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EN-CAS Method No. ENC-22/94

Analytical Method for the Gas Chromatographic Determination of Malathion and Malaoxon Residues in/on Cottonseed When Using Continuous Automated Sample Injections

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1.0 INTRODUCTION

1.1 Scope

This method is used for the determination of malathion and malaoxon residues in cottonseed (undelinted or delinted). The limit of quantitation (LOQ) is 0.01 ppm (µg/g) for malathion and malaoxon. Method validation results for cottonseed are included in this report (see Table I). See Figure 1 for a flowchart of the method.

1.2 Principle

Malathion and its metabolite, malaoxon, are extracted from fuzzy cottonseed by blending with acetonitrile for two minutes. Samples are filtered, partitioned with hexanes, and concentrated to 1 to 2 mL using rotary evaporation prior to the column cleanup step.

Alternately, malathion and malaoxon are extracted from fuzzy cottonseed by blending with 80/20 acetonitrile/water for 2 minutes. All samples are filtered, partitioned with hexanes, evaporated to aqueous and partitioned with DCM. Samples are concentrated to 1-2 mL using rotary evaporation prior to the carbon cleanup step.

Acetone, followed by activated charcoal, is added as a cleanup step. Samples are again filtered and concentrated to incipient dryness using rotary evaporation. The residues are redissolved with dichloromethane (DCM)/acetone and passed through a disposable silica-gel solid phase extraction cartridge.

1.2 Principle (continued)

The eluate is evaporated to dryness, reconstituted with an appropriate volume of acetone-polyethylene glycol, and vortexed. Analyte concentrations are determined by gas chromatography (GC) using a flame photometric detector (FPD) operating in the phosphorus mode.

This method is based on and is essentially identical to American Cyanamid Method M-1886 entitled GC Method for the Determination of Malathion (CL 6,601) and Malaoxon (CL 28,967) Residues in Alfalfa (Green Forage and Hay) When Using Continuous Automated Sample Injections (reference 1). The following modifications have been made:

1. Sample handling procedure: A 10-g representative sample (rather than a 20-g) is weighed into a 16-oz. French square bottle, 200 mL acetonitrile (alternately, 200 mL of 80/20 acetonitrile/water) is added, and the sample is blended for two minutes using an Omni-Mixer homogenizer (instead of a blender). All (80/20 acetonitrile/water) sample extracts are filtered, evaporated to aqueous and partitioned with DCM. Samples are concentrated to 3-5 mL using rotary evaporation prior to the carbon cleanup step. The entire aliquot is processed through the method.

2. Activated carbon: Five grams (rather than 1.0 g), along with 50 mL acetone, is used in the carbon cleanup step.

3. Silica-gel cleanup: The residues are dissolved in 1 mL acetone followed by 9 mL DCM, and a small amount of sodium sulfate is added to absorb water. The entire procedure is conducted using a vacuum box (rather than using positive pressure).

1.2 Principle (continued)

4. Standard solutions (stock, fortification, and GC) are stored in the freezer (vs. a refrigerator) when not in use and are stable for at least six months. High concentration stock solutions, properly stored, are known to be stable for longer periods.

5. An additional transfer, evaporation and reconstitution step is added to adequately dissolve the oily residue remaining prior to the usual final volume step.

6. GC standard solutions: Standards are prepared from higher stock concentrations, using acetone-polyethylene glycol as the dilution solvent, ranging from 0.025 µg/mL up to 1.0 µg/mL.

7. GC column: A J&W DB-5 30-m x 0.32-mm i.d. x 1.0-µm film thickness column is used.

   GC conditions: The initial column temperature is 50°C for 0.5 min ramping at 40°C/min to 175°C for 25 min, then ramping at 35°C/min to 185°C for 6 min, and finally ramping at 35°C/min to 235°C for 5 min. Inlet temperature is 200°C and detector temperature is 250°C.

8. GC analysis: The samples are not analyzed in duplicate, except in selected cases.

2.0 APPARATUS

[**NOTE:** All apparatus listed may be replaced by equivalent apparatus from alternate sources if experimental verification supports such substitutions.]

2.1 Flasks, 250-mL Erlenmeyer or flat-bottomed boiling, with 24/40 ground glass fittings

2.2 Flasks, 500-mL sidearm

2.0 APPARATUS (continued)

2.3 Filtering funnels, Buchner, porcelain, 100-mm plate diameter

2.4 Separatory funnels, 250-mL, with 24/40 ground glass fittings

2.5 Filter paper, 9-cm GF/C and 12.5-cm 934-AH glass fiber filters, Whatman Inc.

2.6 Stoppers, plastic, 24/40

2.7 TurboVap tubes, 15-mL conical, with plastic dry caps, Zymark Corporation, Cat. No. ZA 7519

2.8 Bottles, 16-ounce French squares, wide-mouthed, with Teflon-lined caps

2.9 BAKERBOND spe Disposable Extraction Columns, Silica gel, 500-mg, 3-mL, J. T. Baker Chemical Company, Phillipsburg, New Jersey, Cat. No. 7086-3

2.10 Rotary Evaporator: Büchi Rotavapor, model RE-111, Brinkman Instruments, Inc., Westbury, New York

2.11 Vortexer, Pulser Vortexer Test Tube Mixer, model PV6, Glascol, Terre Haute, Indiana

2.12 Integrator, Hewlett-Packard 3396A

2.13 Omni-Mixer with 35-mm x 195-mm flat-bottom generator, Model No. 17105 Homogenizer, Omni International, Waterbury, Connecticut

2.14 Balance, Analytical, Mettler H20, precision ±0.01 mg

2.15 Balance, Top loading, TL1600 American Scientific Products, precision ±0.01 g

2.16 Microliter syringes, 100-, 250-, 500-, 1000-µL capacity, Hamilton Company

2.17 VacElut SPS24, Analytichem International, Harbor City, California

2.18 TurboVap, LV Evaporator, Zymark Corporation, Hopkinton, Massachusetts

2.0 APPARATUS (continued)

2.19 GC Column, 30-m x 0.32-mm i.d., DB-5 with 1.0-μm film thickness, J&W Scientific, Cat. No. 123-5033

2.20 Cyclosplitter Deactivated Inlet Sleeve, Cat. No. 20706, Restek Corporation, Bellefonte, PA

2.21 GC, HP 5890 equipped with a FPD operating in the phosphorus mode

3.0 REAGENTS

[NOTE: All reagents listed may be replaced by equivalent reagents from alternate sources if experimental verification supports such substitutions.]

