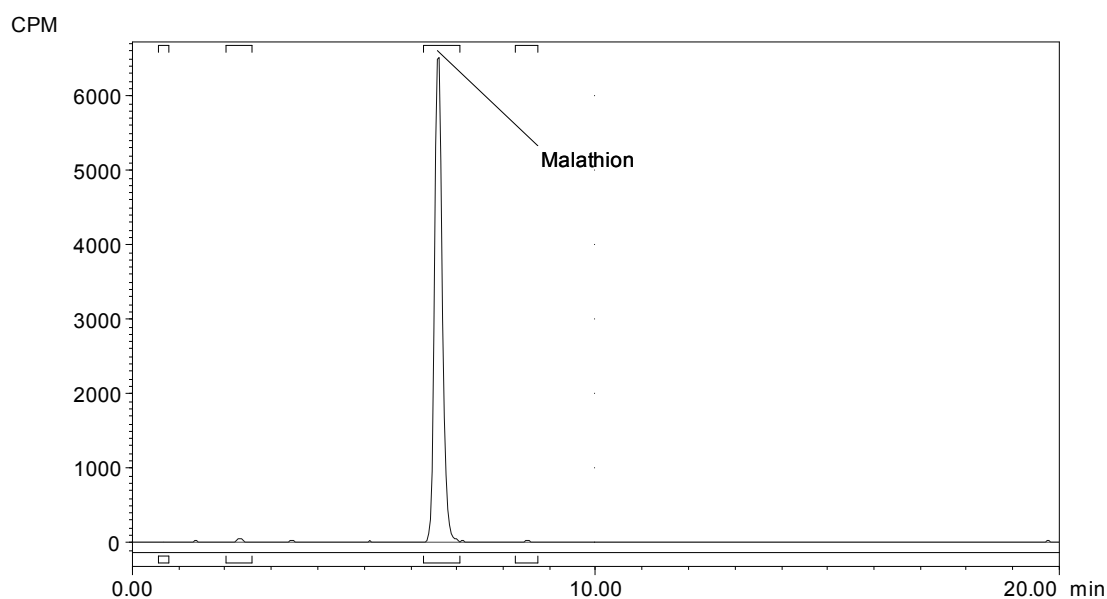


Peak Name	Retention Time (min)	%ROI
-	2.15	0.3
-	5.85	0.3
Malathion	7.50	98.2
-	8.20	0.7
-	10.20	0.4

Appendix 30 Representative HPLC Radiochromatogram for the Radiochemical Purity of [¹⁴C]-Malathion in the 4 mg/kg Intravenous Formulation Post-dose Administration

Sample Name: Postdose ph 4

File Name: 172891_01sep2016Run5Eval1



Peak Name	Retention Time (min)	%ROI
-	2.33	0.8
Malathion	6.58	98.8
-	8.52	0.3

Appendix 31 Individual Concentrations of Total Radioactivity in Whole Blood and Plasma Following a Single Oral Administration of [¹⁴C]-Malathion to Male Rats at a Target Dose Level of 40 mg/kg (Phase 1)

(Results expressed as µg equiv/g or mL)

Sample	Timepoint	001M	002M	003M	004M	Mean	SD
Whole Blood	0.25 h	4.5	7.4	7.0	7.7	6.6	1.5
	0.50 h	6.2	12.3	10.1	10.8	9.8	2.6
	1 h	7.2	22.9	18.2	14.6	15.7	6.6
	2 h	6.0	14.8	17.4	11.3	12.4	4.9
	4 h	3.6	6.6	6.2	5.1	5.4	1.3
	8 h	2.7	2.5	2.3	3.0	2.6	0.3
	12 h	3.1	N.S.	2.0	1.6	2.3	0.8
	24 h	0.3	0.3	0.4	0.3	0.3	0.0
	30 h	0.2	*0.2	0.2	0.3	°0.2	°0.0
	48 h	*0.2	*0.2	*0.2	*0.2	°0.2	°0.0
	72 h	*0.2	*0.1	*0.2	*0.1	°0.2	°0.0
	96 h	*0.1	*0.1	*0.1	*0.1	°0.1	°0.0
Plasma	0.25 h	7.6	15.4	17.1	13.1	13.3	4.1
	0.50 h	9.5	25.9	24.8	18.0	19.5	7.6
	1 h	13.0	36.6	34.2	25.6	27.3	10.7
	2 h	10.7	32.8	27.6	19.4	22.7	9.7
	4 h	6.6	11.6	13.3	27.5	14.7	9.0
	8 h	4.6	3.9	4.3	5.8	4.6	0.8
	12 h	10.1	N.S.	3.3	2.6	5.3	4.1
	24 h	0.4	0.5	0.6	0.5	0.5	0.1
	30 h	0.3	0.3	0.4	0.4	0.3	0.0
	48 h	0.2	0.2	0.3	0.2	0.2	0.0
	72 h	0.1	*0.1	0.2	0.2	°0.1	°0.0
	96 h	*0.1	*0.1	0.1	*0.1	°0.1	°0.0

N.S. = No sample

*=Results calculated from data less than 30 d.p.m. above background

°=Mean includes results calculated from data less than 30 d.p.m. above background

Appendix 32 Individual Concentrations of Total Radioactivity in Whole Blood and Plasma Following a Single Oral Administration of [¹⁴C]-Malathion to Male Rats at a Target Dose Level of 800 mg/kg (Phase 2)

(Results expressed as µg equiv/g or mL)

