•	ABC		. *		
Name	Standard	Lot		· ·	Date
<del>.</del>	<u>Number</u>	<u>Purity</u>	<u>Number</u>	Storage	Received
Malathion	PS-2655	98.4%	AC5561-119-9	•	07-25-88
Malathion	PS-3026	98.4%	5561-119-9	李章	01-11-89
Malaoxon	PS-3311	99.4%	4661-115A	***	
Malaoxon	PS-3516	99.4%	4661-115A	•	05-18-89
Malaoxon	PS-3951			freezer	08-30-89
•		99.4%	4661-115A	refrigerator	04-06-90
Malaoxon	PS-4085	99.4%	4661-115A	freezer	
CYTHION*	NA	91.0%	<del>-</del>	1100201	06-07-90
	2344	71.070	NJ 085-446	room temp.	07-14-80

<sup>\*</sup>freezer until 5/30/89 and then refrigerated

American Cyanamid performed the characterization of these reference materials; these data are available in their files.

During preparation of the stock solutions, a 3.1% error was made in diluting the malathion weighed on August 18, 1989. Where necessary, sample values were corrected on the raw data tables. A 1.1% error was made in diluting malaoxon weighed on May 22, 1989. Again, sample values were corrected where necessary on the raw data tables. Preparation of stock solutions and dilution of working standards are detailed in the analytical raw data of the ABC Laboratories Raw Data Report #38004.

### METHOD OF ANALYSIS

With a few modifications, the methodology used to analyze malathion and malaoxon in the soil and crop samples was the procedure that American Cyanamid Company (M-1923) supplied titled "GC Method for the Determination of Malathion (CL6,601) and Malaoxon (CL28,967) Residues in Grasses (including tall fescue, bermuda, and bluegrass) When Using Continuous Automated Sample Injections." The analytical methodology used for the water samples was developed at ABC Laboratories. Complete methods are given in Appendix I of this report.

## I. Soil Moisture Determination

A soil moisture determination was performed on each soil sample analyzed for malathion/malaoxon. Moisture determinations were performed as described in ABC SOP FC 1.7.1. Each determination consisted of weighing the sample container and then the container plus the wet soil. The sample was dried to a

<sup>\*\*</sup>freezer until 11/14/89 then refrigerated

## B. Crops Extraction

Plant samples were extracted using the same method as the soil with the following exceptions:

- In step one, 20 g of plant matrix were weighed into a 32-oz glass screw cap jar. Two hundred and fifty mL of acetonitrile were added and the jar was capped with a blender blade attachment. The mixture was then blended for two minutes.
- Between steps 3 and 4, the residue was dissolved in 50 mL of acetone. One g of activated carbon was added to the acetone and swirled. The mixture was allowed to stand 30-40 minutes with occasional swirling. It was then filtered through glass fiber filter paper held in a Buchner funnel and the acetone was collected in a 250 mL flat bottom flask. The 500 mL flask, the filter, and the funnel were all rinsed with an additional 50 mL of acetone. The combined acetone was taken to dryness on a rotary evaporator under partial vacuum with a water bath temperature of approximately 40°C.

# C. Water Extraction

The analytical methodology used for the analysis of the water samples was developed at ABC Laboratories. Malathion and malaoxon were extracted from the water as follows:

- One hundred mL of water were added to a 500 mL separatory funnel. Seventy-five mL of methylene chloride were added and the mixture was shaken by hand for 2 minutes. The organic extract was drained through rinsed sodium sulfate in a powder funnel into a 500 mL flat bottom flask.
- The water was extracted again with 75 mL of methylene chieffed. The mixture was shaken for 2 minutes and drained over the tame sodium sulfate into the same 500 mL flask.
- The combined methylene chloride was taken to dryness on a strary evaporator under partial vacuum with a water bath temperature of 40°C.
- The residue was reconstituted with acetone and quantitatively transferred to a 15-mL screw cap test tube. The samples are 1946 in appropriate volumes of 0.02% polyethylene glyppion teams.

### D. Instrumentation

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Instrumentation used for the chromatography of the sample analysis was a Hewlett-Packard 5890 equipped with a flame photometric detector. General GC parameters used were as follows:

Column:

15 m  $\times$  0.53 mm DB1 with a 1.5  $\mu$  film thickness

Injector Temp: Detector Temp:

200°C 240°C

Flow Rate:

Hydrogen: Air:

75 mL/minute 100 mL/minute Helium: ca: 20 mL/minute Nitrogen: ca: 20 mL/minute

Temperatures Program:

Initial Temp.:

Initial Hold: Rate: .