3.1 Acetonitrile, UV, Burdick and Jackson

3.2 Hexanes, pesticide grade, Fisher

3.3 Acetone, Optima, Fisher

3.4 Methylene chloride, pesticide grade, Fisher

3.5 Sodium sulfate (Na_2SO_4), anhydrous, ACS certified, oven-baked at 600°F for 2 hours and cooled to room temperature in a desiccator

3.6 Activated Carbon, Nuchar C-190N, Cat. No. 5790, Eastman Kodak Company (no substitution)

3.7 Polyethylene Glycol, 400, Cat. No. 1369941, Eastman Kodak Company

3.8 Acetone-polyethylene glycol: 0.02% in acetone. 200 μL of polyethylene glycol added to 1,000 mL of acetone

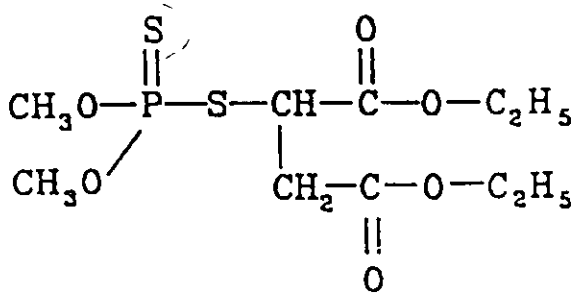
3.9 Analytical Standards: Analytical grade, known purity, Cheminova Agro A/S

a. Malathion: phosphorodithioic acid, S-[1,2-bis(ethoxycarbonyl)ethyl]O,O-dimethyl-dithiophosphate

b. Malaoxon: phosphorothioic acid, S-[1,2-bis (ethoxycarbonyl)ethyl]O,O-dimethyl ester

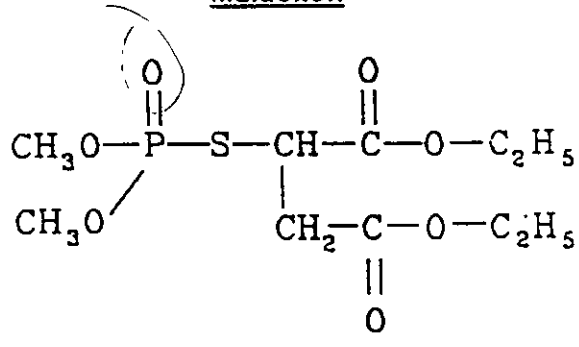
4.0 REFERENCE STANDARDS

Malathion



$C_{10}H_{19}O_6PS_2$
M.W. 330.4

Malaoxon



$C_{10}H_{19}O_7PS$
M.W. 314.3

5.0 PREPARATION OF ANALYTICAL STANDARDS

5.1 Fortification Standards

Weigh 100 mg (corrected for purity) of reference standard (of known purity, lot number and expiration date) separately for both malathion and malaoxon using an analytical balance. Dissolve

5.1 Fortification Standards (continued)

and dilute each to a volume of 100 mL with acetone to prepare 1000 µg/mL stock solutions. Prepare a 100 µg/mL combined malathion and malaoxon standard in acetone by aliquoting appropriately from the individual 1000 µg/mL stock solutions. Serially dilute the 100 µg/mL combined standard in acetone to prepare both a 10 µg/mL and 1.0 µg/mL combined malathion and malaoxon standards in acetone.

Use these solutions to fortify control samples in order to monitor procedural recovery for combined malathion and malaoxon.

For individual fortification standards, serially dilute the individual 1000 µg/mL stock solutions to prepare 100-, 10-, and 1.0 µg/mL malathion standards in acetone as well as 100-, 10-, and 1.0 µg/mL malaoxon standards in acetone. Use these solutions to fortify control samples in order to monitor procedural recovery for malathion and malaoxon individually. The stock and fortification standards are stable for at least 6 months. [NOTE: Store all standards in a freezer at -10°C to -17°C.]

5.2 Gas Chromatographic Standards

Combine the appropriate amounts and serially dilute, with 0.02% polyethylene glycol (PEG) in acetone, the 1000 µg/mL malathion and malaoxon stock solutions to prepare both a 100 µg/mL and 25 µg/mL combined standards. Serially dilute the combined standards to prepare a typical range of standards from 0.025 µg/mL to 1.0 µg/mL, in 0.02% PEG in acetone, to be used as GC calibration standards. The use of PEG is necessary to maintain the malaoxon sensitivity over the course of a GC run. The GC calibration standards are stable for 6 months. [NOTE: Store all standard solutions in a freezer at a temperature of -10°C to -17°C.]

6.0 ANALYTICAL PROCEDURES

6.1 Sample Storage and Processing

6.1.1 Sample Storage

Store residue samples (whole or homogenized) frozen in a freezer at <-5°C.

6.1.2 Sample Processing

Whole cottonseeds are systematically selected to achieve a representative, homogeneous subsample. Often, a typical lab gridding procedure may be used.

6.2 Extraction and Partition

See reference 2 for ^{14}C-extraction validation data.

Cottonseed was validated using the 100% acetonitrile extraction procedure (see Figure 1). However, an alternate 80/20 acetonitrile/water extraction procedure with DCM partition and additional activated charcoal was adopted for routine analysis of cottonseed because it provided better extraction and cleanup of the analytes.

