

Appendix to Report EH 92-06

A TEST OF PROCEDURES FOR DETERMINING THE GROUND WATER PROTECTION LIST

By
Bruce R. Johnson, Chris Collison, Joey Marade and Nancy Miller

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ENVIRONMENTAL HAZARDS ASSESSMENT PROGRAM

STATE OF CALIFORNIA
Environmental Protection Agency
Department of Pesticide Regulation
Environmental Monitoring and Pest Management Branch
1220 N Street, Sacramento, California 95814

EH 92-07

CALIFORNIA DEPT. OF FOOD & AGRIC.
CHEMISTRY LABORATORY SERVICES
ENVIRONMENTAL MONITORING SECTION
2002 Mendocino Dr. #1
Sacramento, CA 95832
(916)+427-4999

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Supercedes: New
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ANALYSIS OF ANTOR, ALACHLOR, METALAXYL
AND METOLACHLOR IN WELL WATER

SCOPE:

This method is for the analysis of antor, alachlor, metalaxyl and metolachlor in well water.

PRINCIPLE:

The water sample is passed through a C18 Sep-Pak™ cartridge. The retained compounds, alachlor and metolachlor, are eluted from the cartridge with ethyl acetate. The eluant is then concentrated and analyzed by gas chromatography with electron capture detection.

The detection limit of the method is 0.1 ppb.

REAGENTS AND EQUIPMENT:

1. Methanol, pesticide grade
2. Ethyl acetate, pesticide grade
3. Sodium sulfate, anhydrous, granular
4. Individual stock standard solutions (1mg/mL): Dissolve 50 mg of each herbicide in acetone and dilute to 50 mL in a volumetric flask.
5. The C18 Sep-Pak™ cartridge, Waters Division of Millipore
6. Vortex mixer, Thermolyne Maxi Mix II, Syborn
7. Centrifuge, Clay-Adams
8. Nitrogen evaporator, Organomation Model #12
9. In-house vacuum manifold: consists of a 4 L side arm filtering flask with a #13 rubber stopper. Eight 4 mm holes are distributed evenly throughout the stopper. Eight 4", 4 mm O.D. glass tubes are inserted into the holes. The glass tubes are then connected to 3 ft, 4 mm I.D. teflon tubing. Another 4" glass tube with tapered ends is connected to the teflon tubing. A vacuum source is connected to the side arm with an in-line flow controller in between. The Sep-Paks are connected to the tapered glass tubing.

ANALYSIS:

1. Weigh 1000 g of well mixed water sample (pH = 5~8) into a 1 L beaker.
2. Connect a C18 Sep-Pac™ cartridge to the in-house vacuum manifold described above.
3. Condition the cartridge by aspirating 5 mL of methanol followed by 10 mL of distilled water through the cartridge. Do not allow the cartridge to run dry. Adjust the flow rate to about 15 mL/minute.
4. Pass the 1000 g water sample through the cartridge by dipping the Sep-Pak into the 1 L beaker. After sample has passed through, allow the vacuum to remain on for 3 minutes.
5. Remove the cartridge from the glass tubing and place in a centrifuge tube.

Centrifuge for 3 minutes at 1200 rpm.

6. Elute the cartridge with 4 mL of ethyl acetate and collect the eluant in a test tube. A small amount of residual water in the eluant is removed by adding 3 g of anhydrous sodium sulfate. Vortex the tube for about 20 seconds.
7. Transfer the dry eluant into a 15 mL graduated test tube. Wash sodium sulfate with 2 mL ethyl acetate and also transfer the solvent into the same test tube.
8. Concentrate the eluant to a final volume of one mL under a gentle stream of nitrogen with no heat applied.

EQUIPMENT CONDITIONS:

PRIMARY ANALYSIS:

Instrument: Varian 3700 GC with Electron Capture Detector
Column: HP-1, methyl silicone gum, 10 m x 0.53 mm x 2.65 um.
Injector temperature: 210°C
Detector temperature: 300°C
Temperature program: 165°C for 1 min. increase at 5°C/min, to 220°C for 4 min.
Carrier gas flow rate: Helium, 20 mL/min.
Sample injected vol.: 2 uL
Retention time: Alachlor - 4.40 ± 0.05 min.
Antor - 8.03 ± 0.05 min.
Metolachlor - 5.34 ± 0.05 min.

CONFIRMATION ANALYSIS:

Instrument: Varian 3700 GC with Thermionic Specific Detector
Column: HP-5, 5% phenyl methyl silicone, 10 m x 0.53 mm x 2.65 um.
Injector temperature: 220°C
Detector temperature: 250°C
Temperature program: 175°C for 1 min, increase at 10°C/min, to 220°C for 4 min.
Carrier gas flow rate: Helium, 20 mL/min
Sample inject vol.: 2 uL
Retention time: Antor - 5.08 ± 0.06 min.
Alachlor - 3.20 ± 0.06 min.
Metalaxyl - 3.26 ± 0.06 min.
Metolachlor - 3.72 ± 0.06 min.

Instrument: varian 6000 GC with Thermionic Specific Detector
Column: DB-210 (50% tri-fluoropropyl methyl polysiloxane) 15 m x 0.537
x 1.0 um

Injector temperature: 210°C
Detector temperature: 250°C
Temperature: isothermal 170°C
Carrier gas flow rate: Helium, 17 mL/min
Sample inject vol.: 2uL
Retention time: Alachlor - 2.44 ± 0.08 min.
Metalaxyl - 3.14 ± 0.08 min.

CALCULATIONS:

$$\text{ppb} = \frac{(\text{sample peak height})(\text{ng standard injected})(\text{ml sample final volume})1000}{(\text{standard peak height})(\text{uL sample injected})(\text{sample weight})}$$

RECOVERIES:

* Recoveries of Alachlor

Spike Levels	* Recovery (mean)	Standard Deviation	n
0.2 ppb	106	4.0	5
0.5 ppb	98.3	2.3	5
1.0 ppb	95.1	2.9	5

* Recoveries of Metolachlor

Spike Levels	* Recovery (mean)	Standard Deviation	n
0.2 ppb	95.8	6.5	5
0.5 ppb	100	3.1	5
1.0 ppb	99.9	4.1	5

The spike recoveries for alachlor and metolachlor were obtained by the above method for five consecutive days.

* Recoveries of Antor

Spike Levels	* Recovery (mean)	n
0.2 ppb	98.0	2
1.0 ppb	96.0	2

* Recoveries of Metalaxyl

Spike Levels	* Recovery (mean)	n
0.2 ppb	104	2
1.0 ppb	94.0	2

DISCUSSION:

Two cartridges in series were used to trap the analytes. However, it was found that all the analytes remained in the first cartridge. No analytes were found in the second cartridge. Even when two liters of spiked solution were passed through the cartridge, there was no significant reduction of spike recovery.

The same spike recoveries were obtained using acetone to elute the cartridge for five consecutive trials. However, the residual water in the eluant could not be removed from acetone by adding the sodium sulfate. Thus, ethyl acetate was used in this method.

In this study, it was shown that the analytes could be trapped in the

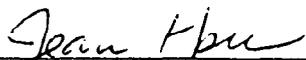
cartridge completely when the pH values of water samples were 5 to 8.

