

Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pesticide Name: Malathion

MRID #: 417277-01

Matrix: Soil

Analysis: GC/FPD

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1961

STATE OF NEW YORK

IN SENATE

JANUARY 10, 1961

REPORT

OF THE

(A) The following is a list of the members of the Board of Regents of the State University of New York, as of January 10, 1961. The Board of Regents is composed of the Governor, the Lieutenant Governor, and the members of the Board of Regents of the State University of New York, as of January 10, 1961. The Board of Regents is composed of the Governor, the Lieutenant Governor, and the members of the Board of Regents of the State University of New York, as of January 10, 1961.

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Analytical MethodologyExtraction

With a few modifications, the methodology used for the analysis of malathion and malaoxon in the soil samples was the procedure supplied by American Cyanamid Company titled "GC Method for the Determination of Malathion (CL6,601) and Malaoxon (CL28,967) Residues in Grasses (including tall fescue, bermunda and bluegrass) When Using Continuous Automated Sample Injections."

Malathion and malaoxon were extracted from the soil as follows:

- 1) Twenty grams of soil were weighed into a 16 oz. screw cap glass jar. Two hundred and fifty ml of acetonitrile were added and the jar covered with an aluminum foil lined cap. The mixture was then shaken at medium speed for 30 minutes on a reciprocal shaker.
- 2) The sample mixture was filtered through glass fiber filter paper in a Buchner funnel. The glass jar was rinsed with 50 ml of acetonitrile and this was poured over the filter paper cake. The 300 ml of acetonitrile were collected in a 500 ml flat bottom flask.
- 3) One hundred and fifty ml of the sample extract were transferred to a 250 ml separatory funnel. Fifty ml of hexane were added and the solution was shaken by hand for 1 minute. The phases were allowed to separate. The acetonitrile was then drained through MeCl_2 /hexane/acetone rinsed sodium sulfate contained in a powder funnel and collected in a 500 ml flat bottom flask. This was evaporated to dryness on a rotary evaporator under partial vacuum with a water bath temperature of approximately 40°C .
- 4) The sample residue was dissolved in 1 ml of acetone and then mixed well with 9 ml of methylene chloride. A disposable silica gel column (SPE) was prepared by rinsing with 3 ml of 10% acetone in methylene chloride. The sample was passed through the column and collected in a screw top glass test tube. The 500 ml flat bottom flask was rinsed with 4 ml of 10% acetone in methylene chloride and this was also passed through the column and collected in the same glass test tube.
- 5) The samples were blown to dryness under a stream of nitrogen, followed by dilution with appropriate volumes of acetone/PEG for GC analysis.

Instrumentation

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:

Percent soil moisture was calculated by the equation:

% soil moisture =

$$\frac{\left(\begin{array}{l} \text{Wet Weight of Soil} \\ \text{and Container (g)} \end{array} - \begin{array}{l} \text{Dry Weight of Soil} \\ \text{and Container (g)} \end{array} \right)}{\left(\begin{array}{l} \text{Wet Weight of Soil} \\ \text{and Container (g)} \end{array} - \begin{array}{l} \text{Container Weight (g)} \end{array} \right)} \times 100$$

Sample Preparation

At the facilities of ABC Laboratories, soil cores from the crop plots were divided into increments of 6-12", 12-18", 18-24", 24-30" and 30-36" through Day 28 post application and 0-6", 6-12", 12-18", 18-24", 24-30" and 30-36" for sampling dates after Day 28. The 0-6" layer through Day 28 post application was hand excavated and composited in the field; no preparation was needed. Soil cores from the bareground plot were divided into increments of 0-6" and 6-12". The five cores from each subplot were composited by depth into a single sample for analysis. This resulted in three replicate samples (A, B and C) from the treated plots and one sample from the nontreated control plot for each depth at each sampling date.

