Appendix D:

Laboratory Analytical Report

Documents
Laboratory Analytical Report
Determination of Chlorpyrifos and its Oxon in XAD Tubes Samples by LC-MS-MS.
Determination of Azinphos-methyl (Guthion) and its Oxon, Phosmet, and Malathion in XAD Tubes Samples by LC-MS-MS.

Chromatograms
Chlorpyrifos oxon
Chlorpyrifos

Stability Study Protocols
Chlorpyrifos and Chlorpyrifos-oxon
Azinphosmethyl, Azinphosmethyl-oxon, Phosmet, and Malathion

Laboratory Analytical Report

A. Introduction

The analytical procedures described below were used for the extraction and analysis of the organophosphorus compounds chlorpyrifos, chlorpyrifos-oxon, azinphosmethyl, azinphosmethyl-oxon, phosmet, and malathion) collected on XAD tubes during air sampling. The extraction method was adapted from NIOSH 5600[1] with the substitution of a solvent compatible with aqueous chromatography. Instrumental analysis was based on published liquid chromatography/mass spectrometry (LC-MS-MS) methods[2-3]. Alder *et al.*[4] compared gas chromatography-mass spectrometry (GC-MS) and LC-MS-MS methods for the analysis of pesticides and reviewed the use of LC-MS-MS in pesticide analysis.

The procedures desorbed the compounds from XAD tubes by ultrasonication of the XAD resin with acetonitrile solution containing stable-isotope labeled internal standards (ISTD). Extracted samples were then analyzed using Liquid Chromatography/Mass Spectrometry (LC-MS-MS) with Multiple Reaction Monitoring (MRM) detection without any sample preparation.

B. Synonyms

Azinphosmethyl = Guthion
Azinphosmethyl oxon = Guthion oxon
Phosmet = Imidan

C. Method

I. Equipment and Apparatus

A. Extraction
1. Labware:
   a. 2-mL disposable polypropylene (PPE) centrifuge tubes
   b. 1000-µL digital pipetter with reusable tip
   c. 1.5-mL GC vials with silicone-PTFE septa crimp caps
   d. Pasteur pipettes, disposable, and bulbs
2. Repipet II® volumetric solvent dispenser or similar
3. Ultrasonic bath

B. Instrumental Analysis
1. Agilent 6410 LC-MS-MS System
2. Column: Gemini, 3µ C18 110A, 150 x 2.00 mm (Phenomenex, 00F-4439-B0)
3. Guard column, Gemini 4 x 2.0 mm, (AJ0-7596) with direct connect holder

II. Instrument Operating Parameters

A. Agilent 6410 LC-MS System
1. Ion Source: ESI+
2. Gas Temp (°C): 350

3. Gas Flow (l/min): 9
4. Nebulizer (psi): 40
5. Capillary (V): 4000

B. Instrument methods included in this appendix.

C. Transitions monitored for assays are in Table 1.

Table 1. MRM used for quantifiers and qualifiers

Compound	ISTD	Type	Precursor Ion	Product Ion	Frag (V)	CE (V)
Chlorpyrifos-$^{13}C_2$-^{15}N-oxon	X	Quantifier	339	283	90	10
Chlorpyrifos-oxon		Quantifier	336	280	90	10
Chlorpyrifos-oxon		Qualifier	336	308	90	10
Chlorpyrifos [diethyl-D_{10}]	X	Quantifier	362	201	90	20
Chlorpyrifos		Quantifier	352	200	90	20
Chlorpyrifos		Qualifier	352	97	90	20
Azinphosmethyl oxon [dimethyl-D_6]	X	Quantifier	308	132	70	5
Azinphosmethyl-oxon		Quantifier	302	132	70	5
Azinphosmethyl-oxon		Qualifier	302	245	70	5
Azinphosmethyl [dimethyl-D_6]	X	Quantifier	324	131	90	10
Azinphosmethyl		Quantifier	318	125	90	10
Azinphosmethyl		Qualifier	318	132	90	10
Phosmet [dimethyl-D_6] X		Quantifier	324	160	90	10
Phosmet		Quantifier	318	160	90	10
Phosmet		Qualifier[a]	X	X	X	X
Malathion-D_{10} X		Quantifier	341	132	90	10
Malathion		Quantifier	331	127	90	10
Malathion		Qualifier	331	99	90	10

[a]No significant transitions were available for use as qualifier.

B. Agilent G1313A Autosampler
   1. 5 µL injection volume

C. Agilent G1312A Binary Pump
   1. Mobile Phase A: 0.1% Formic acid/deionized water
      Mobile Phase B: 0.1% Formic acid/Acetonitrile
   2. Isocratic: 25% A, 75% B
   3. Run Time: 12 minutes for chlorpyrifos and chlorpyrifos-oxon, 13 minutes for other compounds.

III. Reagents

A. Acetonitrile, pesticide grade
B. Acetone, reagent grade, for stock standard preparation
C. Deionized water, Barnstead Nanopure II, 18 mΩ
D. Formic acid, certified ACS, 88%

IV. Standards

A. Primary Standards
   1. Chlorpyrifos, neat, 99.5%, Chem Service Inc., (PS-674)

2. Chlorpyrifos oxon, neat, 98.8%, Chem Services Inc., (MET-674B)
3. Chlorpyrifos, diethyl-D_{10}, 99%, neat, Cambridge Isotope Labs (DLM-4360)
   $^{13}C_2$, ^{15}N-Chlorpyrifos oxon, neat, Dow Agro Sciences
4. Azinphosmethyl oxon, 100 µg/mL solution, Chem Service Inc., (MET-666A)
5. Azinphosmethyl oxon dimethyl-D_6, neat, 99.3%, Bayer Crop Science
6. Azinphosmethyl, 100 µg/mL solution, Chem Service Inc., (F2055S)
7. Azinphosmethyl-D_6, 100 µg/mL solution, EQ Laboratories Inc., (XA10365100AC)
8. Phosmet, 100 µg/mL solution, Chem Service Inc., (F2129S)
9. Phosmet dimethyl-D_6, 100 µg/mL solution, Cambridge Isotope Lab, (DLM-4667-1.2)
10. Malathion, 100 µg/mL solution, Chem Service Inc., (F2118S)
11. Malathion-D_{10}, 100 µg/mL solution, Cambridge Isotope Lab (DLM-4476-1.2)

B. Stock Standards

Stock standards originating from neat material are made in acetone at a concentration at ~ 1 mg/mL.