The validation for antor and metalaxyl has been partially completed. Due to time constraints, further validation will be completed when scheduling allows.

REFERENCES:

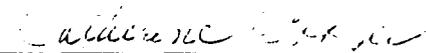
Shibamoto, T. S. and C. R. Mourer, "Report of Method for Analysis of Antor in Water to 0.1 ppb," Trace Analytical Laboratory, Department of Environment Toxicology, University of California, Davis. Jan. 1990.

WRITTEN BY: Jean Hsu



TITLE: Agricultural Chemist I

REVIEWED BY: Catherine Cooper



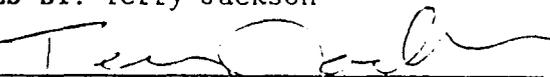
TITLE: Agricultural Chemist III

APPROVED BY: S. Mark Lee



TITLE: Research Agricultural Chemist

APPROVED BY: Terry Jackson



TITLE: Quality Assurance Officer

CALIFORNIA DEPT. OF FOOD AND AGRICULTURE
CHEMISTRY LABORATORY SERVICES
ENVIRONMENTAL MONITORING SECTION
3292 Meadowview Road
Sacramento, CA 95832
(916)-427-4999

Original Date: 5-21-91
Supercedes: NEW
Current Date: 5-21-91
Method #:

DETERMINATION OF DICOFOL FROM WELL WATER

SCOPE:

This method is for the determination of Dicofol from well water.

PRINCIPLE:

Water samples are extracted with methylene chloride. The extracts are rotoevaporated, exchanged into ethyl acetate, concentrated to 2 ml under nitrogen, and analyzed by gas chromatography using electron capture detection.

REAGENTS AND EQUIPMENT:

1. Reagents:

Ethyl acetate, nanograde (checked for chromatographic interferences)
Methylene chloride, pesticide grade
Na₂SO₄, anhydrous

2. Equipment, glassware, and supplies:

Rotary Evaporator, Buchi/Brinkman R110
Nitrogen evaporator (Organomation Model #12)
Separatory funnels, 2 L capacity
Glass wool
Glass filter funnels
Flat-bottom boiling flasks, 500 mL capacity
Graduated conical glass tubes, 15 mL capacity

ANALYSIS:

1. Weigh sample bottles and record each weight before and after transfer of sample. Note whether the sample had been properly adjusted to acidic pH when the sample was taken.
2. Transfer 500 g sample to 2 L separatory funnel.
3. Extract 3 times each with 100 mL methylene chloride as follows.
 - a. Shake gently for 1 min., venting adequately to avoid pressure build-up.
 - b. Allow aqueous and organic layers to separate. Drain methylene chloride layer through glass filter funnel that has been

- plugged with glass wool and filled with anhydrous Na_2SO_4 .
 Collect methylene chloride layers into a 500 mL boiling flask.
- c. After draining methylene chloride layers, wash sodium sulfate with 30 mL methylene chloride, adding to the extract in the boiling flask.
 4. Evaporate samples just to dryness with the rotary evaporator (water temp. - 35° C.) Transfer quantitatively to conical test tube with ethyl acetate.
 5. Place the test tube in a nitrogen evaporator with no heat applied and evaporate to a final volume of 5 mL under a gentle stream of nitrogen.
 6. Stopper the graduated test tube and vortex for about 15 seconds. The sample is ready for GC analysis using electron capture detection.

CHROMATOGRAPHY:

Gas Chromatograph - HP 5880
 Detector - ECD
 Column - 12M x 0.25 mm x 0.33 um HP-1 (methyl silicone)
 Column Temperature - 170° C.
 Injector Temperature - 220° C.
 Detector Temperature - 350° C.
 Helium Carrier Flow - 2.5 cc/min
 Septum Purge - 2 cc/min
 Split vent - 50 cc/min
 Argon-Methane Make-up Flow - 60 cc/min

RECOVERY:

Average recovery for Dicofol is 92.2% at 0.3 ppb (0.28 ppb \pm .04, n = 3), and 91.1% at 1 ppb (0.91 ppb \pm .16, n = 3).

CALCULATION:

$$\mu\text{g}_{\text{dicofol}} = \frac{\text{peak area}_{\text{sample}} \times \text{ng}_{\text{std amount}} \times \text{ml}_{\text{final volume}}}{\text{peak area}_{\text{std}} \times \text{ul}_{\text{injected amount}}}$$

$$\text{ppb}_{\text{dicofol}} = (\mu\text{g}_{\text{dicofol}} / \text{g}_{\text{water}}) \times 1000$$

DISCUSSION:

Check on the sample sheet that the water has been adjusted to acidic pH when the sample was taken. Dicofol can break down to 4,4'-dichlorobenzophenone (DBP) in alkaline media.

This implies checking for DBP in the analytical standard as well, as this will affect quantitation of Dicofol. Typical peak ratios of Dicofol:DBP are 1:3 or 1:4, based on the standard used. For samples which have a higher proportion of DBP, assume breakdown has occurred. If the analytical request is strictly for Dicofol, a blind spike that has been prepared in advance

without being adjusted to acidic pH, will yield a lower recovery of Dicofol than the initial spike level. In that case, peak heights of Dicofol and DBP will need to be adjusted to the appropriate ratio to estimate the original Dicofol spike level. For example, if the sample ratio of Dicofol:DBP is 1:6, with peak heights of 3 and 19 mm, and the Dicofol standard gives a Dicofol:DBP ratio of 1:4, adjust the sample ratio to 1:4 as follows. $1/4 = (3+x)/(19-x)$, solving for "x". The adjusted Dicofol peak height, to be used in the calculation section above, is $3 + x$. Otherwise, the alternative is to report Dicofol and DBP levels separately, noting the different standard and sample ratios on the sample sheet.

"Sorbent" (Analytichem's term), or solid phase extraction (SPE) was explored, but gave inadequate (40 - 50%) recovery with available SPE cartridges (non-polar C-8 and C-18, recommended by SPE manufacturers for organochlorine pesticides as a class), despite structural similarity to DDT. Choice of solvents or solvent combinations for "sorbent" preparation or for analyte elution made no difference in recovery. Another choice of "sorbent" slightly more polar than C-8 or C-18, such as cyclohexyl (CH) may provide the desired selectivity.

REFERENCES AND ACKNOWLEDGMENTS:

The Agrochemicals Handbook, 2nd edition (Royal Society of Chemistry, Cambridge, 1990 update).

"BOND ELUT™ Disposal Extraction Columns for the rapid extraction of ORGANOCHLORINE PESTICIDES from Potable and Surface Waters," *Analytichem International, Inc.*, M206, 2/1/81.

Andrews, Jennifer S. and Thomas J. Good, "Trace Enrichment of Pesticides Using Bonded-Phase Sorbents," *American Laboratory* reprint, *Analytichem International, Inc.*, M151, April 1982.

Van Horne, K.C., ed., *Sorbent Extraction Technology Handbook (Analytichem International, 1985)*.