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A Quaker Mill, a type of grist mill, was used to finely grind and homogenize the sample. Dry ice was passed through the mill to cool it for sample processing. The sample was then run through the mill in the presence of enough dry ice to keep the sample frozen. The rotating plate of the mill was set to allow the maximum gap between rotating and stationary plates. For two successive runs the gap was reduced until on the third and final grind, the gap had been reduced to its smallest opening. Samples were continuously stirred and mixed during grinding. After the final grind the samples were placed in pre-labeled plastic containers and were allowed to sublimate in a small freezer prior to being returned to the walk-in freezer. The samples were labeled as follows:

Example: T1B CA T C 0-6" A
(1) (2) (3) (4) (5) (6)

- (1) Sample Date: PRE, T1B, T1A, T2A, T3A, T4A, T5A, T6A, 1, 3, 7, 14, 28, 60, 90, 120, 150, 180, 210, 270, 330, 390, 450 and 540
- (2) Location: CA=California
- (3) Treatment: T=Treated, U=Nontreated
- (4) Plot: C=Crop, BG=Bareground
- (5) Depth: 0-6", 6-12", 12-18", 18-24", 24-30" and 30-36"
- (6) Replicate: A, B and C

Dates pertaining to the sampling, shipping, receiving, freezing, preparation, extraction and days stored for each sample are listed in Appendix III.

Column: 15 m X 0.53 mm DB1 with a 1.5 μ film thickness
Injector Temp: 200°C
Detector Temp: 240°C
Flow Rate: Hydrogen: 75 ml/minute
Air: 100 ml/minute
Helium: ca: 20 ml/minute
Nitrogen: ca: 20 ml/minute

Temperatures: Program
Initial Temp.: 170°C
Initial Hold: 6 minutes
Rate: 10°C per minute
Final Temp.: 185°C
Final Hold: 1.5 minutes
Injection Volume: 2 λ

Peak height values were obtained with the CALS® system.

Precise records of instrument parameters are contained in each analytical data set in ABC Raw Data Report #38003.

Data Acquisition and Calculations

Peak height counts were obtained using the CALS system. Use of this system is a multi-step process involving the use of four method files. The process of collecting and recording data resulting from a sample injection is carried out according to a set of instructions referred to as the acquisition method. The Acquisition Method (AC) specifies the number of readings/seconds recorded, the signal discrimination level in microvolts and the start and termination times of data acquisition. A GC method can also be specified if analysis is to occur during the acquisition of data.

The information contained in the Schedule File includes the name of the raw data file to be collected, the sample title, the standard (RRF), the sample (RRT), and the number of the AC method which controls data acquisition. The values in the Standard (RRF) and Sample (RRT) columns are multiplication and division factors respectively. The concentration (ng/ml or μ g/ml) value calculated by the computer from the calibration curve is multiplied by the value in the Standard (RRF) column and divided by the value in the Sample (RRT) column. The Standard (RRF) for these analysis is equivalent to the dilution factor for the sample and the Sample (RRT) is a division factor.

After acquisition of the data through the Schedule File and the Acquisition Method, analysis occurs via the General Chromatography (GC) Analysis Method and the scheduling of the program CURVE.

The data acquired from the gas chromatograph are analyzed according to a set of instructions referred to as the General

Chromatography (GC) method. The GC method is a set of directives defining analysis conditions, the time sequence of events during a run (including its termination), and the list of compounds to be named in the analysis report (with their retention times, response factors and calibration compositions). In addition, the information to draw baselines on the chromatograms and calculate peak areas (or heights) is performed through the use of the GC method. A brief description of a GC method used in this analysis and coded values of interest is presented below.

Line #1 Initiates chromatogram analysis start at a specified time (minutes).

Line #2 Fixes the baseline at 0 a specified time (minutes).

Line #3 Resets autobase time threshold (dT) to a specified value, in minutes and hundredths. This defines criteria for automatically establishing the baseline within a chromatogram.

Line #4 Resets autobase amplitude threshold (dH) to a specified value, in microvolts. This defines criteria for peak detection and establishment of baseline.

If the chromatogram remains within dH for at least dT, then that section is considered to be at baseline. The corresponding peak start or end is flagged as starting or ending on baseline. If the chromatogram rises by more than dH above baseline, the peak start is detected. If it crests and then falls by more than dH then a peak is detected. If it falls and then rises by dH without establishing baseline; then peak termination at a valley is flagged. It was experimentally determined that these values optimized peak detection and recording.

Line #5 Terminates chromatograms analysis at a specified time (minutes).

Line #13 Designates the calibration file name.

Line #17 Designates concentration units.

Line #19 & #20 Chromatographic peak identification and the "window" time frame for which peak and baseline characteristics are determined for the metabolite (#19) and compound (#20).