C. Working Standard Solution Diluents

1. Analytical standards solutions were diluted in desorption solution.
2. Spike recovery standards were diluted in acetone.

D. Recovery Spike Solutions (Table 2)
The spiking volume was 50 µL.

Table 2. Recovery Spike Solutions

Name	Compound	Spiking Solution Concentration	Sample Concentration	Units
Low Spike	Chlorpyrifos oxon	105	5.25 ng/mL	
	Chlorpyrifos	101	5.05 ng/mL	
Medium Spike	Chlorpyrifos oxon	1051	52.55 ng/mL	
	Chlorpyrifos	1009	50.45 ng/mL	
High Spike	Chlorpyrifos oxon	20180	1009 ng/mL	
	Chlorpyrifos	21020	1051 ng/mL	
Low Spike Solution	Azinphosmethyl oxon	100	5	ng/mL
	Azinphosmethyl 100		5	ng/mL
	Phosmet 100		5	ng/mL
	Malathion 100 5			ng/mL
Medium Spike Solution	Azinphosmethyl oxon	1000	50	ng/mL
	Azinphosmethyl 100	0	50	ng/mL
	Phosmet 100	0	50	ng/mL
	Malathion 100	0	50	ng/mL
High Spike Solution	Azinphosmethyl oxon	20000	1000	ng/mL
	Azinphosmethyl 200	00	1000	ng/mL
	Phosmet 200	00	1000	ng/mL
	Malathion 200	00	1000	ng/mL

E. Desorption/Dilution Solution.
Table 3 gives the concentrations and identities of internal standards.

Table 3. Internal Standards

Compound	ISTD For	Concentration (ng/mL)
$^{13}C_2$, ^{15}N-Chlorpyrifos -oxon	Chlorpyrifos oxon	27
Chlorpyrifos-D_{10}	Chlorpyrifos 260	
Azinphosmethyl oxon methyl-D_6	Azinphosmethyl oxon	99
Azinphosmethyl-D_6 Azinp	hosmethyl	100
Phosmet dimethyl-D_6 Phosmet		70
Malathion-D_{10} Malath	ion	70

2. Chlorpyrifos oxon, neat, 98.8%, Chem Services Inc., (MET-674B)
3. Chlorpyrifos, diethyl-D_{10}, 99%, neat, Cambridge Isotope Labs (DLM-4360)
   $^{13}C_2$, ^{15}N-Chlorpyrifos oxon, neat, Dow Agro Sciences
4. Azinphosmethyl oxon, 100 µg/mL solution, Chem Service Inc., (MET-666A)
5. Azinphosmethyl oxon dimethyl-D_6, neat, 99.3%, Bayer Crop Science
6. Azinphosmethyl, 100 µg/mL solution, Chem Service Inc., (F2055S)
7. Azinphosmethyl-D_6, 100 µg/mL solution, EQ Laboratories Inc., (XA10365100AC)
8. Phosmet, 100 µg/mL solution, Chem Service Inc., (F2129S)
9. Phosmet dimethyl-D_6, 100 µg/mL solution, Cambridge Isotope Lab, (DLM-4667-1.2)
10. Malathion, 100 µg/mL solution, Chem Service Inc., (F2118S)
11. Malathion-D_{10}, 100 µg/mL solution, Cambridge Isotope Lab (DLM-4476-1.2)

B. Stock Standards

Stock standards originating from neat material are made in acetone at a concentration at ~ 1 mg/mL.

C. Working Standard Solution Diluents

1. Analytical standards solutions were diluted in desorption solution.
2. Spike recovery standards were diluted in acetone.

4. Example calculation:
    i. Sample 3075 chlorpyrifos
    Concentration (ng/mL) = 70 ng/mL V = 1 mL

    Mass (ng) = 70 ng/ mL • 1 mL = 70 ng

    ii. This calculation was performed in the same manner for other analytes.

5. Results were tabulated in an appropriate spreadsheet or report and submitted for evaluation by the QAC.

VIII. Storage

All samples in the EH laboratory were stored at –20 C.

VIII. Method Validation and Analytical Limits

Method validation consisted of fortification of blank XAD tubes at three levels: 5 ng/sample (4 replicates) and 50 ng/sample (4 replicates) and 1,000 ng/sample (4 replicates). The resulting mean percent recoveries, coefficients of variation (%CV), limits of detection (LOD), and limits of quantitation (LOQ) for study compounds are in Table 5.

Table 5. Percent Recovery, Coefficients of Variation (CV%) and Limits of Detection (LOD) and Quantitation (LOQ) for Chlorpyrifos, Chlorpyrifos-oxon, Azinphosmethyl, Azinphosmethyl Oxon, Phosmet and Malathion.

Fortification Level	Recovery %	Chlorpyrifos Oxon	Chlorpyrifos	Azinphosmethyl	Azinphosmethyl Oxon	Phosmet	Malathion
5 ng/sample	Mean	97.6%	94.6%	109%	111%	111%	122%
%CV		3.3%	5.2%	4.8%	2.0%	2.1%	2.2%
50 ng/sample	Mean	93.9%	92.3%	103%	104%	105%	114%
%CV		3.6%	2.1%	1.9%	3.0%	3.1%	2.8%
1000 ng/sample	Mean	85.8%	86.1%	89.7%	94.7%	91.0%	99.1%
%CV		7.7%	3.4%				
LOD	(ng/sample)	1	1	0.5	0.5	0.5	0.4
LOQ	(ng/sample)	2	2	1.8	1.5	1.5	1.2

The limit of quantification (LOQ) is defined as 10 x sd where sd is the standard deviation of determination at a level near the LOD. The limit of detection (LOD) is defined as 3 x sd where sd is the standard deviation of determination at a level near the LOD (NIOSH 2003, Keith *et al.,* 1983).