6.2.1 Weigh 10.0 g of undelinted cottonseed sample (whole seeds) into a 16-oz. French square bottle. If the sample is to be stored frozen prior to processing, the bottle should be capped tightly with a Teflon-lined cap.

6.2.2 Fortify the control sample(s) with the appropriate amount of malathion and/or malaoxon, allowing the solvent to evaporate in a fume hood. Typically, 1 or 2 fortifications are analyzed concurrently with each batch of samples to monitor procedural recovery. The level(s) are chosen to reflect anticipated residues found in the samples. Wait no longer than five minutes before proceeding.

6.2 Extraction and Partition (continued)

6.2.3 Add 200 mL of acetonitrile to the sample and blend for two minutes, using an Omni-Mixer homogenizer, operating at a moderate speed.

NOTE: Special attention must be given at this step because whole cottonseed matrix tends to jam the moving parts in the homogenizer. Repetitive removal of the matrix from the homogenizer rotor may be required to complete the two-minute blend successfully.

6.2.4 Filter the sample, under vacuum, through a 9-cm Whatman GF/C (glass fiber) filter paper placed under a 12.5-cm Whatman 934-AH filter paper, inside a 100-mm Buchner funnel. Collect the extract into a 500-mL sidearm filter flask. Rinse the French square bottle with approximately 50 mL acetonitrile and filter through the Buchner funnel into the same 500-mL filter flask.

6.2.5 Transfer the entire extract to a 500-mL separatory funnel and partition the acetonitrile with 100 mL of hexanes, shaking for approximately one minute. Allow the layers to separate and transfer the lower, acetonitrile layer into a 250-mL Erlenmeyer flask. Discard the hexanes. Evaporate the sample to 1-2 mL using rotary evaporation with a water bath temperature $\leq 40^{\circ}C$.

Go to Section 6.4 for carbon cleanup step.

6.3 Alternate Extraction and Partition

See reference 2 for ^{14}C-extraction validation data.

For some cottonseed samples, difficulties encountered at the extraction step may produce recoveries for malathion and especially malaoxon below 70%. In such cases, the alternate extraction and partition procedure should be used. An alternate carbon clean-up should also be used

6.3 Alternate Extraction and Partition (continued)

in conjunction with the alternate extraction partition.

This alternate procedure is incorporated into the flowchart in Figure 2 and is the preferred procedure for routine analysis of cottonseed.

6.3.1 Weigh a 10-g homogenized cottonseed sample into a 16-oz French Square bottle. Add 200 mL of 80/20 acetonitrile/water to the sample (instead of 200 mL acetonitrile). Blend for two minutes; then filter the extract and rinse the French Square bottle with approximately 50 mL 80/20 acetonitrile/water (instead of acetonitrile).

6.3.2 Partition the extract with two 150-mL portions of hexanes (vs. 1 x 100 mL) using a 500-mL separatory funnel. Shake for one minute, allow the layers to separate and drain the lower layer into a 500-mL Erlenmeyer flask. Discard the hexanes.

6.3.3 Rotary evaporate the sample to <40 mL with a water bath temperature ≤40°C. Add 60 mL water plus 25 mL water (saturated with sodium chloride) to the sample. Partition the aqueous sample with three 50-mL portions of DCM. Collect each DCM fraction (lower layer) into a 250-mL Erlenmeyer flask (discard the aqueous). Rotary evaporate the DCM to 1-2 mL with a water bath temperature ≤40°C.

Go to Section 6.5 for alternate carbon cleanup step.

6.4 Carbon Cleanup

6.4.1 Dissolve the remaining solution from 6.2.5 in 50 mL acetone. Add 1.0 g of activated carbon and swirl. Allow the mixture to stand 30-40 minutes with occasional gentle swirling.

6.4.2 Vacuum-filter the sample through a 9-cm Whatman GF/C filter paper inside a

6.4 Carbon Cleanup (continued)

6.4.2 (continued)

Buchner funnel. Collect the extract into a 500-mL sidearm filter flask. Rinse the Erlenmeyer flask, Buchner funnel and filter paper with 50 mL acetone, collecting the rinse into the same 500-mL filter flask.

6.4.3 Transfer the extract to a 250-mL Erlenmeyer flask and carefully rotary evaporate the sample to incipient dryness at a water bath temperature of ≤40°C. Use a gentle stream of nitrogen to evaporate the solvent just to dryness.

Proceed with silica gel cleanup step (Section 6.6) and the remaining method as written.

6.5 Alternate Carbon Cleanup (Used in Conjunction with the Alternate Extraction and Partition)

6.5.1 Dissolve the remaining solution from 6.3.3 in 50 mL acetone. Add 5.0 g of activated carbon and swirl. Allow the mixture to stand 30-40 minutes with occasional gentle swirling.

6.5.2 Vacuum-filter the sample through a 9-cm Whatman GF/C filter paper inside a Buchner funnel. Collect the extract into a 500-mL sidearm filter flask. Rinse the Erlenmeyer flask, Buchner funnel and filter paper with 50 mL acetone, collecting the rinse into the same 500-mL filter flask.

6.5.3 Transfer the extract to a 250-mL Erlenmeyer flask and carefully rotary evaporate the sample to incipient dryness at a water bath temperature of ≤40°C. Use a gentle stream of nitrogen to evaporate the solvent just to dryness.

Proceed with silica gel cleanup step (Section 6.6) and the remaining method as written.

6.0 ANALYTICAL PROCEDURES (continued)

6.6 Silica Gel Cleanup

6.6.1 Dissolve the residual film from 6.4.3 or 6.5.3 with 1 mL acetone followed by 9 mL of DCM. Add a small amount of Na_2SO_4 (<50 mg) to the sample to absorb water.