The Multi-point Calibration program, CURVE, computes the calibration curve and returns the results of the unknown samples for the completion of the analysis. This program allows the use of linear regression or multi-point polynomial with equal or nonequal weighting. For these analyses, linear regression with equal weighting was used.

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This unknown value was, in the case of the soil samples, then converted to parts per billion in the CALS® program by entering the sample weight in grams and the final dilution volume in ml as follows:

$$\text{found ppb residue} = \frac{\text{ng/ml detected} \times \text{final volume (ml)}}{\text{sample weight (g)}}$$

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

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

Recoveries from fortified samples were determined by the formula:

$$\% \text{ Recovery} = \frac{\text{ppm residue} - \text{average ppm residue in control}}{\text{ppm residue added}} \times 100$$

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

$$\text{corrected ppm, wet basis} = \frac{\text{found ppm residue}}{\text{average \% recovery for the individual compound}} \times 100$$

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 samples was also corrected for moisture content as follows:

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

The methods of analysis used to determine malathion and malaoxon residues in the spray solution aliquots and filter papers are 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 acetone and re-weighing 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 another 100 times with 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. PPM of malathion and malaoxon were calculated by the CALS® program using the following formula:

$$\text{ppm} = \frac{\text{ng/ml detected} \times \text{final volume (ml)}}{\text{sample weight (g)}}$$

The ppm value found for each sample was entered into LOTUS®, converted to pounds of active ingredient per gallon and reported as a percent of the theoretical concentration.

The filter papers were placed in a powder funnel and repeatedly rinsed with acetone. The acetone was collected in a 250 ml volumetric flask. Samples were brought to volume in acetone. Appropriate dilutions, in acetone/PEG, were made and analyzed by GC. Peak heights were obtained using the CALS® system. Values were calculated from peak heights by the CALS® program. Total µg per filter paper of malathion and malaoxon were calculated by the CALS® program using the following formula:

$$\text{Total } \mu\text{g} = \mu\text{g/ml detected} \times \text{final volume (ml)}$$

The total µg value for each filter paper was entered into a LOTUS® worksheet, converted to pounds of active ingredient per acre and reported as a percent of the theoretical application.

Approximately 50 soil samples were reanalyzed due to the occurrence of a peak in close proximity of malaoxon. At certain levels this peak would have masked the detection of malaoxon. The samples were re-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 X 0.25 mm i.d.
0.25 µ film thickness

Injection Temp: 240°C
Detection Temp: 240°C

Flow Rate: N₂ = 40 ml/min He = ca 2.5 ml/min
H₂ = 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

Sample values were not quantitated. Chromatograms are included only as proof that no quantifiable malaoxon existed in the original sample runs.

RESULTS AND DISCUSSION

Study Weather

Applications of the malathion were performed as scheduled. Weather conditions at the six applications were excellent (Table 2). Weather conditions were normal through Day 63 (September 26, 1989) of the study.

with the study average high temperature through Day 63 of 91°F and the ten year average of 95°F. The study average (through Day 63) low temperature was 57°F and the ten year average low temperature was 64°F. Total irrigation and rainfall through sampling Day 63 after the initial application was 9.65 inches.

Method Validation ←

The recovery of malathion and malaoxon from California soil was validated at three fortification levels. Duplicate extractions were made from control soil and control soil fortified at 0.01, 0.5 and 5.00 ppm malathion/malaoxon. Results of the method validation are given in Table 6. An unacceptable recovery of 140% was obtained from one extraction at the 0.01 ppm level. This level was not re-validated but recoveries obtained for the 0.01 ppm level analyzed throughout the study indicated that this was an acceptable detection limit for this method. Copies of the method validation analytical raw data are included in ABC Raw Data Report #38003. The original raw data is included in ABC Raw Data Report #38004 titled "Combined Aquatic Field Dissipation and Accumulation Study on Irrigated Crops for Malathion".