IX. Quality Control

A. Extraction

For each batch of submitted samples, 4 matrix blank and 8 fortified matrix blanks (4 of each at 5 and 50 or 1000 ng/sample) were run to insure batch extraction quality. Fortified matrix blanks were prepared by spiking the material in the front section of the XAD tubes. Field blanks and field spike samples were not distinguished from other samples and were run "blind" by the analyst.

B. Analysis

1. The acceptable minimum number of calibrants was four. No analyte had less than six calibrants. Analyses shall be rejected if less than four calibrants were used.
2. In the event that a sample amount was outside the calibration curve range, the sample was diluted and reanalyzed. Alternatively, higher-level calibrants were added to the calibration curve. This alternative was only acceptable if maximum instrument response was not reached.
3. A mid-level standard (calibration standard from the interior of the curve) was injected at approximately 12 sample intervals during the run as a continuing calibration check. The acceptance criterion for the continuing check standard was RPE of 20% relative to the initial injection. Corrective action shall be determination of root-cause, fixing the problem, and instrumental reanalysis of impacted samples.
4. Calibration performance was based on the linearity of the calibration curve ($r^2 > 0.98$), and the RPE check standard or reference standard (± 20% of the known quantity). Corrective action shall be determination of root-cause, fixing the problem, and instrumental reanalysis of impacted samples.
5. Criteria for the identification of the analyte peaks was MRM, retention time (RT) and the secondary ion (also known as qualifier ion) to primary quantification ion ratio based on the average values for the calibrants (QR). Acceptance criteria were RT ± 0.5 min and QR ± 30% mean calibrant QR. Greater deviations were documented. It was to the discretion of the QAC to reject the identification or flag that data as having failed one criteria of identification. The QAC chose to flag the data in the report as tentatively identified in cases where QR was out of tolerance. RT was not out of tolerance. Failing both criteria shall be construed as misidentification of the peak.
6. The analyst reviewed data for any inference in the peak quantification by closely eluting peaks with the same MRM. The QAC shall provide a secondary review any cases where integration was impacted.

7. If any internal standard area deviated more than a factor of two from the mean of the initial calibrants, the instrument shall be examined for malfunctions and corrective actions taken. The corrective action shall be reanalysis of impacted samples.
8. The acceptable range for matrix blanks <50% of the lowest calibrant, which is usually the LOQ. Higher levels shall be reviewed by the QAC and corrective action taken at the discretion of the QAC. The reporting limit shall be set not less than three times the matrix blank value when the blank value is ≥ 50% of the lowest calibrant.
9. The acceptable range for CV (N > 2) or RPD (N = 2) of instrumental replicates was ≤ 25%. Replicates outside of tolerance shall be examined for root-cause and corrective action taken at the discretion of the QAC.
10. The acceptable range for spiked matrix recovery was between 65–135%. Recoveries outside this range shall be examined by the QAC, who shall determine the appropriate corrective action.
11. Data was reported to the LOD and not flagged as being below the LOQ since LOD and LOQ were listed on each report.

X. Safety and Health

Please consult Material Safety Data Sheet (MSDS) information on pesticides and reagents.

XI. References

1. NIOSH 5600 Organophosphorous Pesticides, *NIOSH Manual of Analytical Methods (NMAM)*, 4th ed., 8/5/94.
2. Direct determination of chlorpyrifos and its main metabolite in human serum and urine by coupled column liquid chromatography/electrospray-tandem mass spectrometry, *Rapid Communications In Mass Spectrometry*, **14**,1485-1490, 2000.
3. Multiresidue Analysis of 301 Pesticides in Food Samples by LC/Triple Quadrupole Mass Spectrometry, Agilent Application Note, Publication Number: 5989-8614EN, Last Updated: 6/27/2008.
4. Residue analysis of 500 high priority pesticides: better by GC- MS or LC - MS / MS ? L Alder, K Greulich, G Kempe, and B Vieth. *Mass Spectrometry Reviews*, 25(6), 838-865, 2006.
5. NIOSH (2003), Measurement Uncertainty And NIOSH Method Accuracy Range, in *NIOSH Manual of Analytical Methods.*
6. Keith *et al.,* Principles of environmental analysis, *Anal. Chem.* **55,** 2210–2218, 1983.
7. EPA Method 8260B Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)

Date: 10/21/2008
Revision Date: 10/24/2008

Title: **Determination of Chlorpyrifos and its Oxon in XAD Tubes Samples by LC-MS-MS.**
Revision: 2

Abstract: This laboratory analytical procedure describes the method of extraction and analysis for chlorpyrifos and its oxon collected on XAD tubes. Analysis is performed by Liquid Chromatography (LC) with Multiple Reaction Monitoring (MRM) Detection.