6.6.2 Precondition a silica gel BAKERBOND spe column by passing 3 mL of 10/90 acetone/DCM through the cartridge, using a VacElut box with a vacuum setting of 5 psi.

6.6.3 Transfer the sample from 6.6.1, leaving the Na_2SO_4 behind, to the preconditioned BAKERBOND spe column, and using a vacuum setting of 5 psi, collect the fraction in a 15-mL TurboVap tube. Rinse the sample flask with 4 mL 10/90 acetone/DCM, transfer to the BAKERBOND spe column, and collect into the same TurboVap tube.

6.7 Final Dilution

6.7.1 Concentrate the sample from 6.5.3 or 6.6.3 to dryness using a TurboVap Evaporator with a gentle stream of nitrogen and a water bath temperature of ≤30°C.

6.7.2 Reconstitute the sample with a known volume (typically 2 mL for a 0.01 ppm screening level or 10 mL for a 0.05 ppm screening level) of 0.02% polyethylene glycol in acetone and vortex the sample for approximately 30 seconds.

NOTE: If the sample evaporates to an oily residue (typically 10-500 µL), and will not go to dryness, quantitatively transfer it nto a graduated centrifuge tube using polyethylene glycol. Concentrate the sample to 1.5 mL using a TurboVap evaporator; then readjust the final volume to 2 mL with polyethylene glycol and vortex for approximately 30 seconds. The sample is now ready for GC analysis. Store samples in a freezer at a temperature of -10°C to -17°C, when necessary, to preserve sample integrity.

6.0 ANALYTICAL PROCEDURES (continued)

6.8 Gas Chromatographic Determinations

Use a 30-m x 0.32-mm, 1.0-µm film thickness, fused silica DB-5 capillary column to achieve GC separation. Use a HP 5890 GC equipped with a FPD operating in the phosphorus mode. GC conditions are listed in Section 7.0 of this method.

6.9 Sequence of Analysis

Inject a series of standards (typically 4 or 5) at the beginning of the GC run to check for linearity. Alternate samples and standards so that at least every third or fourth injection is a standard. Inject 2 or 3 standards at the end of the run. Vary the concentration of standards injected throughout the run to demonstrate detector linearity. See Table II.

6.10 Safety Precautions

Use normal safety precautions, including the wearing of gloves and safety glasses. The use of a fume hood is necessary to minimize exposure to the analytes and organic solvents used in this procedure.

6.11 Time Required for Analysis

A set of 8-10 samples (including controls and recoveries) can be processed and prepared for injection on the GC by one analyst in approximately 1½ 8-hour days. An additional ½ day is required to annotate and calculate the data.

7.0 GAS CHROMATOGRAPHIC ANALYSIS

7.1 Description and Typical Operating Conditions for the HP 5890 GC, using a DB-5 Capillary Column

Instrument: Hewlett-Packard 5890A with a Flame Photometric Detector operating in the phosphorus mode

7.1 Description and Typical Operating Conditions for the HP 5890 GC, using a DB-5 Capillary Column (continued)

Column:	Fused Silica DB-5 Capillary Column, 30-m x 0.32-mm, 1.0-µm film thickness (J & W Scientific)
	(NOTE: The use of a guard column is highly recommended to extend the life of the analytical column. The use of a cyclosplitter inlet is also recommended.)
Gases:	Carrier: He at 50 psi, ≈3.6 mL/min Detector: H_2 at 30 psi, ≈116 mL/min Air at 50 psi, ≈102 mL/min
Injection Volume:	1 µL
Temperature:	Injector: 200°C Detector: 250°C

Column* Temperature Conditions:

Initial Temperature:	50°C
Initial Time:	0.5 min
Rate A:	40°/min
Temperature A: (malaoxon elution)	175°C
Final Time A:	25 min
Rate B:	35°/min
Temperature B: (malathion elution)	185°C
Final Time B:	6 min
Rate C:	35°/min
Temperature C: (burn-off)	250°C
Final Time C:	5 min

* Differences in instrument performance and adjustments to the specific temperature program as a result of column maintenance may yield a shift in a particular analyte's retention time.

7.1 Description and Typical Operating Conditions for the HP 5890 GC, using a DB-5 Capillary Column (continued)

Typical* Retention Times: Malaoxon: 26.7 min
Malathion: 33.7 min

Typical Integrator Parameters: Hewlett-Packard 3396A

RUN PARAMETERS		INTEGRATOR DEFINITIONS
ZERO	= 10	2. SET BASELINE ALL VALLEYS
ATTENUATION	= 3	8 TURN ON START/STOP MARKS
CHART SPEED	= 0 5 cm/min.	9 TURN OFF INTEGRATION
AR REJ	= 0	
THRESHOLD	= 2	
PEAK WIDTH	= 0 35 min.	

TIMETABLE EVENTS	
0 min. CHART SPEED	= 0 0 cm/min
0 min. INTEGRATION #	= 8
0 min. INTEGRATION #	= 2
0 min. INTEGRATION #	= 9
18 min. CHART SPEED	= 0 5 cm/min.
19 min. INTEGRATION #	= -9
36 min CHART SPEED #	= 0 0

* Differences in instrument performance and adjustments to the specific temperature program as a result of column maintenance may yield a shift in a particular analyte's retention time.

7.2 Calibration

Use the combined malathion and malaoxon standards in polyethylene glycol-acetone in concentrations ranging from 0.025 µg/mL to 1.0 µg/mL to calibrate the instrument. Inject 4 or 5 standards at the beginning of the run, after approximately every 2 or 3 samples throughout the run, and at least 2 standards at the end of the run. Generate a linear regression curve using the resulting peak height (obtained from the integrator) vs. nanograms injected. The correlation coefficient for the line should be equal to or greater than 0.990. The nanograms found are determined by inserting the sample peak height values into the standard curve linear regression equation.