Sample	Timepoint	005M	006M	007M	008M	Mean	SD
Whole Blood	0.25 h	79	69	99	38	71	25
	0.50 h	72	63	114	49	75	28
	1 h	56	74	95	39	66	24
	2 h	126	55	50	43	68	39
	4 h	67	48	109	N.S.	74	31
	8 h	60	53	78	95	71	19
	12 h	34	33	57	N.S.	41	14
	24 h	8	13	60	17	24	24
	30 h	5	8	22	8	11	7
	48 h	3	4	6	5	5	2
	72 h	3	2	4	3	3	1
	96 h	*2	2	3	*2	°2	°0
Plasma	0.25 h	135	105	153	84	119	31
	0.50 h	168	133	186	119	152	31
	1 h	99	132	157	87	119	32
	2 h	258	86	91	72	127	88
	4 h	115	109	225	N.S.	150	65
	8 h	87	91	138	168	121	39
	12 h	52	53	98	N.S.	68	26
	24 h	13	23	90	23	37	36
	30 h	7	10	34	10	15	13
	48 h	4	5	7	5	5	1
	72 h	3	3	5	4	4	1
	96 h	1	2	2	2	2	0

N.S. = No sample

*=Results calculated from data less than 30 d.p.m. above background

°=Mean includes results calculated from data less than 30 d.p.m. above background

Appendix 33 Individual Concentrations of Total Radioactivity in Whole Blood and Plasma Following a Single Oral Administration of [¹⁴C]-Malathion to Male Rats at a Target Dose Level of 1200 mg/kg (Phase 3)

(Results expressed as µg equiv/g or mL)

Sample	Timepoint	009M	010M	011M	012M	Mean	SD
Whole Blood	0.25 h	95	81	75	99	88	11
	0.50 h	172	155	105	150	146	29
	1 h	131	153	105	114	126	21
	2 h	58	91	80	76	76	13
	4 h	76	47	116	72	78	28
	8 h	140	85	104	53	96	36
	12 h	64	43	74	48	57	14
	24 h	31	37	30	20	29	7
	30 h	17	19	17	13	17	3
	48 h	8	10	10	8	9	1
	72 h	5	6	6	6	6	1
	96 h	5	5	5	4	5	1
Plasma	0.25 h	171	181	161	179	173	9
	0.50 h	250	292	208	244	249	34
	1 h	183	261	178	201	206	38
	2 h	84	190	177	126	144	49
	4 h	139	78	214	150	145	56
	8 h	254	139	174	101	167	65
	12 h	103	68	109	72	88	21
	24 h	50	60	46	34	47	11
	30 h	28	27	27	20	26	4
	48 h	9	11	12	10	11	1
	72 h	5	5	7	6	6	1
	96 h	3	4	5	4	4	1

Appendix 34 Individual Concentrations of Total Radioactivity in Whole Blood and Plasma Following a Single Intravenous Administration of [¹⁴C]-Malathion to Male Rats at a Target Dose Level of 4 mg/kg (Phase 4)

(Results expressed as µg equiv/g or mL)

Sample	Timepoint	013M	014M	015M	016M	Mean	SD
Whole Blood	5 min	9.61	12.27	6.64	8.89	9.35	2.32
	0.25 h	5.11	7.69	2.96	5.29	5.26	1.93
	0.50 h	3.51	4.19	1.43	2.67	2.95	1.19
	1 h	1.30	2.28	0.43	1.00	1.25	0.78
	2 h	0.37	1.00	0.14	0.33	0.46	0.37
	4 h	0.12	0.20	0.08	0.12	0.13	0.05
	8 h	0.08	0.10	0.05	0.06	0.07	0.02
	12 h	0.07	0.09	0.05	0.08	0.07	0.02
	24 h	0.03	0.04	0.02	0.03	0.03	0.01
	30 h	*0.02	0.03	*0.01	*0.02	°0.02	°0.01
	48 h	*0.01	*0.02	*0.01	*0.02	°0.01	°0.00
	72 h	*0.01	*0.01	*0.01	*0.01	°0.01	°0.00
	96 h	N.S.	*0.01	*0.01	*0.00	°0.01	°0.00
Plasma	5 m	18.21	21.70	12.66	17.68	17.56	3.72
	0.25 h	10.09	12.71	5.52	10.71	9.76	3.04
	0.50 h	6.42	7.87	2.38	6.21	5.72	2.34
	1 h	2.10	4.41	0.71	1.81	2.26	1.55
	2 h	0.61	1.70	0.18	0.49	0.75	0.66
	4 h	0.16	0.35	0.08	0.20	0.20	0.11
	8 h	0.10	0.17	0.07	0.09	0.11	0.05
	12 h	0.09	0.13	0.08	0.12	0.10	0.02
	24 h	0.03	0.05	0.03	0.04	0.04	0.01
	30 h	0.03	0.04	0.02	0.03	0.03	0.01
	48 h	0.02	0.02	0.02	0.02	0.02	0.00
	72 h	*0.01	0.01	0.01	*0.01	°0.01	°0.00
	96 h	N.S.	0.01	0.01	*0.01	°0.01	°0.00

N.S. = No sample

*=Results calculated from data less than 30 d.p.m. above background

°=Mean includes results calculated from data less than 30 d.p.m. above background

Appendix 35 Individual Concentrations of Total Radioactivity in Whole Blood, Red Blood Cells and Plasma Following a Single Oral Administration of [¹⁴C]-Malathion to Male Rats at Target Dose Levels of 800 or 1200 mg/kg (Phase 5 and 6)**(Results expressed as µg equiv/g or mL)****Phase 5 - 800 mg/kg**

Sample	Timepoint (h)	017M	018M	Mean
Plasma	1.5	173	288	231
Red Blood Cells	1.5	75	105	90
Whole Blood	1.5	91	152	122

Phase 6 - 1200 mg/kg

Sample	Timepoint (h)	019M	020M	Mean
Plasma	1.5	154	193	173
Red Blood Cells	1.5	40	64	52
Whole Blood	1.5	79	100	89