I. Equipment and Apparatus
- A. Extraction
  1. Labware:
     a. 2-mL disposable polypropylene (PPE) centrifuge tubes
     b. 1000-µL digital pipettor with reusable tip
     c. 1.5-mL GC vials with silicone-PTFE septa crimp caps
     d. Pasteur pipettes, disposable, and bulbs
  2. Repipet II® volumetric solvent dispenser or similar
  3. Sonicator
- B. Instrumental Analysis
  1. Agilent 6410 LC-MS-MS System
  2. Column: Gemini, 3µ C18 110A, 150 x 2.00 mm (Phenomenex, 00F-4439-B0)
  3. Guard column, Gemini 4 x 2.0 mm, (AJ0-7596) with direct connect holder

III. Instrument Operating Parameters
- A. Agilent 6410 LC-MS System
  1. Ion Source: ESI+
  2. Gas Temp (°C): 350
  3. Gas Flow (l/min): 9
  4. Nebulizer (psi): 40
  5. Capillary (V): 4000

MRM Table

Compound	ISTD	Type	Prec Ion	Prod Ion	Frag (V)	CE (V)
Chlorpyrifos-$^{13}C_2$-^{15}N-oxon	X	Quantifier	339	283	90	10
Chlorpyrifos-oxon		Quantifier	336	280	90	10
Chlorpyrifos-oxon		Qualifier	336	308	90	10
Chlorpyrifos-d_{10}	X	Quantifier	362	201	90	20
Chlorpyrifos		Quantifier	352	200	90	20
Chlorpyrifos		Qualifier	352	97	90	20

E. Agilent G1313A Autosampler
   1. 5 µL injection volume
F. Agilent G1312A Binary Pump
   1. Mobile Phase A: 0.1% Formic acid/Deionized water
      Mobile Phase B: 0.1% Formic acid/Acetonitrile
   2. Isocratic: 25% A, 75% B
   3. Run Time: 12 minutes

III. Reagents
A. Acetonitrile, pesticide grade
B. Acetone, reagent grade, for Stock standard preparation
C. Deionized water, Barnstead Nanopure II, 18 mΩ
D. Formic acid, certified ACS, 88%

IV. Standards
A. Primary Standards
   2. Chlorpyrifos, neat, 99.5%, Chem Service Inc.
   2. Chlorpyrifos oxon, neat, 98.8%, Chem Services Inc.
   3. Chlorpyrifos, diethyl- d_{-10}, 99%, Cambridge Isotope Labs
      $^{13}C_2$, ^{15}N-Chlorpyrifos oxon, neat, Dow Agro Sciences

C. Stock Standards
Stock standards are made in acetone at a concentration at ~ 1mg/mL.

C. Working Standard Solution Diluents
   1. Analytical standards solutions are diluted in desorption solution.
   2. Spike recovery standards are diluted in acetone.

D. Recovery Spike Solutions

Name	Compound	Spiking Solution Concentration	Sample Concentration	Units
Low Spike	Chlorpyrifos oxon	105	5.25 ng/mL	
	Chlorpyrifos	101	5.05 ng/mL	
Medium Spike	Chlorpyrifos oxon	1051	52.55 ng/mL	
	Chlorpyrifos	1009	50.45 ng/mL	
High Spike	Chlorpyrifos oxon	20.18	1.009 µg/mL	
	Chlorpyrifos	21.02	1.051 µg/mL	

Spiking volume: 50µL

E. Desorption/Dilution Solution:
1 ml of acetonitrile containing ISTD's:

Compound	ISTD For	Concentration (ng/mL)
$^{13}C_2$, ^{15}N-Chlorpyrifos -oxon	Chlorpyrifos oxon	27
Chlorpyrifos-d_{10}	Chlorpyrifos	260

V. Sample Preparation

A. Extraction

1. Transfer the front and back sections of the sample tube into separate 2-mL PPE tubes. The front filter is extracted with the front section. The foam between front and back is extracted with the back section. The foam after the back section is discarded.
9. Fortify blank samples for recovery with low, medium or high recovery spike solutions (50 µl) using a micro-pipettor and appropriate glass tip.
10. Add 1.0 mL desorption solution.
11. Cap the PPE tubes tightly and let them sit for ~30 minutes.
12. Sonicate for 30 minutes.
13. Remove tubes from sonicator, let stand for another 10 minutes.
14. Centrifuge for 10 minutes @ 3,500 rpm.
15. Using a Pasteur pipette, transfer aliquots to labeled vials, seal, and analyze on LC-MS-MS or store in the freezer if they can't be analyzed immediately. Holding time should not be more than 4 weeks.

VII. Calculations

1. The calibration curves for chlorpyrifos and chlorpyrifos oxon are calculated using the response ratio between the chemical and corresponding internal standard.
2. Concentrations are computed by the Agilent Mass-Hunter software using the internal standard method.
3. Tabulate results in an appropriate spreadsheet or report and submit for evaluation by the QAC.

VIII. Quality Control

A. Extraction

For each batch of 80 submitted samples, 4 matrix blank and 8 fortified matrix blanks (4 of each at 5 and 50 or 1000 ng/sample) will be run to insure batch extraction quality. Fortified matrix blanks are prepared by spiking the material from the front section of the XAD tubes. Field blanks and field spike samples are not distinguished from other samples and are run "blind" by the analyst.

B. Analysis

12. Each batch run will consist at minimum of a 4-point calibration curve with concentrations bracketing the various field fortification levels.
13. In the event that a sample amount is outside the calibration curve range, the sample will be diluted and reanalyzed. Alternatively, higher-level calibrants may be added to the calibration curve provided that maximum instrument response is not reached.
14. A mid-level standard (calibration standard from the interior of the curve) will be injected at 12 sample intervals (minimum) during the run as a continuing calibration check. The acceptance criterion for the continuing check standard is a RPE of 20% relative to the initial injection. Greater deviations will be documented and corrective action taken at the direction of the QAC.
15. Calibration performance will be based on the linearity of the calibration curve ($r^2 > 0.98$), and the RPE check standard or reference standard (± 20% of the known quantity). Greater deviations will be documented and corrective action taken at the direction of the QAC .
16. Criteria for the identification of the analyte peaks will be MRM, retention time (RT) and the secondary ion (also known as qualifier ion) to primary quantification ion ratio based on the average values for the calibrants (QR). Acceptance criteria are RT ± 0.5 min and QR ± 30% mean calibrant QR. Greater deviations will be documented. The QAC will decide whether to reject the identification or flag that data as having failed one criteria of identification. Failing both criteria will be construed as misidentification of the peak.
17. The analyst will review data for any inference in the peak quantification by closely eluting peaks with the same MRM. The QAC will review any cases where integration may be impacted.
18. If any internal standard area deviates more than a factor of two from the mean of the initial calibrants, the instrument should be examined for malfunctions and corrective actions taken. Reanalysis of impacted samples will be necessary.
19. Matrix blanks should be less than 50% of the lowest calibrant, which is usually the LOQ. Higher levels will be reviewed by the QAC and corrective action taken at the discretion of the QAC. The reporting limit is set not less than three times the matrix blank value when the blank value is ≥ 50% of the lowest calibrant.
20. The CV of replicates must be ≤ 25%. If higher the QAC will investigate and decide on appropriate action.
21. Spiked matrix recoveries must be between 65–135%. If these recoveries are outside this range the QAC will investigate and decide on appropriate action.
22. Data reported between the LOD and LOQ will be flagged as such.