7.0 GAS CHROMATOGRAPHIC ANALYSIS (continued)

7.3 Representative Chromatograms

Typical chromatograms illustrating GC calibration standards as well as controls and recoveries are shown in Figures 3 to 5. (The chromatogram of the cottonseed control sample, Figure 4, indicates low-level contamination of this sample with malathion). Typical calibration curves for malathion and malaoxon are shown in Figures 6 and 7.

7.4 GC/MS Confirmation

Use a DB-5 or DB-17 capillary column to achieve separation of samples with malathion and/or malaoxon residues that require confirmation other than on the HP 5890 GC with a FPD detector. A GC equipped with a mass selective detector can be calibrated to allow detection at 0.05 ppm for both analytes.

7.5 Acceptance Criteria

7.5.1 Retention Time

The retention time of the analytes in the fortified samples and the actual samples should be within ± 0.15 minutes of the retention time of the nearest standard in the analytical set to be accepted for the analyte--contingent on the column used and the GC conditions described in this method.

7.5.2 Standard Curve Range

The standard curve range is 0.025 µg/mL to 1.0 µg/mL for both analytes. If a sample peak height exceeds the peak height of the highest standard in the standard curve, the final extract will be diluted so that its peak height will fall within the standard curve upon reinjection. Evaluate analytes individually for dilutions needed for a given sample.

7.5 Acceptance Criteria (continued)

7.5.3 Recovery From Fortified Samples

Recovery from fortified samples should be in the range of 70% to 120%. Low levels of analyte in the control matrix should be subtracted from the results when calculating recoveries.

7.5.4 Reproducibility

Reproducibility can be measured by results from the validation. Duplicate analyses at a given fortification level should not vary more than 20%.

7.5.5 Peak Shape and Width

Sample peak shape and width are evaluated manually to determine that they are similar to that of the fortified samples.

7.5.6 Calibration Curves

As stated in Section 7.2, the correlation coefficient of the regression line determined for each analyte must be greater than 0.990.

7.5.7 Analysis Time Limits

Samples should be analyzed within 60 days of being homogenized. The final extract should be chromatographed within 7 days after extraction of the homogenized sample.

8.0 CALCULATIONS

8.1 Calculation of ng Found

The ng of analyte found are determined from the standard curve as follows:

$$\text{ng Found} = \frac{\text{Peak height (counts)} - \text{standard curve y intercept (counts)}}{\text{standard curve slope (counts/ng)}}$$

8.2 Calculation of mg-Equivalent Injected

$$\text{mg-equiv injected} = \frac{\text{Sample wt (g)} \times \text{aliquot vol (mL)} \times \mu\text{L inj.} \times 1000\ \text{mg/g}}{\text{Extraction vol (mL)} \times \text{mL final vol} \times \text{dilution factor} \times 1000\ \mu\text{L/mL}}$$

8.3 Calculation of ppm Found

$$\text{ppm found} = \frac{\text{ng analyte found}}{\text{mg-equiv. injected}}$$

8.4 Calculation of Corrected ppm (for Fortifications Only)

$$\text{ppm (corrected)} = \text{ppm (fortification)} - \text{ppm (control)}$$

8.5 Calculation of Percent Recovery

$$\%\ \text{Recovery} = \frac{\text{ppm found}^{*}}{\text{fortification level (ppm)}} \times 100$$

* Laboratory fortifications were corrected for any control contribution

8.0 CALCULATIONS (continued)

8.6 Example Calculation

EM1112-S1, malathion, GC Run # 45886, dated 3/07/94, Cottonseed, Figure 5

Where:

sample weight	= 10 g
extraction volume	= 200 mL
aliquot volume	= 200 mL
final volume	= 2.0 mL
µL injected	= 1.0 µL
dilution factor	= 1
peak height (sample)	= 11624 counts
y-intercept	= -349.16 counts
slope	= 152060.1 counts/ng
fortification level (ppm)	= 0.01 ppm
control contribution (ppm)	= 0.0064 ppm

$$\text{ng found} = \frac{11624 \text{ counts} - (-349.16 \text{ counts})}{152060.1 \text{ counts/ng}} = 0.0787 \text{ ng}$$

$$\text{mg equiv injected} = \frac{10 \text{ g} \times 200 \text{ mL} \times 1 \text{ µL} \times 1000 \text{ mg/g}}{200 \text{ mL} \times 2.0 \text{ mL} \times 1000 \text{ µL/mL}} = 5.0 \text{ mg}$$

$$\text{ppm Found} = \frac{0.0787 \text{ ng}}{5.0 \text{ mg}} = 0.0157 \text{ ppm}$$

$$\text{ppm corrected} = 0.0157 \text{ ppm} - 0.0064 \text{ ppm} = 0.0093 \text{ ppm}$$

$$\% \text{ Recovered} = \frac{0.0093 \text{ ppm}}{0.01 \text{ ppm}} \times 100 = 93\%$$

9.0 LIMIT OF QUANTITATION

Adjust the instrument sensitivity, GC calibration standards and final sample volumes to allow detection of malathion and malaoxon at 50% of the LOQ. The LOQ for cottonseed was 0.01 ppm for each analyte based on the signal-to-noise ratio being greater than or equal to 5, but was adjusted to 0.05 ppm during the course of the actual RAC analyses for cottonseed due to variability in low level recoveries.

10.0 ANALYTICAL NOTES

For some cottonseed samples, difficulties encountered at the extraction step may produce recoveries for malathion and especially malaoxon below 70%. In such cases, the alternate extraction and partition procedure outlined in Section 6.3 should be used. An alternate carbon clean-up should also be used in conjunction with the alternate extraction partition.

This alternate procedure (Section 6.3) is incorporated into the flowchart in Figure 2 and is the preferred procedure for routine analysis of cottonseed.

11.0 VALIDATION RESULTS

See Table I for method validation results. The mean and standard deviation for malathion and malaoxon were 91% ± 4.2 (n=10) and 109% ± 8.6 (n=10), respectively.