WRITTEN BY: Billy Fong

TITLE: *Billy Fong*
Agricultural Chemist II

REVIEWED BY: Catherine Cooper

TITLE: *Catherine Cooper*
Agricultural Chemist III

APPROVED BY: Terry Jackson

TITLE: *Terry Jackson*
Quality Assurance Officer

APPROVED BY: Mark Lee

TITLE: *Mark Lee*
Principal Agricultural Chemist

CALIFORNIA DEPT. OF FOOD AND AGRICULTURE
CHEMISTRY LABORATORY SERVICES
ENVIRONMENTAL MONITORING SECTION
3292 Meadowview Road
Sacramento, CA 95832
(916)-427-4999

Original Date: 5-20-91
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Current Date: 5-20-91
Method #:

DETERMINATION OF OMITE FROM WELL WATER

SCOPE:

This method is for the determination of Omite from well water.

PRINCIPLE:

Water samples are passed through two conditioned Sep-Pak™ C-18 cartridges in series. The retained Omite is eluted with ethyl acetate, evaporated to 1 mL, and analyzed by gas chromatography using electrochemical detection in the sulfur mode.

REAGENTS AND EQUIPMENT:

1. Reagents:

Ethyl acetate, nanograde (checked for chromatographic interferences)
Methanol, HPLC grade
Water, distilled
Na₂SO₄, anhydrous

2. Equipment, glassware and supplies:

Nitrogen evaporator (Organomation Model #12)
Glass culture tubes, 13 x 100 mm (Pyrex)
Graduated conical glass tubes, 15 mL capacity
Luer-lok tip syringe, 5 mL capacity
Vortex mixer (Thermolyne Maxi-Mix II)
Sep-Pak™ C-18 Cartridges
In-house vacuum manifold: consists of a 4 L side arm filtering flask with a #13 rubber stopper. A vacuum source is connected to the side arm with an in-line controller. Eight 4 mm holes are distributed evenly throughout the stopper. Eight 4", 4 mm O.D. glass tubes are inserted into the holes. The glass tubes are then connected to 3 ft of 4 mm I.D. Tygon™ tubing. Another 4" glass tube with tapered ends is connected to the Tygon™ tubing on the side arm. The Sep-Paks are connected to the tapered glass tubing.

ANALYSIS:

1. Weigh sample bottles and record each weight.
2. For each sample, attach two Sep-Pak™ C-18 cartridges in series to the glass tube attached to the in-house vacuum manifold.
3. Condition the cartridges by slowly aspirating 10 mL of methanol, followed by 20 mL of distilled water. Do not allow the cartridges to go dry.
4. Place the conditioned cartridges into the sample bottle containing the sample. Adjust the flow so that the sample is aspirated at 15 mL/minute. After all the sample has passed through the cartridges, allow the vacuum to remain on for 3-4 minutes.
5. Remove the cartridges from the glass tubing and attach them to the barrel of a luer-lok tip syringe. Elute with 2 rinses of ethyl acetate, 4 mL per rinse, combining the eluants in a glass conical tube.
6. Add 2-3 g anhydrous Na₂SO₄ to the extract to absorb any water present. Place on a vortex mixer for 20 seconds. Allow the Na₂SO₄ to settle and transfer the eluant quantitatively, washing the Na₂SO₄ with several rinses, to a 15 mL graduated test tube.
7. Place the test tube in a nitrogen evaporator with low heat applied and evaporate to a final volume of 1 mL under a gentle stream of nitrogen.
8. Stopper the graduated test tube and vortex for about 15 seconds. The sample is ready for GC analysis using electrochemical detection in the sulfur mode.

CHROMATOGRAPHY:

Gas Chromatograph - Varian 3700
 Detector - Hall™ 700A ELCD, Sulfur mode
 (Atten - 1, Range -1, Vent - 1.75 min.)
 Column - 10 M x 0.53 mm x 2.65 um HP-1 (methyl silicone)
 Column Temperature - 200° C.
 Injector Temperature - 230 ° C.
 Detector Temperature - 240 ° C.
 Helium Carrier Flow - 25 cc/min
 Air Reaction Gas Flow - 100 cc/min

RECOVERY:

Average recovery for Omite is 78% at 0.3 ppb (.23 ppb ±.03, n = 3), and 70% at 1 ppb (.70 ppb ±.10, n = 3).

CALCULATION:

$$\mu\text{g}_{\text{omite}} = \frac{\text{peak area}_{\text{sample}} \times \text{ng}_{\text{std amount}} \times \text{mL}_{\text{final volume}}}{\text{peak area}_{\text{std}} \times \mu\text{L}_{\text{injected amount}}}$$

$$\text{ppb}_{\text{omite}} = (\mu\text{g}_{\text{omite}}/\text{g}_{\text{water}}) \times 1000$$

DISCUSSION:

Some breakthrough (but not more than 0.2 ppb) of Omite was observed when passing the sample from one C-18 SPE cartridge to the next. This is why two cartridges are needed.

WRITTEN BY: Billy Fong

TITLE: Billy Fong
Agricultural Chemist II

REVIEWED BY: Catherine Cooper

TITLE: Catherine Cooper
Agricultural Chemist III

APPROVED BY: Terry Jackson

TITLE: Terry Jackson
Quality Assurance Officer

APPROVED BY: Mark Lee

TITLE: Mark Lee
Principal Agricultural Chemist

CALIFORNIA DEPT. OF FOOD & AGRIC.
CHEMISTRY LABORATORY SERVICES
ENVIRONMENTAL MONITORING SECTION
5275 Meadowview Road
Sacramento, Ca. 95832
(916) 427-4649/4999

Original Date: 06/09/89
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Method #:

DEF IN WELL WATER

SCOPE:

This method is for the determination of Def in well water.

PRINCIPLE:

The samples of water were extracted by shaking in a separatory funnel with methylene chloride. The extract was filtered and evaporated to dryness. It was then transferred and brought up to final volume with acetone. The extract was analyzed by gas chromatography using a flame photometric detector (FPD).

REAGENTS AND EQUIPMENT:

Methylene chloride and acetone (pesticide residue grade)
Sodium sulfate (anhydrous)
Rotary evaporator (Büchi/Brinkmann, R110)
Nitrogen evaporator (Organomation Model # 12)
Vortex mixer for test tubes
Balance (Mettler PC 4400)

ANALYSIS:

- 1) Remove samples from refrigerated storage and allow them to come to room temperature. Samples consist of approximately 1 L and are stored in 1 L amber glass bottles to prevent any photodegradation from occurring.
- 2) Record weight of the sample by weighing sample bottle before and after transfer.
- 3) Extract sample by shaking with 100 mL of methylene chloride for 2 min. Pressure builds up during extraction so venting is necessary.
- 4) Allow layers to separate and filter the organic layer through 25 g anhydrous sodium sulfate and filter paper. Collect extract in a 500 mL boiling flask.
- 5) Repeat steps 3 & 4 two more times using 80 mL of methylene chloride each time.
- 6) Rinse sodium sulfate with 20 mL additional methylene chloride and collect in the same 500 mL boiling flask.

RECOVERIES:

3 Recoveries of Def

Levels	Def(mean)	Standard Deviation(+/-)
0.3 ppb (n=3)	102	8.54
1.0 ppb (n=3)	101	1.15

MINIMUM DETECTABLE LEVEL:

The minimum detectable level was 0.1 ppb (1 liter volume of sample used.)
S/N=3

DISCUSSION:

A FFAP column was used in the TSD because another project was going on at the same time and used that column as its primary. HP-17 and HP-1 will work just as well. Minimum validation was done because there were only seven samples and short notice. Complete validation will be done when time permits.