IX. Validation

Method validation consisted of fortification of blank XAD tubes at three levels: 5 ng/sample (4 replicates) and 50 ng/sample (4 replicates) and 1,000 ng/sample (4 replicates). The resulting mean percent recoveries and coefficients of variation (%CV) are:

Fortification Level	Recovery %	Chlorpyrifos Oxon	Chlorpyrifos
5 ng/sample	Mean	97.6% 94.6%	
	%CV	3.3% 5.2%	
50 ng/sample	Mean	93.9% 92.3%	
	%CV	3.6% 2.1%	
1000 ng/sample	Mean	85.8% 86.1%	
	%CV	7.7% 3.4%	
	LOD	1 ng/sample	1 ng/sample
	LOQ	2 ng/sample	2 ng/sample

The limit of quantification (LOQ) is defined as 10 x sd where sd is the standard deviation of determination at a level near the LOD . The limit of detection (LOD) is defined as 3 x sd where sd is the standard deviation of determination at a level near the LOD (NIOSH 2003, Keith *et al.,* 1983).

X. Safety and Health

Please consult Material Safety Data Sheet (MSDS) information on pesticides and reagents.

XI. References

1. NIOSH Organophosphorous Pesticides, #5600.
2. Direct determination of chlorpyrifos and its main metabolite in human serum and urine by coupled column liquid chromatography/electrospray-tandem mass spectrometry, *Rapid Communications In Mass Spectrometry*, **14**,1485-1490(2000)
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5. Keith *et al.,* Principles of environmental analysis, *Anal. Chem.* **55,** 2210–2218, 1983.
6. EPA Method 8260B Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)

XII. Attachments

1. Instrumental Method

Written by Jianbo Yu, Ph.D, Research Scientist
Reviewed by: Russell Dills, 10/23/08

Date: 12/11/2008
Revision Date:

Title: **Determination of Azinphos-methyl (Guthion) and its Oxon, Phosmet, and Malathion in XAD Tubes Samples by LC-MS-MS.**
Revision: 2

Abstract: This laboratory analytical procedure describes the method of extraction and analysis for guthion and its oxon, phosmet, and malathion collected on XAD tubes. Analysis is performed by Liquid Chromatography (LC) with Multiple Reaction Monitoring (MRM) Detection.

I. Equipment and Apparatus
- A. Extraction
  1. Labware:
     a. 2-mL disposable polypropylene (PPE) centrifuge tubes
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     c. 1.5-mL GC vials with silicone-PTFE septa crimp caps
     d. Pasteur pipettes, disposable, and bulbs
  2. Repipet II® volumetric solvent dispenser or similar
  3. Sonicator
- B. Instrumental Analysis
  1. Agilent 6410 LC-MS-MS System
  2. Column: Gemini, 3µ C18 110A, 150 x 2.00 mm (Phenomenex, 00F-4439-B0)
  3. Guard column, Gemini 4 x 2.0 mm, (AJ0-7596) with direct connect holder

IV. Instrument Operating Parameters
- A. Agilent 6410 LC-MS System
  1. Ion Source: ESI+
  2. Gas Temp (°C): 350
  3. Gas Flow (l/min): 9
  4. Nebulizer (psi): 40
  5. Capillary (V): 4000

MRM Table

Compound	ISTD	Type	Prec Ion	Prod Ion	Frag (V)	CE (V)
Guthion oxon-methyl-d_6	X	Quantifier	308	132	70	5
Guthion-oxon		Quantifier	302	132	70	5
Guthion-oxon		Qualifier	302	245	70	5
Guthion-d_6	X	Quantifier	324	131	90	10
Guthion		Quantifier	318	125	90	10
Guthion		Qualifier	318	132	90	10

Phosmet-dimethyl-d_6 X		Quantifier	324	160	90	10
Phosmet		Quantifier	318	160	90	10
Phosmet		Qualifier	X	X	X	X
Malathion-d_{10} X		Quantifier	341	132	90	10
Malathion		Quantifier	331	127	90	10
Malathion		Qualifier	331	99	90	10

G. Agilent G1313A Autosampler
   1. 5 µL injection volume

H. Agilent G1312A Binary Pump
   1. Mobile Phase A: 0.1% Formic acid/Deionized water
      Mobile Phase B: 0.1% Formic acid/Acetonitrile
   2. Gradient: 45% A, 55% B

Time (min)	%B
i.	55
3	55
7	100
8	100
9	55

   3. Run Time: 13 minutes

III. Reagents
   A. Acetonitrile, pesticide grade
   B. Acetone, reagent grade, for Stock standard preparation
   C. Deionized water, Barnstead Nanopure II, 18 mΩ
   D. Formic acid, certified ACS, 88%

IV. Standards
   A. Primary Standards
      3. Guthion oxon, commercial standard solution, Chem Service Inc.
      4. Guthion oxon-methyl-d_6, neat, 99.3%, Bayer CropScience
      3. Guthion, commercial standard solution, Chem Service Inc.
      4. Guthion-d_6, commercial standard solution, Dr. Ehrenstorfer
      5. Phosmet, commercial standard solution, Chem Service Inc.
      6. Phosmet-dimethyl-d_6, commercial standard solution, CIL
      7. Malathion, commercial standard solution, Chem Service Inc.
      8. Malathion-d_{10}, commercial standard solution, CIL

   D. Stock Standards
      Stock standards originating from neat material are made in acetone at a concentration at ~ 1mg/mL.