12.0 REFERENCES

1. American Cyanamid Method M-1886 entitled GC Method for the Determination of Malathion (CL 6,601) and Malaoxon (CL 28,967) Residues in Alfalfa (Green Forage and Hay) When Using Continuous Automated Sample Injections, issued March 7, 1989.

2. EN-CAS Project # 92-0106 entitled Accountability Study of the Proposed Method for the Determination of Malathion (O,O-Dimethyl Phosphorodithioate of Diethyl Mercaptosuccinate) and its Metabolite, Malaoxon, in/on Alfalfa Hay Treated with 14C-Radiolabelled Malathion (MRID # 42894601), issued August 19, 1993.

Table I

Cottonseed Method Validation Results for Malathion and Malaoxon

E #	Fort Level (ppm) a	Analyte Fortified	ppm Found or % Recovery Malathion	Malaoxon
EM1112-C	Control	NA	0.01[b]	<0.01[b]
EM1112-C2[c]	Control	NA	<0.01[b]	<0.01[b]
EM1112-S1[c]	0.01	Both analytes	93	115
EM1112-S2[c]	0.01	Both analytes	89	118
EM1112-S3[c]	0 01	Both analytes	91	111
EM1112-S4	0.05	Both analytes	85	112
EM1112-S5	0 05	Both analytes	95	121
EM1112-S6	0.05	Both analytes	84	113
EM1112-S7	0 50	Both analytes	97	104
EM1112-S8	0 50	Both analytes	94	98
EM1112-S9	0 50	Both analytes	92	95
EM1112-S10	0.50	Malaoxon only	<0.01[b]	103
EM1112-S11	0.50	Malathion only	92	<0.01[b]
		Mean:	91	109
		Standard Deviation: (n=10)	4 2	8.6

a - The fortification level is expressed as the individual analyte, not as the parent equivalent.
b - Validation samples were screened to 0 01 ppm but the RAC sample analyses were ultimately screened to 0.05 ppm, as per Sponsor approval.
c - Initial recoveries at the 0 01 ppm level were outside the 70-120% recovery range specified by the protocol EM1112-C2, EM1112-S1, EM1112-S2 and EM1112-S3 were reanalyzed as a supplemental validation set to achieve recoveries in the range specified However, due to unacceptable and variable recovery of malathion and malaoxon at 0 01 ppm encountered during routine analysis of samples, an LOQ of 0 05 ppm was adopted

NOTE. These data were obtained using the 100% acetonitrile extraction procedure (1 0 g carbon), but analysis should be conducted using the 80/20 acetonitrile/water extraction procedure with DCM partition (5 0 g carbon) as described in the text (Section 5.3) and Figure 2

Table II

Typical Series of Injections Used for the Quantitation of a Set of Samples

Sample	Number of Field Samples
4 standards 1 control sample 2 fortified samples, 2 levels	
1 standard 3 field samples	3
1 standard 3 field samples	3
1 standard 3 field samples	3
1 standard 1 reagent blank 2 standards	
Total of 23 injections	Total of 9 field samples

NOTE: To prevent possible carryover from samples containing high levels of residues, acetone may be injected following selected samples anticipated by the Principal Anlaytical Investigator to contain high residues.

FIGURE 1

Flowchart of Analytical Method for Cottonseed

Determination of Malathion and Malaoxon Residues in Cottonseed When Using Continuous Automated Sample Injections

Weigh a 10.0-g sample into an 16-oz French square bottle

↓

Blend for 2 minutes with 200 mL acetonitrile using an Omni-Mixer homogenizer

↓

Vacuum-filter and rinse with acetonitrile

↓

Partition the sample with 100 mL hexanes (discard hexanes)

↓

Concentrate the extract to 1-2 mL using rotary evaporation with a water bath temperature ≤40°C

↓

Add 50 mL acetone - 1.0 g carbon Nuchar C-190N

↓

Vacuum-filter and rinse with 50 mL acetone

↓

Concentrate the extract to incipient dryness using rotary evaporation with a water bath temperature ≤40°C. Dry sample with a gentle stream of nitrogen. Add 1 mL acetone, followed by 9 mL dichloromethane (DCM). Add a small amount of Na_2SO_4 to absorb the water

↓

Precondition a silica gel BAKERBOND spe column with 3 mL 10/90 acetone/DCM, transfer the sample to the BAKERBOND spe column, and collect in a TurboVap tube. Rinse the sample flask with 4 mL 10/90 acetone/DCM and collect in the same TurboVap tube

↓

Concentrate the sample to dryness using a TurboVap evaporator with a gentle stream of nitrogen and a water bath temperature of ≤30°C

↓

Add an appropriate volume of 0.02% polyethylene glycol in acetone and vortex for 20-30 seconds

↓

(continued on next page)

FIGURE 1 (continued)

Flowchart of Analytical Method for Cottonseed

Determination of Malathion and Malaoxon Residues in Cottonseed When Using Continuous Automated Sample Injections

↓

When sample evaporates to an oily residue, quantitatively transfer into a graduated centrifuge tube using polyethylene glycol. Concentrate the sample to 1.5 mL using a TurboVap evaporator then readjust the final volume to 2 mL with polyethylene glycol and vortex for 20-30 seconds

↓

Analyze the sample on a GC using a 30-m x 0.32-mm DB-5 capillary column (1.0-µm film thickness) and a flame photometric detector (FPD) operating in the phosphorus mode

FIGURE 2

Flowchart of Alternate Analytical Method for Cottonseed*

Determination of Malathion and Malaoxon Residues in/on Cottonseed When Using Continuous Automated Sample Injections

Weigh a 10.0-g sample into a 16-oz French square bottle

↓

Blend for 2 minutes with 200 mL 80/20 acetonitrile/water using an Omni-Mixer homogenizer