6 minutes

Final Temp.:

15°C per minute

185°C

2λ

Final Hold:

2.0 minutes

Injection Volume:

Rice and irrigated plot soil samples exhibited a peak in proximity to that of malaoxon. At certain levels this peak would have masked the detection of malaoxon. The samples were extracted, partitioned, and columned as stated in the method. Changes were made in the instrumentation to allow for better separation of the malaoxon from the interference peak. The new GC parameters are as follows:

Column:

DB5 30 m  $\times$  0.25 m i.d.

 $0.25 \mu$  film thickness

Injection Temp.:

240°C

Detection Temp.:

240°C

Flow Rate:

Nitrogen = 40 mL/min Helium = ca: 2.5 mL/min

Hydrogen = 75 mL/min

Air = 100 mL/min

Temperature Program: >

Initial Temp.:

120°C

Initial Hold:

. 0 minutes

Rate:

18°/min to 210°C then 5°/min

Final Temp.:

225°C

Final Hold:

5.5 minutes

ABC LABS NO. 38004-41

Peak height values were obtained with the Computer Automated Laboratory System (CALS<sup>®</sup>) and Multichrom™ systems.

Precise records of instrument parameters are contained in each analytical data set in the analytical raw data of ABC Raw Data Report #38004.

# IV. Data Acquisition and Calculations

### A. Data Acquisition

The chromatographic data for the study were acquired, analyzed, and reported on two computer systems during the course of the study. The original system acquired the chromatographic data in a HP-1000 computer. Peak response measurements, standard curve generation, data analysis, chromatograms, and results were performed by the CALS® software. The peak response for each component (known and unknown) was measured and written into a calibration file containing the concentration values for each standard. The computer generated a standard curve for each component of concentration versus peak height. The concentration for each unknown was calculated from the standard curve. The total residue concentration that was found was calculated from the concentration detected, using the STD (RRF) and SMP (RRT) factors from the schedule file.

The VG Multichrom<sup>®</sup> data system was also used to acquire and analyze data obtained from the HP-5890 GC instrument. The central processing unit was a Digital Equipment Corporation (DEC) MicroVax 3800 equipped with 32 Mbytes of memory and 800 Mbytes of disk. The operating system was Vax VMS version 5.3-1. IBM model 4019 laser printers were used as printing and plotting devices. The network, based on the DECnet (Ethernet) protocol, consists of a fiber optic network between buildings, a twisted-pair network (10 Base-T) within buildings, and a Thinnet segment within each of the instrument labs for attachment of the chromatography servers. Chipcom fiber optic controllers, twisted-pair transceivers, 3Com personal computer Ethernet controllers, and DEC server 300 controllers are some of the network components used.

The Chromatography Servers are four channel, high resolution analog to digital converters that connect directly to the Ethernet network. They are controlled by the Multichrom software according to parameters set up by the users as described in the Multichrom User Reference.

### B. Calculations

Parts per billion concentrations of individual residues were calculated in CALS® and Multichrom using the following formula:

found ppb residue = 
$$\frac{ng|mL}{detected \times final \ volume \ (mL)}$$
  
sample weight (g)

final volume 
$$(mL) = \frac{\text{extract volume } (mL) \times \text{chromatographic volume } (mL)}{\text{aliquot volume } (mL)}$$

Concentrations of individual residues, expressed in ppm units, were then entered into Quattro® spreadsheets.

All samples analyzed for malathion/malaoxon were double injected. The average of the two injections was used to calculate % recovery and total ppm of residue in the sample.

Recoveries from fortified samples were determined by the following formula:

Residues found in the treated samples were corrected for procedural recoveries by the following calculation:

If the average % recovery was 100 or greater, 100% was used. Residue values were not corrected for control values.

The residue level in the treated soil samples was also corrected for moisture content as follows:

corrected ppm, dry basis = 
$$\frac{\text{corrected ppm, wet basis}}{(100\% - \% \text{ soil moisture})} \times 100$$
expressed as a decimal

ABC LABS NO. 38004-43

The sample average and average of the replicates are included. When residues were found in some but not all of the replicates above the LOD. 0.005 ppm replaced < 0.01 in the calculation of averages.