Application Verification

Table 7 presents the results from the spray solution sample analyses. To determine the expected concentration (mg/ml) of the application samples the following calculations were required:

$$\frac{9.33 \text{ lbs ai}}{\text{gal formulation}} \times \frac{453.6 \text{ g}}{\text{lb}} \times \frac{1 \text{ gal}}{3785 \text{ ml}} = 1.12 \text{ g ai/ml}$$

Spray solution (example: Application 1)

$$\frac{325.7 \text{ g (malathion formulation)}}{\text{tank mix}} \times 1.12 \text{ g ai/ml} = 331.2 \text{ g ai}$$

$$\frac{330.6 \text{ g ai}}{25 \text{ gal H}_2\text{O}} = 13.26 \text{ g ai/gal}$$

$$\frac{13.26 \text{ g ai}}{\text{gal}} \times \frac{1 \text{ gal}}{3785 \text{ ml}} = 0.00350 \text{ g ai/ml}$$

$$= 3.50 \text{ mg ai/ml}$$

Also included in Table 7 are the associated sample identifications and significant dates. The average percent of the theoretical concentration for the six applications ranged from 62% to 116%. The overall average of percent theoretical for all applications was 90% with a standard deviation of 19.0%.

The results from the application measurement analyses are presented in Tables 8 and 9. To calculate the expected amount of malathion (mg/sample) required in an application measurement sample (filter paper) to obtain 1.16 lb ai/acre (16 ounces/acre), the following calculations were required:

analyzed, uncorrected values in ppm, average percent procedural recovery for the fortification samples analyzed concurrently with any given sample, the average percent moisture determined for each sample, and a "corrected for procedural recovery" ppm value on a dry weight basis. Replicate analyses were performed on some samples, in which case more than one value for a given sample may be listed. The sample replicate average and the average of the three sub-plots (A, B and C) are also included. Samples were analyzed for both malathion and malaoxon through Day 28 following the final application to a depth of 24 inches.

Malathion

The results from the analysis of soil samples from the treated crop plot for malathion are presented in Table 11. In the treated crop plot 0-6" layer, malathion residues first appeared in samples taken immediately after the initial application (TIA) at an average concentration of 0.055 ppm. Average residue concentrations found in samples taken after the second and third applications were 0.072 ppm and 0.13 ppm, respectively. Samples taken after the fourth and fifth applications contained 0.11 and 0.13 ppm, respectively. Samples taken after the sixth application had an average concentration of 0.082 ppm. On Day 1 following the final application, the average residue concentration was 0.14 ppm. By Day 3 following the final application residues had dissipated to below the 0.01 ppm limit of detection.

A half-life calculation could not be performed for the dissipation of malathion in the treated crop plot 0-6" layer. Graph 1 depicts the average residue concentration vs. time of malathion detected in the treated crop plot 0-6" layer.

Malathion residues were first detected in 6-12" soil samples taken after the initial application (TIA) at an average concentration of 0.064 ppm. Malathion residues were above the 0.01 ppm limit of detection in samples taken after the five subsequent applications at average concentrations of 0.047, 0.14, 0.072 and 0.066 ppm, respectively. On Day 1 following the final application the average concentration of malathion was 0.13 ppm. On Day 7 malathion was above the limit of detection in only one replicate at 0.13 ppm. No residues were found at or above limit of detection in 6-12" soil samples after Day 7.

Malathion residues were not found at or above the limit of detection in any of the 12-18" soil samples. Malathion residues were found in 18-24" soil samples taken after the second application at an average concentration of 0.023 ppm. No residues were found at or above the limit of detection on any subsequent sampling dates.

The results from the analysis of soil samples from the treated bareground plot are presented in Table 12. In the treated bareground plot 0-6" samples residues first appeared in samples taken immediately after the initial application (TIA) at an average concentration of 0.088 ppm. Average residue concentrations detected in samples taken after the second and third applications were 0.037 ppm and 0.087 ppm, respectively. Samples taken after the fourth and fifth applications

$$\begin{aligned} \text{Area} &= (3.1416)r^2 \\ &= 3.1416 \times (0.246 \text{ ft})^2 \\ &= 0.1902 \text{ ft}^2 \end{aligned}$$

$$1.16 \text{ lb ai} \times 453.6 \text{ g/lb} = 526.2 \text{ g ai}$$

$$1.16 \text{ lb ai malathion/A} = 526.2 \text{ g a.i./43,560 ft}^2$$

$$\frac{526.2 \text{ g ai}}{43,560 \text{ ft}^2} = \frac{Xg}{0.1902 \text{ ft}^2}$$

$$\begin{aligned} X &= 0.00230 \text{ g/filter paper} \\ &= 2.30 \text{ mg/filter paper} \end{aligned}$$

Included in Table 8 are the averages of the fifteen samples taken from the treated crop plot at each application. The averages for applications 1, 2, 3, 4, 5 and 6 are 0.58, 0.75, 0.68, 0.89, 0.67 and 0.32 lb ai/acre, respectively. These values represent an average percent of the theoretical rate applied of 50%, 64%, 58%, 76%, 57% and 27%, respectively, with an overall average value of 55 ± 19%.