   C. Working Standard Solution Diluents

1. Analytical standards solutions are diluted in desorption solution.
2. Spike recovery standards are diluted in acetone.

D. Recovery Spike Solutions

Name	Compound	Spiking Solution Concentration	Sample Concentration	Units
Low Spike Solution	Guthion oxon	100	5	ng/mL
	Guthion 100		5	ng/mL
	Phosmet 100		5	ng/mL
	Malathion 100		5	ng/mL
Medium Spike Solution	Guthion oxon	1000	50	ng/mL
	Guthion 100	0	50	ng/mL
	Phosmet 100	0	50	ng/mL
	Malathion 100	0	50	ng/mL
High Spike Solution	Guthion oxon	20	1	µg/mL
	Guthion 20		1	µg/mL
	Phosmet 20		1	µg/mL
	Malathion 20		1	µg/mL

Spiking volume: 50µL

E. Desorption/Dilution Solution:
1 ml of 1% acetone in acetonitrile containing ISTD's:

Compound	ISTD For	Concentration (ng/mL)
Guthion-oxon-methyl-d_6	Guthion oxon	99
Guthion-d_6	Guthion 100	
Phosmet-dimethyl-d_6 Phosmet		70
Malathion-d_{10} Malath	ion	70

V. Sample Preparation

A. Extraction

1. Transfer the front and back sections of the sample tube into separate 2-mL PPE tubes. The front filter is extracted with the front section. The foam between front and back is extracted with the back section. The foam after the back section is discarded.
16. Fortify blank samples for recovery with low, medium or high recovery spike solutions (50 µl) using a micro-pipettor and appropriate glass tip.
17. Add 1.0 mL desorption solution.
18. Cap the PPE tubes tightly and let them sit for ~30 minutes.
19. Sonicate for 30 minutes.
20. Remove tubes from sonicator, let stand for another 10 minutes.
21. Centrifuge for 10 minutes @ 3,500 rpm.
22. Using a Pasteur pipette, transfer aliquots to labeled vials, seal, and analyze on LC-MS-MS or store in the freezer if they can't be analyzed immediately. Holding time should not be more than 4 weeks.

VII. Calculations

4. The calibration curves for guthion and its oxon, phosmet, and malathion are calculated using the response ratio between the chemical and corresponding internal standard.
5. Concentrations are computed by the Agilent Mass-Hunter software using the internal standard method.
6. Tabulate results in an appropriate spreadsheet or report and submit for evaluation by the QAC.

VIII. Quality Control

A. Extraction

For each batch of 80 submitted samples, 4 matrix blank and 8 fortified matrix blanks (4 of each at 5 and 50 or 1000 ng/sample) will be run to insure batch extraction quality. Fortified matrix blanks are prepared by spiking the material from the front section of the XAD tubes. Field blanks and field spike samples are not distinguished from other samples and are run "blind" by the analyst.

B. Analysis

23. Each batch run will consist at minimum of a 4-point calibration curve with concentrations bracketing the various field fortification levels.
24. In the event that a sample amount is outside the calibration curve range, the sample will be diluted and reanalyzed. Alternatively, higher-level calibrants may be added to the calibration curve provided that maximum instrument response is not reached.
25. A mid-level standard (calibration standard from the interior of the curve) will be injected at 12 sample intervals (minimum) during the run as a continuing calibration check. The acceptance criterion for the continuing check standard is a RPE of 20% relative to the initial injection. Greater deviations will be documented and corrective action taken at the direction of the QAC.
26. Calibration performance will be based on the linearity of the calibration curve ($r^2 > 0.98$), and the RPE check standard or reference standard (± 20% of the known quantity). Greater deviations will be documented and corrective action taken at the direction of the QAC .
27. Criteria for the identification of the analyte peaks will be MRM, retention time (RT) and the secondary ion (also known as qualifier ion) to primary quantification ion ratio based on the average values for the calibrants (QR). Acceptance criteria are RT ± 0.5 min and QR ± 30% mean calibrant QR. Greater deviations will be documented. The QAC will decide whether to reject the identification or flag that data as having failed one criteria of identification. Failing both criteria will be construed as misidentification of the peak.
28. The analyst will review data for any inference in the peak quantification by closely eluting peaks with the same MRM. The QAC will review any cases where integration may be impacted.
29. If any internal standard area deviates more than a factor of two from the mean of the initial calibrants, the instrument should be examined for malfunctions and corrective actions taken. Reanalysis of impacted samples will be necessary.
30. Matrix blanks should be less than 50% of the lowest calibrant, which is usually the LOQ. Higher levels will be reviewed by the QAC and corrective action taken at the discretion of the QAC. The reporting limit is set not less than three times the matrix blank value when the blank value is ≥ 50% of the lowest calibrant.
31. The CV of replicates must be ≤ 25%. If higher the QAC will investigate and decide on appropriate action.
32. Spiked matrix recoveries must be between 65–135%. If these recoveries are outside this range the QAC will investigate and decide on appropriate action.
33. Data reported between the LOD and LOQ will be flagged as such.