REFERENCE:

- 1) White, Jane, *Malathion and Malaoxon in Water*, 1990, Environmental Monitoring Methods, California Department of Food and Agriculture.

WRITTEN BY: Jane White

Jane White

TITLE: Agricultural Chemist I

REVIEWED BY: Catherine Cooper

Catherine Cooper

TITLE: Agricultural Chemist III

APPROVED BY: Terry Jackson

Terry Jackson

TITLE: Quality Assurance Officer

APPROVED BY: S. Mark Lee

S. Mark Lee

TITLE: Research Agricultural Chemist

CALIFORNIA DEPT. OF FOOD & AGRIC.
ENVIRONMENTAL MONITORING SECTION
CHEMISTRY LABORATORY SERVICES
3292 Meadowview Road
Sacramento, CA 95832
(916)+427-4999

Original Date: 5/23/91
Supercedes: New
Current Date: 6/04/91
Method #: ??

Study GW-90 Water Screen for Metasystox-R

SCOPE:

This method has been developed and used to screen well water for metasystox-r.

PRINCIPLE:

Well waters were extracted for metasystox-r with dichloromethane. The dichloromethane was rotary evaporated to dryness and the residue dissolved in 2 mL of acetone. The metasystox-r was then oxidized by potassium permanganate to its sulfone form and extracted with dichloromethane. The dichloromethane was rotary evaporated to near dryness and brought to volume in acetone for GLC analysis.

REAGENTS AND EQUIPMENT:

1. Dichloromethane, pesticide grade
2. Acetone, pesticide grade
3. 2,2,4-Trimethyl pentane (iso-octane), pesticide grade
4. Sodium sulfate (anhydrous)
5. Magnesium sulfate - 20% solution w/v
6. Potassium permanganate - 0.1 N solution
7. Separatory funnel - (250 & 1000 mL)
8. Funnels, 60 degree short stem, 3-4 inch diameter
9. Flask, flat-bottomed boiling - (250 & 500 mL)
10. Graduated conical centrifuge tube - (15 mL)
11. Rotary evaporator - (Büchi-Brinkmann, R110)
12. Meyers N-EVAP - (Organomation Model # 12)
13. Vortex mixer for test tubes
14. Balance (Mettler PC 4400)
15. Glass wool - (Pyrex[®] Silver 8 micron - used as a filtering aid with the sodium sulfate)

ANALYSIS:

- 1) Remove samples from refrigerated storage and allow them to come to room temperature. Samples are approximately 1 L in size. They have been stored in amber glass bottles to prevent any photodegradation from occurring.
- 2) Record weight of the sample by weighing sample bottle before and after transfer. Weigh ~800 g (± 1 g) of sample into the 1 L separatory funnel.
- 3) Extract sample three times with equal 100 mL volumes of dichloromethane by shaking for 2 minutes each time. *Vent frequently to prevent any pressure from building up.*
- 4) Allow layers to separate and drain the organic layer through 20 g anhydrous sodium sulfate that has been prewashed with ~ 10mL of dichloromethane. Collect extract in a 500 mL boiling flask.

FORTIFICATION:

Metasystox-R was spiked into separate 800 mL volumes of water at the levels listed below.

RECOVERIES:

8 Recoveries of Metasystox-R:

<u>Levels</u> (ppb)	<u>Metasystox-R</u> (mean)	<u>Standard Deviation</u> (±)	<u>CV</u>
0.2 ppb (n=5)	86	1.3×10^{-2}	7.58
1.0 ppb (n=5)	91	8.35×10^{-2}	9.14
5.0 ppb (n=5)	84	8.69×10^{-1}	20.70
10.0 ppb (n=5)	83	6.30×10^{-1}	7.60

Recovery validation was done prior to samples.

MINIMUM DETECTABLE LEVEL:

The minimum detectable level was 0.1 ppb (800 mL volume of sample used) at a signal to noise ratio of 4.

DISCUSSION:

At the beginning and end of each set standards were run consisting of 0.1, 1, 2.5, 5 and 10 ng/uL. 1, 2.5 and 5 ng/uL standards were run after every 10-12 samples. A blank and a 10 ppb spike for metasystox-r was run with each set of samples.

REFERENCE:

- 1) Margetich, Sheila. *Determination of Dislodgeable Metasystox-R Residues from Leaf Punch Surfaces*, 1984, Worker Health and Safety, California Department of Food and Agriculture.
- 2) Lee, Paul. *Oxidation of Metasystox-R Residues for Gas Chromatography*, 1990, Environmental Monitoring Methods, California Department of Food and Agriculture.

CALIFORNIA DEPT. OF FOOD & AGRIC.
ENVIRONMENTAL MONITORING SECTION
CHEMISTRY LABORATORY SERVICES
3292 Meadowview Road
Sacramento, CA 95832
(916)+427-4999

Original Date: 5/21/91
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Method #: ??

Study GW-90 Water Screen for Fenvalerate (Pydrin)

SCOPE:

This method has been developed and used to screen well water for fenvalerate.

PRINCIPLE:

Well waters were extracted for fenvalerate with dichloromethane. The dichloromethane was rotary evaporated to dryness and brought to volume in acetone for GLC analysis.

REAGENTS AND EQUIPMENT:

1. Dichloromethane, pesticide grade
2. Acetone, pesticide grade
3. Sodium sulfate (anhydrous)
4. Separatory funnel - (1000 mL)
5. Funnels, 60 degree short stem, 3-4 inch diameter
6. Flask, flat-bottomed boiling - (500 mL)
7. Graduated conical centrifuge tube - (15 mL)
8. Rotary evaporator - (Büchi-Brinkmann, R110)
9. Meyers N-EVAP - (Organomation Model # 12)
10. Vortex mixer for test tubes
11. Balance (Mettler PC 4400)
12. Glass wool - (Pyrex[®] Silver 8 micron - used as a filtering aid with the sodium sulfate)

ANALYSIS:

- 1) Remove samples from refrigerated storage and allow them to come to room temperature. Samples are approximately 1 L in size. They have been stored in amber glass bottles to prevent any photodegradation from occurring.
- 2) Record weight of the sample by weighing sample bottle before and after transfer. Weigh ~800 g (\pm 1 g) of sample into the 1 L separatory funnel.
- 3) Extract sample by shaking with 100 mL of dichloromethane for 2 minutes. *Vent frequently to release pressure.*
- 4) Allow layers to separate and drain the organic layer through 20 g anhydrous sodium sulfate that has been prewashed with ~ 10mL of dichloromethane. Collect extract in a 500 mL boiling flask.
- 5) Repeat steps 3 & 4 two more times using 100 mL and 80 mL of dichloromethane for the second and third extractions respectively.
- 6) Rinse sodium sulfate three times with 10 mL additional dichloromethane and collect in the same 500 mL boiling flask.