Included in Table 9 are the averages of the fifteen samples taken from the treated bareground plot at each application. The averages for applications 1, 2, 3, 4, 5 and 6 are 0.70, 0.69, 1.0, 0.95, 0.76 and 0.56 lb ai/acre, respectively. These values represent an average percent of the theoretical rate applied of 60%, 59%, 85%, 91%, 65% and 48%, respectively, with an overall average value of 66 ± 29%.

Fortified Control Samples ←

Recovery from fortified samples processed and analyzed concurrently with field samples for malathion and malaaxon are reported in Table 10. The overall average percent recovery and the standard deviation for each fortification level are also included. Control soil samples were fortified at levels of 0.01 ppm, 0.10 ppm and 1.0 ppm for both malathion and malaaxon. Overall average percent recovery for malathion was 98 ± 7%, while malaaxon overall average percent recovery was 93 ± 7%. These data indicate good recovery through the extraction and silica gel cleanup procedure.

Authentic Field Samples

Table 11 and 12 presents the results from the analysis of soil samples from the treated, and bareground plots, respectively, for malathion and malaaxon. Table 13 lists the results from the analysis of samples from the control plot. Included in the tables are the sample I.D.'s, the assigned analytical laboratory numbers for each sample

contained 0.047 ppm and 0.062 ppm, respectively. Samples taken after the sixth application had an average concentration of 0.067 ppm. On Day 1 following the final application, the average residue concentration was 0.11 ppm. By Day 3 following the final application residues had dissipated to below the 0.01 ppm limit of detection.

A half-life calculation could not be performed for the dissipation of malathion in the treated bareground 0-6" layer. Graph 2 depicts the average residue concentration of malathion detected in the treated bareground plot 0-6" layer vs. time. No residues were detected in any samples analyzed from any subsequent sampling dates or the 6-12" depths, with the exception of the T5A 6-12" soil samples which contained an average malathion concentration of 0.0044 ppm.

Malaoxon

Table 11 and 12 also present the results from the analysis of soil samples from the treated and bareground plots, respectively, for malaoxon. No residues of malaoxon were detected at or above the 0.01 ppm limit of detection in any sample analyzed.

Control Samples

Table 13 lists the results of the analysis of the soil samples from the control plot. No residues were detected at or above the 0.01 ppm limit of detection for malathion or malaoxon in any sample analyzed.

GC/Mass Spec Confirmation ←

Four soil extracts were submitted for GC/Mass Spectroscopy confirmation of malathion and malaoxon. One standard containing both malathion and malaoxon at 10 µg/ml in acetone was also injected. No malathion or malaoxon were observed above the detection limit (~0.2 µg/ml) in the control sample (38003-8000). Both malathion and malaoxon were found in each spiked extract. The mass spectra of ABC Sample Nos. 38003-8002 and 38003-8003 demonstrate that the compound identified as malathion by GC is malathion. No malaoxon was observed above the detection limit (~0.3 µg/ml). However, a very small peak (~0.113 µg/ml) can be observed in the ion chromatogram of the sample (38003-8003) with m/e 127, and RT 7.532 min. This peak is very close to the peak of the malaoxon standard (RT 7.439 min). The ion was not there in sufficient abundance for positive identification. All raw data may be found in ABC Raw Data Report #38003.

Stability Studies

All samples received at ABC Laboratories were stored in a freezer at -20°C. Samples were maintained frozen except when an aliquot for analysis was removed. After removal of the material for analysis the sample was returned to the freezer.

Analytical raw data for field spikes and freezer stability spike samples are included in the ABC Raw Data Report #38003.