IX. Validation

Method validation consisted of fortification of blank XAD tubes at three levels: 5 ng/sample (4 replicates) and 50 ng/sample (4 replicates) and 1,000 ng/sample (4 replicates). The resulting mean percent recoveries and coefficients of variation (%CV) are:

Fortification Level	Recovery %	Guthion Oxon	Guthion	Phosmet	Malathion
5 ng/sample	Mean	111% 109%	111% 122%		
	%CV	2.0% 4.8%	2.1% 2.2%		
50 ng/sample	Mean	104% 103%	105% 114%		
	%CV	3.0% 1.9%	3.1% 2.8%		
1000 ng/sample	Mean	94.7% 89.7%	91.0% 99.1%		
	%CV	1.5% 1.6%	1.6% 3.0%		
	LOD ng/sample 0.5		0.5	0.5	0.4
	LOQ ng/sample 1.5		1.8	1.5	1.2

The limit of quantification (LOQ) is defined as 10 x sd where sd is the standard deviation of determination at a level near the LOD . The limit of detection (LOD) is defined as 3 x sd where sd is the standard deviation of determination at a level near the LOD (NIOSH 2003, Keith *et al.,* 1983).

X. Safety and Health

Please consult Material Safety Data Sheet (MSDS) information on pesticides and reagents.

XI. References

1. NIOSH Organophosphorous Pesticides, #5600.
2. Direct determination of chlorpyrifos and its main metabolite in human serum and urine by coupled column liquid chromatography/electrospray-tandem mass spectrometry, *Rapid Communications In Mass Spectrometry*, **14**,1485-1490(2000)
3. Multiresidue Analysis of 301 Pesticides in Food Samples by LC/Triple Quadrupole Mass Spectrometry, Agilent Application Note, Publication Number: 5989-8614EN, Last Updated: 6/27/2008.
4. NIOSH (2003), Measurement Uncertainty And NIOSH Method Accuracy Range, in *NIOSH Manual of Analytical Methods.*
5. Keith *et al.,* Principles of environmental analysis, *Anal. Chem.* **55,** 2210–2218, 1983.

6. EPA Method 8260B Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)

XII. Attachments

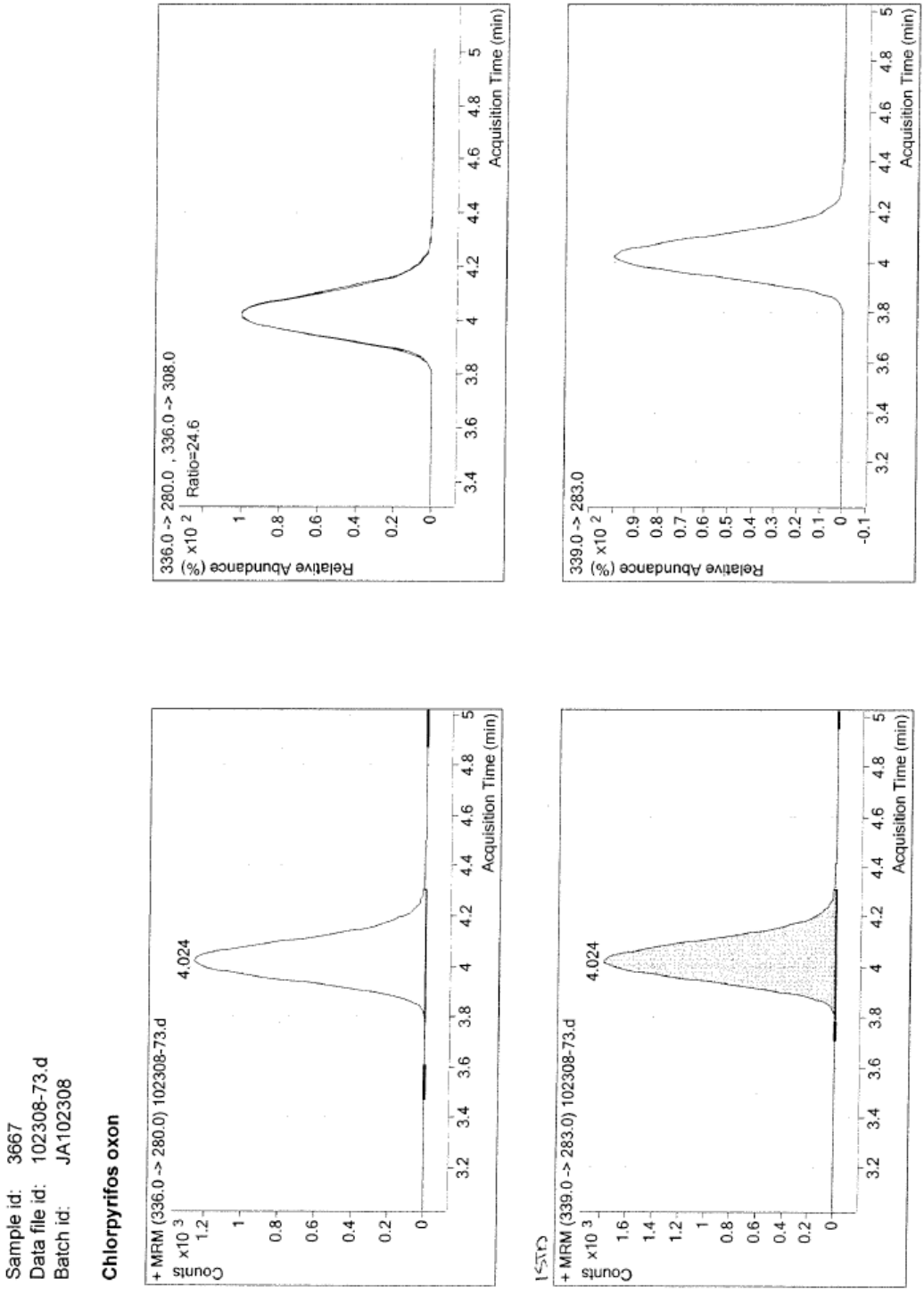
1. Instrumental Method

Written by Jianbo Yu, Ph.D, Research Scientist / Jacqui Ahmad, Research Scientist
Reviewed/Approved by: Russell Dills, Laboratory Director

Chromatogram
Chlorpyrifos-oxon

Sample ID 3667.