↓

Vacuum-filter and rinse with 80/20 acetonitrile/water

↓

Partition the sample with two 150 mL portions of hexanes (discard hexanes)

↓

Rotary evaporate sample to aqueous with a water bath temperature ≤40°C

↓

Add 60 mL d.i. water - 25 mL d.i. water saturated with sodium chloride. Partition the sample with three 50-mL portions of DCM (discard aqueous)

↓

Concentrate the extract to 1-2 mL using rotary evaporation with a water bath temperature ≤40°C

Add 50 mL acetone - 5.0 g carbon Nuchar C-190N

↓

Vacuum-filter and rinse with 50 mL acetone

↓

Concentrate the extract to incipient dryness using rotary evaporation with a water bath temperature ≤40°C. Dry sample with a gentle stream of nitrogen. Add 1 mL acetone, followed by 9 mL DCM. Add a small amount of Na_2SO_4 to absorb the water

↓

Precondition a silica gel BAKERBOND spe column with 3 mL 10/90 acetone/DCM, transfer the sample to the BAKERBOND spe column, and collect in a TurboVap tube. Rinse the sample flask with 4 mL 10/90 acetone/DCM and collect in the same TurboVap tube

↓

(continued on next page)

* This method flowchart reflects the preferred procedure as applied to routine analyses.

FIGURE 2 (continued)

Flowchart of Alternate Analytical Method for Cottonseed*

<u>Determination of Malathion and Malaoxon Residues in/on Cottonseed When Using Continuous Automated Sample Injections</u>

↓

Concentrate the sample to dryness using a TurboVap evaporator with a gentle stream of nitrogen and a water bath temperature of ≤30°C

↓

Add an appropriate volume of 0.02% polyethylene glycol in acetone and vortex for 20-30 seconds

↓

Analyze the sample on a GC using a 30-m x 0.32-mm DB-5 capillary column (1.0-µm film thickness) and a FPD operating in the phosphorus mode

* This method flowchart reflects the preferred procedure as applied to routine analyses.

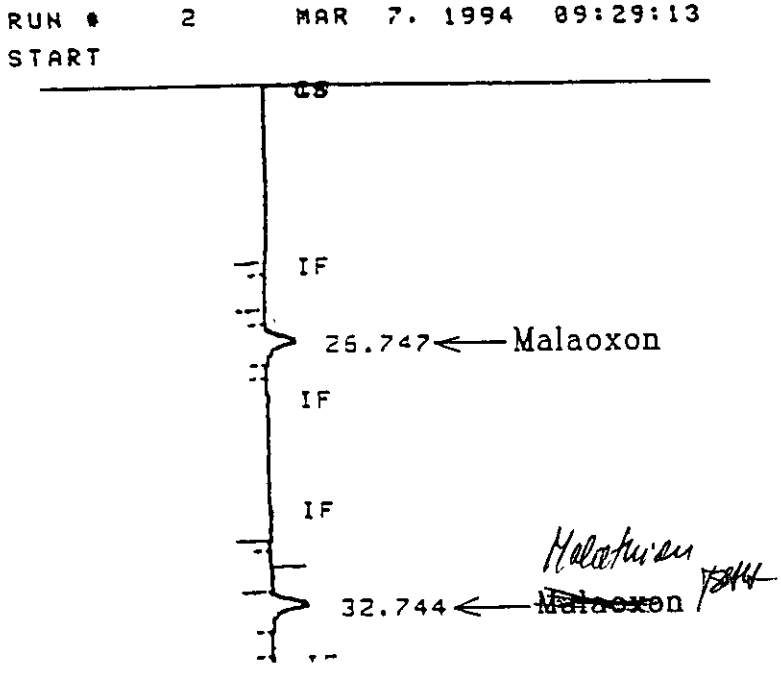
FIGURE 3

Typical Chromatogram

Malathion and Malaoxon

GC Standard

0.025 µg/mL



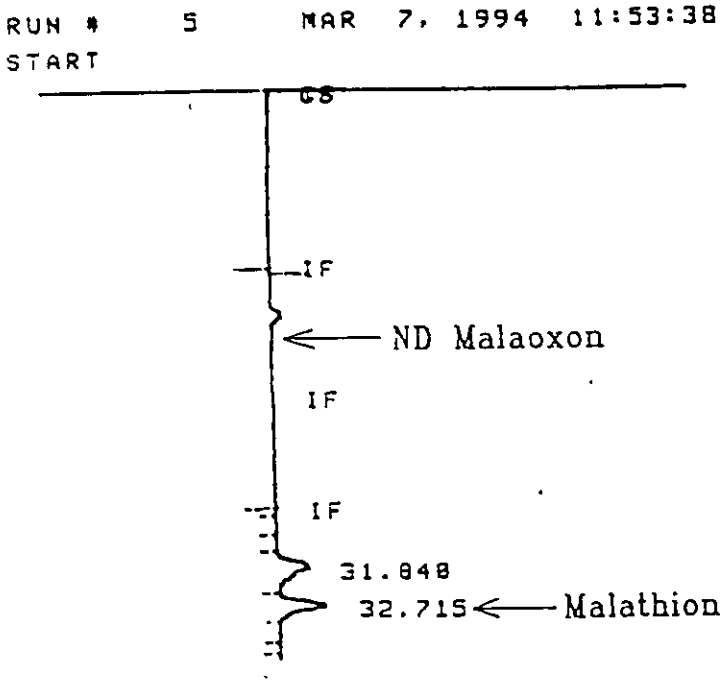
0.025 ng injected
GC run # 45886, set # MV-1R, dated 3/07/94