ANALYSIS: continued

- 7) The extract was rotary evaporated to just dryness at 35°C under approximately 17 inches of Hg vacuum. Add ~ 1-2 mL acetone to the flask to rinse down the sides.
- 8) Transfer extract to a calibrated centrifuge test tube. Rinse flask several times (~10 mL total) with acetone. Transfer each wash to the same test tube.
- 9) Evaporate extract in a nitrogen evaporator with waterbath set at 35°C to a final volume of 3 mL under a gentle stream of nitrogen.
- 10) Stopper the graduated test tube and mix contents by placing on a vibrating mixer for about 15 seconds. Submit sample for gas chromatographic analysis.

EQUIPMENT CONDITIONS:

PRIMARY ANALYSIS

Varian: 6000 GC with ECD

Column: HP-1 (100% methyl polysiloxane) 25 m x 0.2 mm x 0.33 um

Carrier gas: Helium, Flow rate: 2 mL/minute

Make-up gas: Argon (95%)/ Methane(5%), Flow rate: 25mL/minute

Injector: 240°C

Detector: 300°C

Temperature program: Initial Temp: 175°C held for 1 minutes

Rate: 25°C/minute

Intermediate Temp: 245°C held for 20 minutes

Rate: 25°C/minute

Final Temp: 275°C held for 5 minutes

Injection volume: 2 uL

Range 10, Attenuation 32

Retention times: Fenvalerate = 23.20 ± 0.1 minute and 24.5 ± 0.1 minute

Linearity checked: 0.2 ng - 20 ng

CONFIRMATION ANALYSIS

Hewlett Packard 5880 A GC with ECD

Column: HP-17 (50% phenyl, 50% methyl-polysiloxane) 12 m x 0.20 mm x 0.33 um

Carrier gas: Helium, flow rate: 15 mL/minute

Split vent: 50 mL/minute

Septum purge: 2 mL/minute

Make-up gas: Argon (95%)/ Methane(5%), Flow rate: 25mL/minute

Injector: 250°C

Detector: 350°C

Temperature program: Initial temp: 200°C held for 1 minute

Rate: 6°C/minute

Final temp: 240°C held for 25 minutes

Injection volume: 2 uL

Attenuation: 24

Retention times: Fenvalerate = 11.81 ± 0.1 minute and 12.69 ± 0.1 minute

Linearity checked: 0.2 ng - 20 ng

PPB FENVALURATE (PYDRIN)

$$\text{ppb in sample} = \frac{(\text{peak height sample})(\text{ng/ul std})(\text{ul injected std})(\text{final volume mLs})(1000)}{(\text{peak height std})(\text{ul injected sample})(\text{weight of sample g})}$$

FORTIFICATION:

Fenvalerate was spiked into separate 800 mL volumes of water at the levels listed below.

RECOVERIES:

Recoveries of Fenvalerate:

<u>Levels</u> (ppb)	<u>Fenvalerate</u> (mean)	<u>Standard Deviation</u> (±)	<u>% CV</u>
0.3 ppb (n=3)	103	1.0×10^{-2}	3.23
1.0 ppb (n=3)	100	4.0×10^{-2}	4.06
5.0 ppb (n=3)	99	9.0×10^{-2}	1.83

Recovery validation was done prior to samples.

MINIMUM DETECTABLE LEVEL:

The minimum detectable level was 0.1 ppb (800 mL volume of sample used) at a signal to noise ratio of 4.

REFERENCE:

- 1) Johnson, Darrel E., *Determination of Synthetic Pyrethroids in Selected Vegetables by Gas Chromatography*, Laboratory Information Bulletin June 1990, Vol. 6, No. 6, art.# 3474, DHHS, Public Health Services, Food and Drug Administration, Rockville, MD, 20857.

WRITTEN BY: Jim Echelberry

Jim D. Echelberry

TITLE: Laboratory Technician

REVISED BY: Karen Hefner

Karen Hefner

TITLE: Agricultural Chemist II

REVIEWED BY: Catherine Cooper

Catherine Cooper

TITLE: Agricultural Chemist III

APPROVED BY: Terry Jackson

Terry Jackson

TITLE: Quality Assurance Officer

APPROVED BY: S. Mark Lee

S. Mark Lee

TITLE: Research Agricultural Chemist

CALIFORNIA DEPT. OF FOOD & AGRIC.
CHEMISTRY LABORATORY SERVICES
ENVIROMENTAL MONITORING SECTION
3292 Meadowview Road
Sacramento, CA 95832
(916)-427-4999

Original Date: May/24/1991
Supersedes: none
Current Date: June/06/1991
Method #:

Bromoxynil in Groundwater by GC/MSD

SCOPE:

This method is for the determination of bromoxynil (3,5-dibromo-4-hydroxybenzotrile) in groundwater samples. The detection limit of this method is 0.1 ppb for bromoxynil.

PRINCIPLE:

The water sample is acidified below pH 2. Bromoxynil is extracted with dichloromethane. The residues are derivatized with diazomethane, and analyzed by gas chromatography on a capillary column using a mass selective detector (MSD).

REAGENTS AND EQUIPMENT:

1. Dichloromethane, pesticide grade
2. Hexane, pesticide grade
3. Sulfuric acid, concentrated, A.C.S. reagent grade
4. Sodium sulfate (anhydrous)
5. Diazomethane (Aldrich Technical Information Bulletin #AL-131 (cat #210,025-0) *Diazomethane is explosive and a carcinogen, use with care.*)
6. Separatory funnel - (2000 mL)
7. Funnels, 60 degree short stem, 3-4 inch diameter
8. Flask, flat-bottomed boiling - (500 mL)
9. Graduated conical centrifuge tube - (15 mL) *Check calibration*
10. Rotary evaporator - (Büchi-Brinkmann, R110)
11. Meyers N-EVAP - (Organomation Model # 12)
12. Vortex mixer for test tubes
13. Balance (Mettler PC 4400)
14. Glass wool - (Pyrex[®] Silver 8 micron - used as a filtering aid with the sodium sulfate)

ANALYSIS:

- 1) Remove samples from refrigerated storage and allow them to come to ambient temperature. Samples are approximately 1 L in size. They have been stored in amber glass bottles to prevent any photodegradation from occurring.
- 2) Record weight of the sample by weighing sample bottle before and after transfer. Weigh entire sample (~ 1000 g ± 1 g if possible) into the 2 L separatory funnel.

ANALYSIS: continued

- 3) Add 2.5 mL of concentrated sulfuric acid to the water sample and mix well.
- 4) Extract sample three times with 100, 80 and 80 mL volumes of dichloromethane by shaking for 2 minutes each time. *Vent frequently to prevent any pressure from building up.* Allow layers to separate and drain the organic layer through 20 g anhydrous sodium sulfate that has been prewashed with ~ 10mL of dichloromethane. Collect extract in a 500 mL boiling flask.
- 5) Rinse sodium sulfate three times with 10 mL additional dichloromethane and collect in the same 500 mL boiling flask.
- 6) Rotoevaporate the extract to ~ 2 mL at 35°C under approximately 17 inches of Hg vacuum.
- 7) Add 5 mL of hexane, used as a keeper, and evaporate extract to ~ 2 mL.
- 8) Add 2 mL hexane to the flask to rinse down the sides.
- 9) Add 3 mL of diazomethane to flask and swirl to mix. *Diazomethane is explosive and a carcinogen, wear gloves and use with care.*
- 10) Allow the reagent to contact the inside surface of the flask by swirling gently and let the reaction mixture sit in fume hood covered with aluminum foil for 20 minutes. (If the yellow color has disappeared within 20 minutes, add additional diazomethane and let the reaction mixture sit for another 20 minutes.)
- 11) Evaporate the solvent and the excess reagent to just dryness at ambient temperature using a gentle stream of nitrogen.
- 12) Rinse flask several times (~10 mL total) with hexane to dissolve the residue and quantitatively transfer each wash to the same calibrated test tube.
- 13) Evaporate extract in a nitrogen evaporator with waterbath set at 35°C to a final volume of 3 mL under a gentle stream of nitrogen.
- 14) Stopper the calibrated test tube and mix contents by placing on a vibrating mixer for about 15 seconds. Submit sample for gas chromatographic analysis.