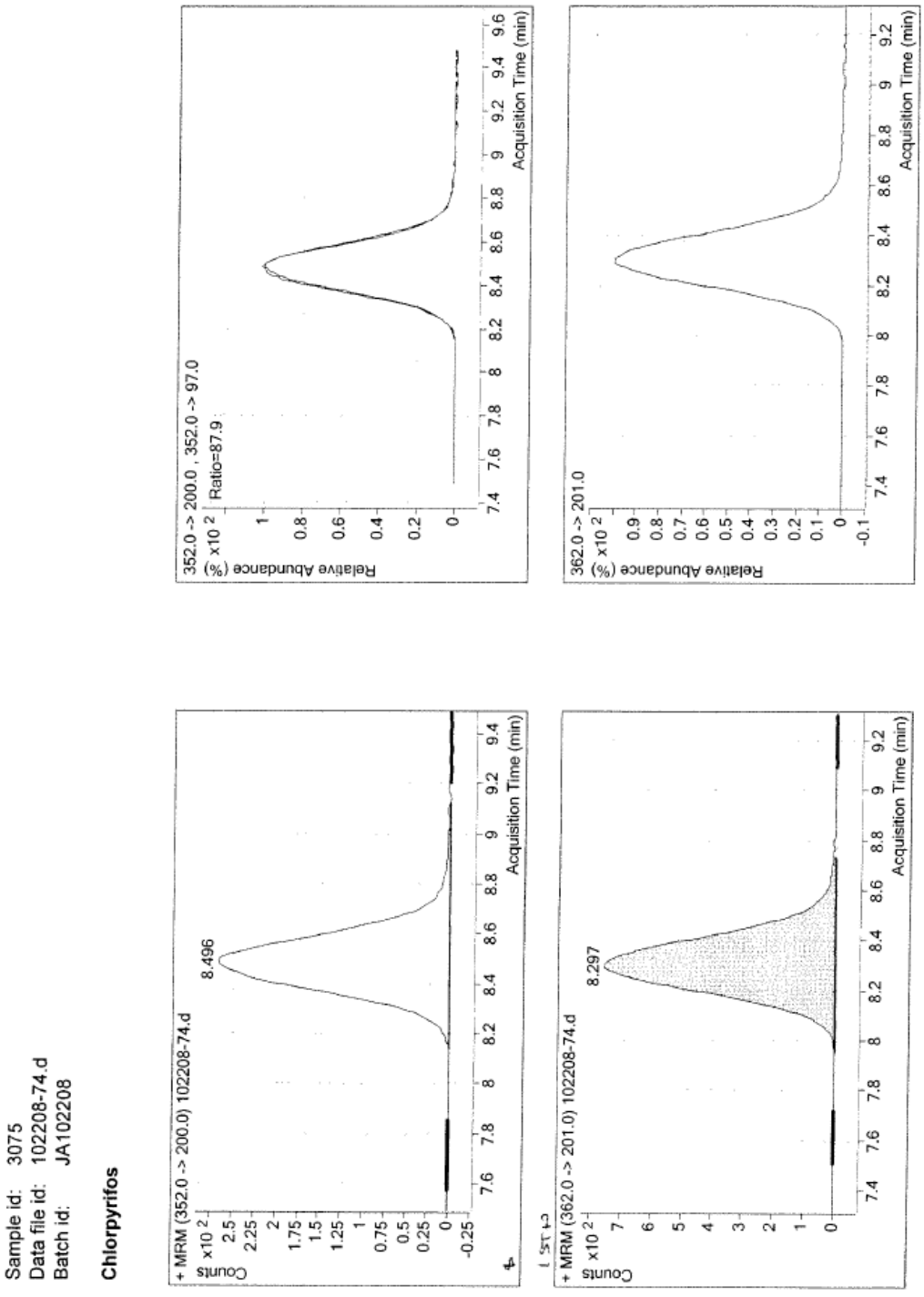
The panel on the upper right is the MRM plot for the quantifier ion. The panel on the upper left is the MRM plot for the qualifier ion. The panel on the lower left is the MRM plot for the quantifier ion of the oxon's internal standard. The lower right panel is not pertinent.



Chromatogram
Chlorpyrifos

Sample ID 3075.

The panel on the upper right is the MRM plot for the quantifier ion. The panel on the upper left is the MRM plot for the qualifier ion. The panel on the lower left is the MRM plot for the quantifier ion of chlorpyrifos' internal standard. The lower right panel is not pertinent.



Stability Study Protocol
Chlorpyrifos and Chlorpyrifos-oxon

The OVS XAD tubes were spiked into the front bed (25 µl of 1.0 µg/mL solution). The solvent for the spike was acetone. All samples were stored at -20°C. Analysis protocol was same as other samples. Each time point was done in triplicate. Both compounds were spiked at 25 ng/sample. Table 6 gives the storage durations.

Table 6. Storage Duration

Spiking date	Analysis Date	Days in Storage
11/13/08	12/15/08	32
11/13/08	1/13/09	61
11/13/08	2/13/09	92
11/13/08	3/13/09	120
11/13/08	4/13/09	151
11/13/08	5/13/09	181
11/13/08	7/13/09	242
11/13/08	11/13/09	360

Stability Study Protocol
Azinphosmethyl, Azinphosmethyl-oxon, Phosmet, and Malathion

The OVS XAD tubes were spiked into the front bed (25 µl of 2.0 µg/mL solution). The solvent for the spike was acetone. All samples were stored at -20°C. Analysis protocol was same as other samples. All compounds were spiked at 50 ng/sample. Table 7 gives the storage durations and the number of samples (N) analyzed at each time point.

Table 7. Storage Duration

Spiking date	**Analysis Date**	**Days in Storage**	**N**
5/20/08	12/15/08	209	6
5/20/08	1/13/09	238	6
5/20/08	2/13/09	269	3
5/20/08	3/13/09	297	3
5/20/08	5/20/09	365	3