The analytical method used to determine malathion and malaoxon residues in the spray solution aliquots is stated below.

The aliquot amount from the spray tank was determined by weighing the vial plus the sample, quantitatively transferring the entire sample to a 250 mL volumetric flask with acctone, and reweighing the empty dry vial. The samples were brought to volume in acetone. A 1-mL aliquot was removed from the 250-mL volumetric flask and diluted again with appropriate amounts of acetone/PEG. A portion of this dilution was analyzed by GC. Peak heights were obtained using the CALS® system. Values were calculated from peak heights by the CALS® program. Levels of malathion and malaoxon (ppm) were calculated by the CALS® program using the following formula:

# $ppm = \mu g/mL$ detected × final volume (mL) sample weight (g)

The ppm value found for each sample was entered into Quattro®, converted to lb a.i./gal, and reported as a percent of the theoretical

A summary of all analyses is included in data summary tables in the Analytical Raw Data of the Raw Data Report #38004.

The following information is included in Appendix V:

- Representative standard curves for malathion and malaoxon
- retention times for both malathion and malaoxon;
- a typical control chromatogram;
- a typical spiked control chromatogram;
- representative authentic water, soil, and plant chromatograms are included in Appendix V.



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J. Boyd

AMERICAN CYANAMID COMPANY AGRICULTURAL RESEARCH DIVISION CHEMICAL DEVELOPMENT P. O. BOX 400 PRINCETON, NEW JERSEY 08540

Recommended Method of Analysis

Malathion (CL 6,601): GC Method for the Determination of Malathion (CL 6,601) and Malaoxon (CL 28,967) Residues in Grasses (including tail fescue, bermuda and bluegrass) when using continuous automated sample injections.

### A. Principle

Residues of malathion (CL 6,601) and malaoxon (CL 23,967) are extracted from finely ground plant tissue with acetonitrile. The filtered extracts are subjected to cleanup procedures involving treatment with activated charcoal and passage of a methylene chloride-acetone solution through a disposable silica-gel solid phase extraction cartridge. The malathion (CL 6,601) and malaoxon (CL 28,967) concentrations are determined by gas chromatography using an instrument equipped with a flame photometric detector operating in the phosphorus mode. Results are calculated using linear regression from external standards. The validated sensitivity of the method is

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B. Apparatus (Items from other manufacturers may be used provided they are functionally equivalent).

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- 1. Gas Chromatograph: Tracor Model 540 equipped with a flame photometric detector.
- Waring Blendor: Model 31BL46 with 1-quart capacity glass blender jar (Waring Products Division, Dynamics Corporation of American, New Hartford, Connecticut).
- 3. Balance: Analytical, Mettler H3SAR, precision ± 0.05 mg.
- 4. Balance: Pan, Sartorius, Model 2254, precision = 5 mg.
- 5. Assorted Glassware: General laboratory, flasks, beakers, assorted volumetric flasks, pipets, etc.
- 6. Microliter Svringe: Hamilton #701-N, 10-mcL capacity.
- 7. Rotary Evaporator: Buchler Instruments (Model DBL-10GN), equipped with a warm water bath (about 30°C) in which evaporation flasks can be partially submerged.
- 8. Filtering Funnel: Buchner, Porcelain, 100 mm plate diameter.
- 9. Filter Paper: 7-cm diameter, glass fiber filter, Whatman, Incorporated.
- 10. Recorder: Spectra-physics Model SP 4270 recording integrator.
- 11. GC-Column: 90 cm x 2 mm ID giass, packed with 10% OV-101 on 80/100 mesh Supelcoport.
- 12. Solid Phase Extraction Columns: Silica gel, 500 mg, 3-mL (J. T. Baker Chemical Company, Phillipsburg, New Jersey, Cat. No. 7086-3).
- 13. <u>Mini-Column Vacuum Manifold</u>: Analytichem A1600 10-place vacuum manifold or equivalent (ie. Baker SPE-10).
- C. Reagents (Items from other manufacturers may be used provided they are functionally equivalent).
  - 1. Analytical Standards: Analytical grade, known purity, American Cyanamid, Agricultural Research Division, P.O. Box 400, Princeton, New Jersey 08540. 199.0440
    - a. Malathion: phosphorodithioic acid, S-[1,2-bis (ethoxycarbonyi) ethyl] 0,0-dimethyldithiophosphate.