Instrument Conditions:

Hewlett-Packard Model 5890 Gas Chromatograph equipped with a series 5970 Mass Selective Detector, a Model 9000-340 Computer System, and a Model 7673A Autosampler.

Mass Selective Detector operated in selective ion monitoring mode (SIM). This mode was switched on 6 minutes after injection.

Column: HP-1 (cross-linked methyl silicon), 25 m X 0.2 mm X 0.33 um film.
 Carrier: Helium, 50 cm/sec
 Column Temperature: Initial 60°C 0.5 minute
 Program Rate 20°C/min
 Final 250°C 5 minutes
 Volume injected: 2 microliter
 Injector Temperature: 250°C
 Detector Temperature: 250°C
 Ions Selected for SIM Acquisition: (m/z) 195, 198, 248, 250, 274, 276, 278, 289, 291, 293.
 Solvent delay: 6 minutes
 Retention time: Bromoxynil = 10.10 +/- 0.1 minutes

PPB Bromoxynil

$$\text{ppb in sample} = \frac{(\text{peak height sample})(\text{ng/uL std})(\text{uL injected std})(\text{final volume mLs})(1000)}{(\text{peak height std})(\text{uL injection sample})(\text{weight of sample g})}$$

FORTIFICATION

Bromoxynil was spiked into separated 1000 ml volume of water at the levels listed below.

Recovery:

% Recoveries of Bromoxynil:

<u>Levels</u> (ppb)	<u>Bromoxynil</u> (mean)	<u>Data Range</u>
0.5 ppb (n=3)	91	0.37 - 0.51
1.0 ppb	103	1.02 - 1.04

Recovery validation was done prior to samples.

MINIMUM DETECTABLE LEVEL:

The minimum detectable level was 0.1 ppb (980-1000 ml. volume of sample used) at a signal to noise ratio of 4:1.

DISCUSSION:

Standards were run consisting of 0.2, 0.4, and 0.8 ng/uL at the beginning and end of each set. A blank and a 1 ppb spike for bromoxynil accompanied each set of samples.

We chose 10 ions for our detection throughout the entire analysis, they are (m/z) 195, 198, 248, 250, 274, 276, 278, 289, 291 and 293. The expected ratio of the ion intensities were very consistent, giving a high degree of confidence. Further confirmation with a full mass spectrometric scan is almost unnecessary.

This method was developed only for six samples and a rush analysis was requested. It is the author's opinion that the method is very decent, although one of the recoveries at the 0.5 ppb spike level appeared low (74%). Further validation will be undertaken at a future date.

The standard was prepared in a blank sample extract for better quantitation. A standard prepared in solvent gave a diminished response resulting in recoveries larger than 150%. This is probably due to the effect of the water matrix.

REFERENCE:

- 1) *Additional Principles and Methods of Analysis*; Zweig, G., and Sherma, J., Ed.; Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives; Academic Press: New York and London, 1967; Vol. 5, p347-362.
- 2) *Gas Chromatographic Analysis*; Zweig, G., and Sherma, J., Ed.; Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives; Academic Press: New York and London, 1972; Vol. 6, p605-610.
- 3) Lee, Paul, *DCPA (Dacthal), MTP AND TPA in Groundwater by GC/MSD* 1990, Environmental Monitoring Methods, California Department of Food and Agriculture.

WRITTEN BY: Jim Echelberry

Jim Echelberry

TITLE: Laboratory Technician

REVISED BY: Paul Lee

Paul Lee

TITLE: Agricultural Chemist III

REVIEWED BY: Catherine Cooper

Catherine Cooper

TITLE: Supervising Chemist III

APPROVED BY: Terry Jackson

Terry Jackson

TITLE: Quality Assurance Officer

APPROVED BY: S. Mark Lee

S. Mark Lee

TITLE: Research Agricultural Chemist

CALIFORNIA DEPT. OF FOOD & AGRIC.
ENVIRONMENTAL MONITORING SECTION
CHEMISTRY LABORATORY SERVICES
3292 Meadowview Road
Sacramento, CA 95832
(916)+427-4998/4999

Original Date: 5/23/91
Supercedes: New
Current Date: 6/04/91
Method #: ??

Study GW-90 Water Screen for Cypermethrin

SCOPE:

This method has been developed and used to screen well water for cypermethrin.

PRINCIPLE:

Well waters were extracted for cypermethrin with dichloromethane. The dichloromethane was rotary evaporated to dryness and brought to volume in acetone for GLC analysis.

REAGENTS AND EQUIPMENT:

1. Dichloromethane, pesticide grade
2. Acetone, pesticide grade
3. Sodium sulfate (anhydrous)
4. Separatory funnel - (1000 mL)
5. Funnels, 60 degree short stem, 3-4 inch diameter
6. Flask, flat-bottomed boiling - (500 mL)
7. Graduated conical centrifuge tube - (15 mL)
8. Rotary evaporator - (Büchi-Brinkmann, R110)
9. Meyers N-EVAP - (Organomation Model # 12)
10. Vortex mixer for test tubes
11. Balance (Mettler PC 4400)
12. Glass wool - (Pyrex[®] Silver 8 micron - used as a filtering aid with the sodium sulfate)

ANALYSIS:

- 1) Remove samples from refrigerated storage and allow them to come to room temperature. Samples are approximately 1 L in size. They have been stored in amber glass bottles to prevent any photodegradation from occurring.
- 2) Record weight of the sample by weighing sample bottle before and after transfer. Weigh ~800 g (\pm 1 g) of sample into the 1 L separatory funnel.
- 3) Extract sample by shaking with 100 mL of dichloromethane for 2 minutes. *Vent separatory funnel frequently to release any pressure.*
- 4) Allow layers to separate and drain the organic layer through 30 g anhydrous sodium sulfate that has been prewashed with ~ 10 mL of dichloromethane. Collect extract in a 500 mL boiling flask.
- 5) Repeat steps 3 & 4 two more times using 100 mL and 80 mL of dichloromethane for the second and third extractions respectively.
- 6) Rinse sodium sulfate three times with 10 mL additional dichloromethane and collect in the same 500 mL boiling flask.