TABLE 1

Soil Characteristics for the Terrestrial Field Dissipation
for Malathion - Crop Bareground Application Study

	Soil Depth (inches)			
	0-12	12-24	24-36	36-48
% Organic Matter	0.6	0.4	0.3	0.2
pH	6.1	6.5	6.9	7.1
CEC (meq/100 g)	3.0	2.9	3.1	4.2
Water Holding Capacity (%)	8.19	7.60	7.52	9.90
Bulk Density (g/cc)	1.40	1.39	1.42	1.39
% Sand	67	69	71	63
% Sil-	24	23	19	27
% Clay	9	8	10	10
Texture	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Loam

TABLE I

(a) *Staphylococcus aureus* (ATCC 12228) on 10% tryptic soy agar
 (b) *Staphylococcus aureus* (ATCC 12228) on 10% tryptic soy agar
 (c) *Staphylococcus aureus* (ATCC 12228) on 10% tryptic soy agar

Time (hr)	Optical Density (600 nm)	Optical Density (700 nm)	Optical Density (800 nm)	Notes
0	0.00	0.00	0.00	Initial inoculum
1	0.10	0.08	0.06	
2	0.20	0.16	0.12	
3	0.30	0.24	0.18	(a) <i>Staphylococcus aureus</i> (ATCC 12228)
4	0.40	0.32	0.24	(b) <i>Staphylococcus aureus</i> (ATCC 12228)
5	0.50	0.40	0.30	(c) <i>Staphylococcus aureus</i> (ATCC 12228)
6	0.60	0.48	0.36	
7	0.70	0.56	0.42	
8	0.80	0.64	0.48	
9	0.90	0.72	0.54	
10	1.00	0.80	0.60	
11	1.10	0.88	0.66	



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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 tall 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 0.05 ppm for each compound.

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ABC LABS # 8003

PG 0161

Attachment 1

ABC LABS #C 38003

pg 0160

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

1. Gas Chromatograph: Tracor Model 540 equipped with a flame photometric detector.
2. Waring Blender: Model 31BL46 with 1-quart capacity glass blender jar (Waring Products Division, Dynamics Corporation of America, New Hartford, Connecticut).
3. Balance: Analytical, Mettler H35AR, 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 Syringe: Hamilton #701-N, 10-mL 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 glass, 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. Mini-Column Vacuum Manifold: 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. 609-799-0462
- a. Malathion: phosphorodithioic acid, S-[1,2-bis (ethoxycarbonyl) ethyl] 0,0-dimethyldithiophosphate.

- 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, Bellefonte, Pennsylvania 16823-0048.
 3. Solvents, High Purity: B & J Brand, Baxter, Burdick and Jackson, Incorporated, McGaw Park, Illinois 60085:
acetone
acetonitrile
methylene chloride
hexane
 4. Activated Carbon: Nuchar C-130N, Cat. No. 5790, Eastman Kodak Company, Rochester, New York 14650.
 5. Polyethylene Glycol, 400: P-165, Fisher Scientific Company, Fair Lawn, New Jersey, 07410.
 6. Acetone-PEG: 0.02% PEG in acetone, 200 ml 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.

Pipet 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.

Pipet a 1-mL aliquot of Solution A into a 100-mL volumetric flask, dilute to the mark with acetone 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 acetone-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 acetone-PEG. Mix well. These solutions, designated Solutions G, H, and I, 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 acetone-PEG and mix well. These solutions, designated Solutions J and K, contain 0.125 and 0.050 mcg/mL, respectively, of each compound.

E. Preparation and Conditioning of the Chromatographic Column (Commercial packed columns may be used provided they are functionally equivalent).

Place a loosely compressed pledget of sterilized 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 malathion peak is about 20% of full-scale deflection when 5-mL aliquots of Solution J are injected. Usually the new column is ready for malathion analysis immediately following overnight conditioning mentioned above.

Malaoxon, 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.
2. Use acetone 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 extracts. 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	190° C
Inlet Temperature	250° C
Detector Temperature	275° C
Helium Flow Rate	30 mL/minute
Hydrogen Flow Rate	100 mL/minute
Air Flow Rate	150 mL/minute

G. Linearity Check

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-mL 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.

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.

I. 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 activated 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 N₂ to

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 acetone 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 eluate 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 eluate through the column as described above. Use a gentle stream of N_2 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.

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.