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- b. Malaoxon: phosphorothioic acid, S-1,2-bis (ethoxycarbonyl) ethyl 0,0-dimethyl ester.
- 2. GC Packing: 10% OV-101 on 80/100 mesh Supelcoport, Cat. No. 1-1753, Supelco, Incorporated, Belfonte, Pennsylvania 16823-0048.
- 3. Solvents, High Purity: B & J Brand, Baxter, Burdick and Jackson, Incorporated, acetone acetonitrile methylene chloride hexane
- 4. Activated Carbon: Nuchar C-190N, Cat. No. 5790, Eastman Kodak Company, Rochester, New York 14650.
- 5. Polvethylene Giveor, 400: P-165, Fisher Trientific Company, Fair Lawn, New Jersey, 07410.
- 6. Acetone-PEG: 0.02% PEG in acetone, 200 mcl of polyethylene glycol 400 was added to 1,000 mL of acetone.

### D. Preparation of Standard Solutions

Standard Solutions described below are stable for at least one month if kept tightly capped and refrigerated overnight and during periods when they are not being used; allow the solutions to warm to room temperature before opening. The Stock Solutions are stable for at least three months under the same conditions.

### 1. Stock Solutions

Tare a 50-mL class A volumetric flask with its stopper. Into the flask weigh accurately (to the nearest 0.1 milligram) approximately 50 to 70 milligrams of malathion analytical standard. Fill to the mark with acetone and mix well. Make appropriate dilutions with class A volumetric glassware to yield a stock standard solution containing 1.00 mg/mL malathion. Prepare a 1.00 mg/mL stock standard solution of malaoxon in the same manner.

### 2. Fortification Solutions

Pipet 5-mL aliquots of each of the 1.00 mg/mL stock standard solutions in a single 50-mL volumetric flask, dilute to the mark with acetone and mix well. This solution, designated as Solution A, contains 100 mcg/mL each of malathion and malaoxon.

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Piper a 5-mL aliquot of Solution A into a 50-mL volumetric flask, dilute to the mark with acetone and mix well. This solution, designated Solution B, contains 10 mcg/mL each of malathion and malaoxon.

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Pipet a 1-mL aliquot of Solution A into a 100-mL volumetric flask, dilute to the mark with acctone and mix well. This solution, designated Solution C, contains 1 mcg/mL each of malathion and malaoxon.

# 3. Gas Chromatography Standard Solutions

Pipet 3, 2, and 1-mL aliquots of Solution A into separate 100-mL volumetric flasks and dilute each to the mark with acctone-PEG. Mix well. These solutions, designated Solutions D, E, and F, contain 3.00, 2.00 and 1.00 mcg/mL, respectively, of each compound. Pipet 5, 3, and 2-mL aliquots of Solution B into separate 100-mL volumetric flasks and dilute each to the mark with acctone-PEG. Mix well. These solutions, designated Solutions G. H. and L contain 0.500, 0.300 and 0.200 mcg/mL, respectively, of each compound. Pipet 25 mL of Solution F into a 200-mL volumetric flask and 5 mL of Solution C into a 100-mL volumetric flask; dilute each to the mark with acctone-PEG and mix well. These solutions, designated Solutions J and K, contain 0.125 and 0.050 mcg/mL, respectively, of each compound.

E. <u>Preparation and Conditioning of the Ceromatographic Column</u> (Commercial packed columns may be used provided they are functionally equivalent).

Place a loosely compressed pledget of silanized glass wool in the exit end of the column and attach a funnel to the inlet end by means of a short length of rubber tubing. Pour a small amount of packing into the funnel and tap the column gently to start the flow of packing. Apply gently suction to the exit end of the column and continue tapping the column until the packing is complete. Remove the funnel and vacuum tubing from the column and do not place a pledget of glass wool in the inlet end of the column.

Condition the column in the instrument oven overnight at a temperature about 25°C above the expected operating temperature. In the conditioning step connect the column to the injection port with the normal flow of carrier gas. Do not connect the column to the detector during conditioning. After the conditioning period, connect the column to the detector.