ANAYSIS: continued

- 7) The extract was rotary evaporated to just dryness at 35°C under approximately 17 inches of Hg vacuum. Add ~ 1-2 mL acetone to the flask to rinse down the sides.
- 8) Transfer extract to a calibrated centrifuge test tube. Rinse flask several times (~10 mL total) with acetone. Transfer each wash to the same test tube.
- 9) Evaporate extract in a nitrogen evaporator with waterbath set at 35°C to a final volume of 3 mL under a gentle stream of nitrogen.
- 10) Stopper the graduated test tube and mix contents by placing on a vibrating mixer for about 15 seconds. Submit sample for gas chromatographic analysis.

EQUIPMENT CONDITIONS:

PRIMARY ANALYSIS

Varian: 6000 GC with ECD

Column: HP-1 (100% methyl polysiloxane) 25 m x 0.2 mm x 0.33 um

Carrier gas: Helium, Flow rate: 2 mL/minute

Make-up gas: Argon (95%)/ Methane (5%), Flow rate: 25 mL/minute

Injector: 240°C

Detector: 300°C

Temperature program: Initial Temp: 150°C held for 1 minutes

Rate: 15°C/minute

Final Temp: 245°C held for 23 minutes

Injection volume: 2 uL

Range 10, Attenuation 32

Retention times: Cypermethrin = 25.98, 26.63, 27.18 and 27.47 (all ± 0.1 minute)

Linearity checked: 0.2 ng - 20 ng

CONFIRMATION ANALYSIS

Hewlett Packard 5880 A GC with ECD

Column: HP-17 (50% phenyl, 50% methyl-polysiloxane) 12 m x 0.20 mm x 0.33 um

Carrier gas: Helium, flow rate: 15 mL/minute

Split vent: 50 mL/minute

Septum purge: 2 mL/minute

Make-up gas: Argon (95%)/ Methane (5%), Flow rate: 25mL/minute

Injector: 230°C

Detector: 350°C

Temperature program: Initial temp: 175°C held for 1 minute

Rate: 6°C/minute

Final temp: 235°C held for 15 minutes

Injection volume: 2 uL

Attenuation: 2⁴

Retention times: Cypermethrin = 14.72, 15.10, 15.25 and 15.41 (all ± 0.1 minute)

Linearity checked: 0.2 ng - 20 ng

PPB CYPERMETHRIN

$$\text{ppb in sample} = \frac{(\text{peak height sample})(\text{ng/ul std})(\text{ul injected std})(\text{final volume mLs})(1000)}{(\text{peak height std})(\text{ul injected sample})(\text{weight of sample g})}$$

FORTIFICATION:

Cypermethrin was spiked into separate 800 mL volumes of water at the levels listed below.

RECOVERIES:

% Recoveries of Cypermethrin:

<u>Levels</u> (ppb)	<u>Cypermethrin</u> (mean)	<u>Standard Deviation</u> (±)	<u>% CV</u>
0.3 ppb (n=3)	116	1.53×10^{-2}	4.41
1.0 ppb (n=3)	94	3.6×10^{-2}	3.84

Recovery validation was done prior to samples.

MINIMUM DETECTABLE LEVEL:

The minimum detectable level was 0.1 ppb (800 mL volume of sample used) at a signal to noise ratio of 4.

REFERENCE:

- 1) Johnson, Darrel E., *Determination of Synthetic Pyrethroids in Selected Vegetables by Gas Chromatography*, Laboratory Information Bulletin June 1990, Vol. 6, No. 6, art.# 3474, DHHS. Public Health Services. Food and Drug Administration. Rockville, MD. 20857.
- 2) *Pesticide Analytical Manual*; U.S. Department of Health, Education, and Welfare. Food and Drug Administration. U.S. Government Printing Office: Washington DC. June 1982, Vol. 1, Pesticide Reg. Sec. 180-418.

CALIFORNIA DEPT. OF FOOD AND AGRICULTURE
CHEMISTRY LABORATORY SERVICES
ENVIRONMENTAL MONITORING SECTION
3292 Meadowview Road
Sacramento, CA 95832
(916)-427-4999

Original Date: 5-20-91
Supercedes: NEW
Current Date: 5-20-91
Method #:

DETERMINATION OF CAPTAN FROM WELL WATER

SCOPE:

This method is for the determination of Captan from well water.

PRINCIPLE:

Water samples are passed through a conditioned Sep-Pak™ C18 cartridge. The retained Captan is eluted with ethyl acetate, evaporated to 1 mL, and analyzed by gas chromatography using electron capture detection.

REAGENTS AND EQUIPMENT:

Ethyl acetate, nanograde (checked for chromatographic interferences)
Methanol, HPLC grade
Water, distilled
Na₂SO₄, anhydrous
Nitrogen evaporator (Organomation Model #12)
Centrifuge (Clay-Adams)
Glass culture tubes, 13 X 100 mm (Pyrex)
Luer-lok tip syringes, 10 mL
Graduated conical glass tubes, 15 mL capacity
Vortex mixer (Thermolyne Maxi-Mix II)
Sep-Pak™ C18 Cartridges
In-house vacuum manifold: consists of a 4 L side arm filtering flask with a #13 rubber stopper. A vacuum source is connected to the side arm with an in-line controller. Eight 4 mm holes are distributed evenly throughout the stopper. Eight 4", 4 mm O.D. glass tubes are inserted into the holes. The glass tubes are then connected to 3 ft of 4 mm I.D. Tygon™ tubing. Another 4" glass tube with tapered ends is connected to the Tygon™ tubing on the side arm. The Sep-Paks™ are connected to the tapered glass tubing.

ANALYSIS:

1. Weigh sample bottles and record each weight.
2. For each sample, attach a Sep-Pak™ C18 cartridge to the glass tube attached to the in-house vacuum manifold.
3. Condition the cartridge by slowly aspirating 10 mL of methanol, followed

- by 20 mL of distilled water. Do not allow the cartridge to go dry.
4. Place the conditioned cartridge into the sample bottle containing the sample. Adjust the flow so that the sample is aspirated at 15 mL/minute. After all the sample has passed through the cartridge, allow the vacuum to remain on for 3-4 more minutes.
 5. Remove the cartridge from the glass tubing and place in a centrifuge tube. Centrifuge for 3 minutes at 1200 rpm.
 6. Connect the cartridge to a 10 mL luer-lok syringe. Pass 4 mL of ethyl acetate through it and collect the eluant in a conical tube.
 7. Add 3 g of Na_2SO_4 to the tube and vortex for about 15 seconds. Allow the Na_2SO_4 to settle and transfer the eluant quantitatively, washing the Na_2SO_4 with several rinses, to a 15 mL graduated test tube.
 8. Place the test tube in a nitrogen evaporator with no heat applied and evaporate to a final volume of 1 mL under a gentle stream of nitrogen.
 9. Stopper the graduated test tube and vortex for about 15 seconds. The sample is ready for GC analysis using electron capture detection.

CHROMATOGRAPHY:

Gas Chromatograph - Varian 3700
 Detector - ECD
 Column - 10 M x 0.53 mm x 2.65 μm HP-1 (methyl silicone)
 Column Temperature - 185° C.
 Injector Temperature - 220° C.
 Detector Temperature - 300° C.
 Helium Carrier Flow - 22.5 cc/min
 Argon-Methane Detector Flow - 12 cc/min

Manual injections were made using the solvent plug technique (drawing up 1 μL solvent, 1 μL air, 2 μL sample).