K. Calculations

Calculate the concentration of malathion (CL 6,601) or malathion (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:

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

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 mL)

V5 = volume of standard solution injected (in mL)

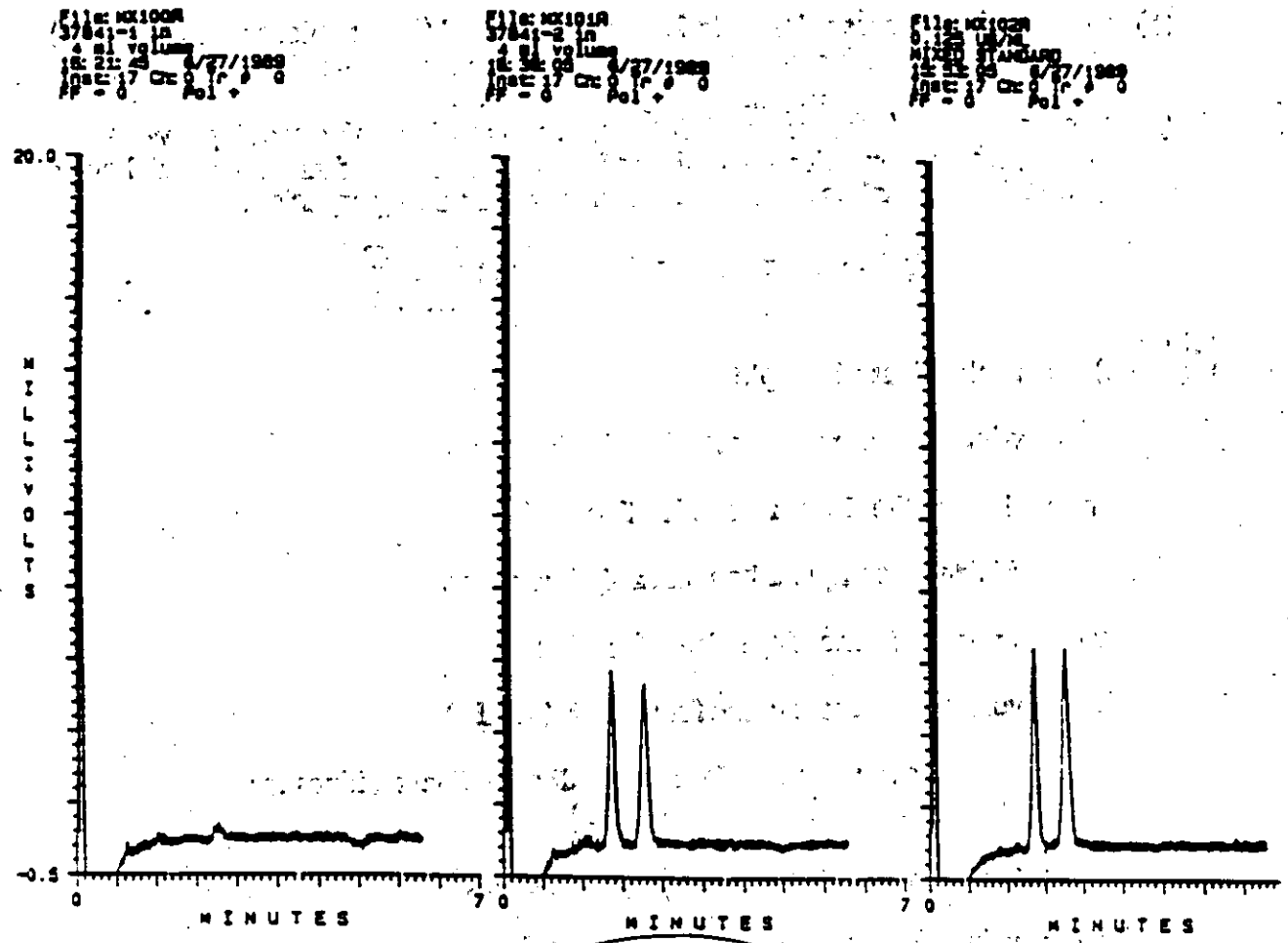
DF = dilution factor (DF = 1 if additional dilution is not needed in Section J)

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 M-1923.A: Typical Chromatograms



File: MX100R
57841-1 in
4 ml vol
8: 21: 23 8/27/1989
17 0 0
AP: 0 Pol

File: MX101R
57841-2 in
4 ml vol
8: 21: 23 8/27/1989
17 0 0
AP: 0 Pol

File: MX102R
57841-3 in
4 ml vol
8: 21: 23 8/27/1989
17 0 0
AP: 0 Pol

Tall Fescue Forage-
Control

Tall Fescue Forage
+0.05 ppm Fortification
Injection equivalent to
12.5 µg of Tall Fescue Forage

0.125 mcg/mL Mixed
Standard