Using as guides the approximate gas chromatographic conditions listed in the next section and the typical chromatograms shown in the attached figure, adjust the instrument to give adequate peak shape, resolution from interfering peaks, and sensitivity such that the maiathion peak is about 20% of full-scale deflection when 5-mol aliquots of Solution J are injected. Usually the new column is ready for maiathion analysis immediately following overnight conditioning mentioned above.

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Malaction, on the other hand, is prone to low sensitivity and poor stability compared to malathion if the following are not done:

- 1. Keep the column inlet free of baked on sample extract. Remove discolored packing and clean the inside of the column with acetone and a pipe cleaner. Add new packing material as needed मक्ति है। ते हैंत, विदेशकेंद्री
- 2. Use acctone with 0.02% PEG as diluent for all standards and samples analyzed by GC. This helps maintain the malaoxon sensitivity when standards are injected. The absence of PEG results in lower sensitivity for standards when compared to malaoxon injected with sample extract.
  - 3. Use an ordered sample/standard sequence Every third injection should be a standard. This maintains long term stability making it possible to use linear

regression.

It is usually necessary to make several injections of Solution A and a processed sample extract to condition the column. This should be done immediately before analyzing extraces. The peak height ratios of malaoxon to malathion can be used to determine whether or not a column is sufficiently conditioned to begin testing for linearity (Section G). A column is well conditioned if the malaoxon to malathion ratio is greater than or equal to 80%. As the column is used the ratio will slowly drop, depending on the amount of sample extract injected into the column. Replacing a few centimeters of packing material at the column inlet and several alternating injections of Solution A and processed sample extract will quickly revive the malaoxon response.

# F. Approximate Gas Chromatographic Conditions

Column Temperature
Inlet Temperature
Detector Temperature
Helium Flow Rate Helium Flow Rate

30 mL/minute

Hydrogen Flow Rate

Air, Flow Rate

100 mL/minute G. Linearity Check of the state of the state

A linearity check must be performed prior to GC analysis which is often included in the standard curve of each set of processed sample extracts. Inject 5-mcL aliquots of at least Solutions G through K (0.050 mcg/mL to 0.500 mcg/mL), or greater to match the high standard to be used on the standard curve during sample analysis. Plot the peak height for each compound versus its concentration to demonstrate linearity of response. Concentration to demonstrate li

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Significant departure from linearity either prior to or during processed sample extracts (a correlation coefficient of less than 0.995) indicates instrumental or operational difficulties which must be corrected before proceeding.

### H. Recovery Test

The ability of the analyst to perform these procedures satisfactorily must be demonstrated by recovery tests before analysis of unknown samples is attempted. In addition, at least one recovery sample must be run concurrently with each batch of samples to demonstrate that the overall operation of the procedure for that batch of samples was satisfactory. Acceptable recovery may range from 70 to 120% with overall average recovery expected to agree with that found during method validation.

Weigh a 20-g portion of untreated sample into a Waring Blender cup and add by pipet an appropriate aliquot of a fortification solution to yield the desired level. For example, a 1-mL aliquot of the 1-mcg/mL standard added to a 20-gm sample will give a fortification level of 0.05 ppm.

Let the sample stand for no more than 5 minutes. Analyze the sample by the procedure described in the following section.

### L Sample Handling Procedure

### 1. Blender Cup Conditioning

Prior to extraction add 80 to 100 mL of acetonitrile to a dry blender cup, blend for two minutes and discard.

### 2. Extraction and Partitioning

Weigh a frozen representative 20-gram portion of the sample into a blender cup. Add 300 mL of acetonitrile and blend for 2 minutes at moderate speed. Filter the mixture with vacuum through a glass-fiber filter paper held in a Buchner funnel. Transfer a 150-mL aliquot of the filtrate to a 250-mL separatory funnel, add 50 mL of hexane, and shake for 1 minute. Allow the phases to separate and draw off the lower phase into a 500-mL evaporation flask. Concentrate the solution to about 1-2 mL of solvent on the rotary evaporator.