FIGURE 4

Typical Chromatogram

Malathion and Malaoxon

Cottonseed Control



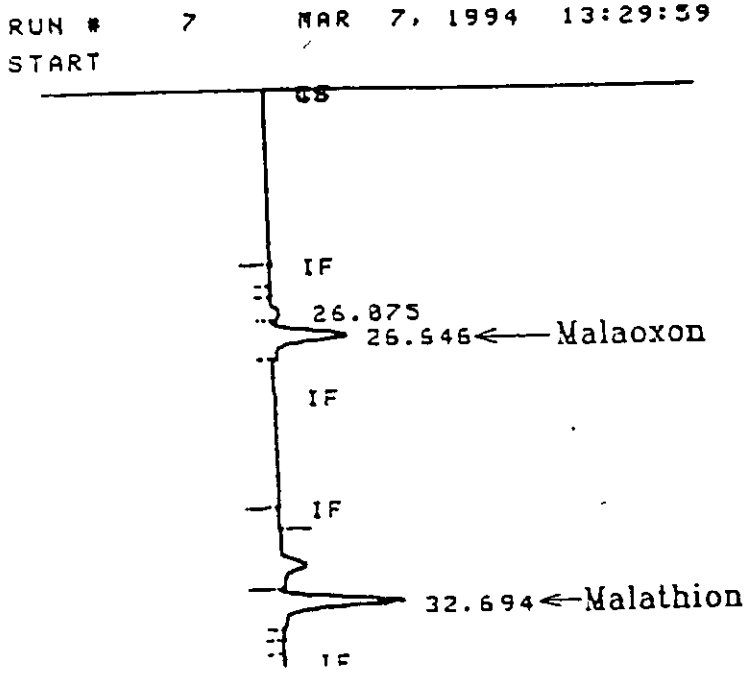
EN-CAS Sample ID #: EM1112-C2
Malaoxon ppm Found: <0.01 ppm
Malathion ppm Found: <0.01 ppm (0.0064 ppm detected)
GC run # 45886, set # MV-1R, dated 3/07/94

FIGURE 5

Typical Chromatogram

Malathion and Malaoxon

Cottonseed Control + 0.01 ppm
(Malathion and Malaoxon)

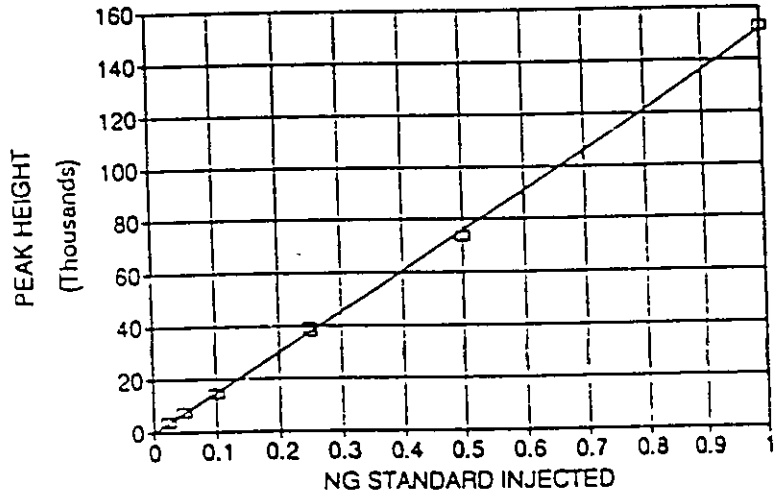


EN-CAS Sample ID #: EM1112-S1
Malaoxon Recovered: 115%
Malathion Recovered: 93%
GC run # 45886, set # MV-1R, dated 3/07/94

FIGURE 6

Typical Calibration Curve

Malathion



X	Y
0 25	39059
0 025	3627
0 1	14251
0.05	7247
0 025	3909
0 25	37771
0.5	73291
1	152574

STATISTICS

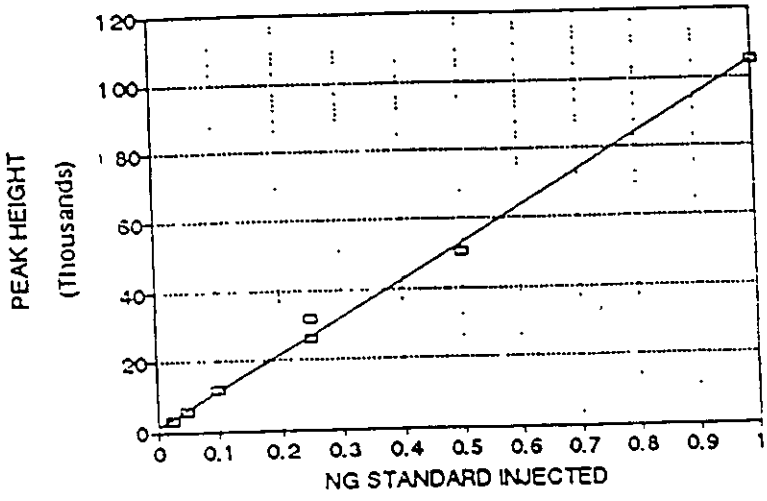
Regression Output.			
Constant		-349.16	(y-int)
Std Err of Y Est		1227 528	
R Squared		0 999501	(variance)
No. of Observations		8	
Degrees of Freedom		6	
X Coefficient(s)	152060.1		,slope)
Std Err of Coef.	1386 686		
Corr Coef	0 999751		

GC run # 45886, set # MV-1R, dated 3/07/94

FIGURE 7

Typical Calibration Curve

Malaoxon



X	Y
0 25	32009
0 025	3024
0.1	11653
0.05	5856
0.025	3158
0 25	26451
0 5	50444
1	105033

STATISTICS

Regression Output:			
Constant		1228 627	y-int)
Std Err of Y Est		2323 725	
R Squared		0 996161	(variance)
No. of Observations		8	
Degrees of Freedom		6	
X Coefficient(s)	103567 7		slope)
Std Err of Coef	2624 799		
Corr Coeff	0 998079		

GC run # 45886, set # MV-1R, dated 3/07/94