### 3. Cleanup

Dissolve the remaining solution in 50 mL of acetone, add 1 g of activaters carbon and swirl. Allow the mixture to stand 30-40 minutes with occasional swirling. With the aid of vacuum, filter the mixture through a glass-fiber filter held in a Buchner funnel. Rinse the flask, filter and funnel with 50 mL of acetone. Collect the acetone solution in an evaporation flask and evaporate to near dryness. Use a gentle stream of N<sub>2</sub> to

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evaporate the solvent just to dryness. Prepare a disposable silica-gel column in the following manner: attach a 10 mL disposable syringe to the column and force 3 mL of a 10% solution of acctone in methylene chloride through the column.

For grass green forage and hay samples, do the following:

Dissolve the residual film in one mL of acetone and mix well. Add nine mL of methylene chloride and mix well. Pass the solution through the column, collecting the cluate in a test tube. Follow with a 4 mL rinse of 10% acetone in methylene chloride. Add the rinse to the sample flask before adding it to the column, and allow most of the sample solution to pass through the column before adding the rinse without letting the column bed go dry. Alternatively, a vacuum box (i.e. Vac-Elut or Baker SPE-10) may be used to draw rinse and cluate through the column as described above. Use a gentle stream of N<sub>2</sub> to evaporate the solvent just to dryness. Dissolve in 4 mL of acetone-PEG for GC analysis.

# J. Gas Chromatographic Analysis

Condition column (Section E, last paragraph) immediately before analyzing extracts. Begin the automated GC set with several standards to determine linearity at the beginning of the run (Section G). Follow the curve with samples and standards arranged on the autosampler tray so that every third injection is a standard. Vary the concentration of subsequent standards injected so that the range of the detector linearity will be demonstrated throughout the run.

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Analyze all samples in duplicate. If the duplicate injections for a given sample differ by more than 10%, analyze the sample again by GC. When duplicate injections differ by more than 10% a second time, either make appropriate adjustments in the operating system or reextract and reanalyze the appropriate sample. If a sample peak height exceeds the peak height of the most concentrated standard in the standard curve, dilute the sample solution with acetone-PEG so that its peak height will fall within the standard curve and reinject; record the dilution factor for use in calculations as described below.

Replace the column packing at the injection-port end of the column whenever the response to malaoxon drops off by 25% or more from the response obtained initially after equilibration of the column as described in Section E.

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### K. Calculations

Calculate the concentration of malathion (CL 6,601) or malation (CL 28,967) as follows:

- (1) Compile the concentrations of all standards injected (independent variable, x-axis) and their corresponding peak heights (dependent variable, y-axis).
- (2) Use a calculator/computer and linear regression to determine the slope, y-intercept, and correlation coefficient of standard concentration versus peak height. Back-calculate analyte concentrations using the following equation:

$$ppm = \frac{R (samp) - b}{m} \times \frac{V1 \times V3 \times V5 \times DF}{W \times V2 \times V4}$$

Where:

R (Samp) = peak height of sample

b = y-intercept of the linear regression line

m = slope of the linear regression line

W = Weight of sample taken for analysis in grams

V1 = volume of extracting solvent (in mls)

V2 = volume of extract taken for analysis (in mLs)

V3 = volume of acetone-PEG added to dissolve final residues for chromatographic analysis (in mLs)

V4 = volume of sample solution injected (in mcL)

V5 = volume of standard solution injected (in mcL)

DF = dilution factor (DF = 1 if additional dilution is not needed in Section J)

NOTE: The Computer Aided Laboratory System (CALS) automatically calculates the slope, y-intercept, correlation coefficient, and plots all pertinent standards and the linear regressed line. From the linear regressed line, concentrations of sample residues (mcg/g) are automatically interpolated. All data entered into the computer to accomplish this task are presented with their corresponding curves in each report.

CALS groups V3 and DF into a multiplier named Std.(RRF). Likewise, V1, V5, W. V2, and V4 are grouped into a divisor named Smp(RRT). These are entered by the analyst and are printed out as part of the Schedule File. All Schedule Files are presented in each report.

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### FIGURE 1

Chemical Structures of Malathion and Malaoxon .

### Malathion

Sucinic acid, mercapto-diethyl ester, S-ester with 0.0-dimethyl phosphorodithiate  $^{\rm C_{10}B_{19}O_6PS_2}$ 

$$(CH_3O)_2 P - S - CH - C - O - C_2H_5$$

$$CH_2 - C - O - C_2H_5$$

### Malaoxon

Butanedioic acid, (dimethoxyphosphinyl)thio)-diethyl ester  $C_{10}B_{19}C_{6}PS$