RECOVERY:

<u>spike level,</u> ppb		<u>result,</u> ppb	<u>recovery,</u> %	
0.5	A	0.52	104	
	B	0.46	92	
	C	0.49	99	
	D	0.47	95	$\bar{x} = 97.5\% \pm 5.2$
1.5	A	1.38	92	
	B	1.33	89	
	C	1.18	79	
	D	1.35	90	$\bar{x} = 87.5\% \pm 5.8$
5.0	A	3.95	79	
	B	4.55	91	
	C	5.10	102	
	D	4.45	89	$\bar{x} = 90.2\% \pm 9.4$

These validation spikes confirm initial trial work (Fong, Hsu) using SPE (solid phase extraction) with several pairs of spike levels (0.2, 1.0 and 10 ppb) which gave 80 to 90% recoveries.

CALCULATION:

$$\text{ug}_{\text{captan}} = \frac{\text{peak area}_{\text{sample}} \times \text{ng}_{\text{std amount}} \times \text{ml}_{\text{final volume}}}{\text{peak area}_{\text{std}} \times \text{ul}_{\text{injected amount}}}$$

$$\text{ppb}_{\text{captan}} = (\text{ug}_{\text{captan}}/\text{g}_{\text{water}}) \times 1000$$

DISCUSSION:

Captan standards should be made up in ethyl acetate for GC analysis. Captan is unstable in other solvents, such as methanol, and cannot give reproducible responses. Previous investigations (Quan, Margetich) have shown recoveries from Captan standards made in methanol are only about 50% by GC. However, Captan standards made in ethyl acetate give reproducible responses on GC even by manual injection, using the solvent plug technique.

Captan is not stable in water. It appears to break down in a matter of hours. Even under refrigeration, only 10 - 20% remains after 1 week; in the freezer, 10 - 20% remains after 2 weeks, based on a storage study. If the samples are taken a few days prior to extraction, any Captan that might be present will already be broken down. Samples should be extracted immediately upon arrival. Preliminary recovery spikes, blank matrix and matrix spikes, as well as validation spikes, should also be extracted without delay after preparation. A storage study of shipping spikes should be further pursued.

Spike recoveries will be adversely affected by the use of tap water due to the presence of chlorine. Recoveries are reduced to about 20% in tap water, under acidic (pH = 6.74) or alkaline conditions (pH = 8.45). Distilled water is suitable for spike preparation, providing the pH is checked. Rapid hydrolysis of Captan occurs in alkaline conditions, slow hydrolysis in neutral or acidic conditions (*Agrochemicals Handbook*).

Hall 700A™ detectors may not have sufficient sensitivity to provide a suitable alternative by GC. Initial work with Captan analyzed by HPLC with a borrowed Tracor 965™ photoconductivity detector (Lee, 1989) showed promising results. However, the PCD experienced some difficulties in translating the electronic signal to the recording integrator without becoming saturated, even after the cell had been cleaned by sonication. Further development should be pursued under more controlled conditions.

REFERENCES AND ACKNOWLEDGMENTS:

The *Agrochemicals Handbook*, 2nd edition (Royal Society of Chemistry, Cambridge, 1990 update).

Acknowledgments to: P. Lee (1989), S. Margetich and V. Quan (1990) for background work; J. Hsu for trial work by solid phase extraction; J. White for apparatus descriptions, J. Echelberry and J. Temple for validation work.

WRITTEN BY: Billy Fong

TITLE: Billy Fong
Agricultural Chemist II

REVIEWED BY: Catherine Cooper

TITLE: Catherine Cooper
Agricultural Chemist III

APPROVED BY: Terry Jackson

TITLE: Terry Jackson
Quality Assurance Officer

APPROVED BY: Mark Lee

TITLE: Mark Lee
Principal Agricultural Chemist

CALIFORNIA DEPT. OF FOOD & AGRIC.
CHEMISTRY LABORATORY SERVICES
ENVIRONMENTAL MONITORING SECTION
3292 Meadowview Road
Sacramento, CA 95832
(916)+427-4998/4999

Original Date:11/15/1990
Supercedes:
Current Date:10/17/1991
Method #:

RESIDUE ANALYSIS OF BENOMYL IN WELL WATER.

SCOPE:

This method was developed to analyze Benomyl in well water.

PRINCIPLE:

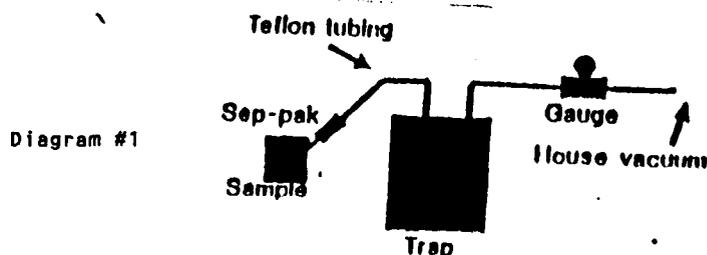
A conditioned C18 reverse phase Sep-Pak[®] is used to trap Benomyl from well water samples. Centrifugation is used to eliminate any remaining water from the Sep-Pak[®] before a methanol elution and analysis by LC/UV.

REAGENTS AND EQUIPMENT:

Methanol, pesticide grade or equivalent.
Working standards in Methanol (Diluted from stock standard.)
In house vacuum manifold.
C18 reverse phase Sep-pak,[®] Waters, Division of Millipore. 1 gram.
Nylon acrodisc, 25mm diameter; 0.2 micron, Gelman Sciences.
Centrifuge: Clay Adams.
Beakers, 600 mL.
Graduated test tubes, 10 mL.
Micro-Mate Syringes, 10 cc - Popper & Sons Inc.
N-EVAP - Meyers Organomation Associates Incorporated
Vibrating mixer.
Sodium Sulfate, anhydrous, granular (ACS).
Acetic acid, glacial.
Pipettes.

ANALYSIS:

1. Weigh 500.0 grams of well water into a 600 mL beaker.
2. Connect a C18 reverse phase Sep-Pak[®] to the in house vacuum manifold as follows in diagram #1.



ANALYSIS:

3. Condition the Sep-Pak[®] with about 5 mL of methanol followed by about 10 mL of distilled water using the in-house vacuum. Do not let the Sep-Pak[®] go to dryness.
4. Attach the conditioned Sep-Pak[®] to 15 cm x 5 mm glass tubing and dip into the beaker containing the 500g of sample. Adjust the flow rate to about 3-5 mL/minute (about 6 in Hg).
5. After the water sample has passed through the Sep-Pak[®], leave the vacuum on allowing air to pass through the Sep-Pak[®] for ~3-5 minutes.
6. Remove the Sep-Pak[®] from the glass tube and insert the Sep-Pak[®] into a centrifuge tube and centrifuge for 2 minutes at ~1200 rpm, a setting of 4 on the centrifuge speed dial.
7. Elute all chemicals with 10 mL of methanol, using a 10 cc syringe, into a 15 mL graduate test tube.
8. Concentrate the eluting solvent to 1.0 mL by using a nitrogen evaporator which set at 35°C. Mix it well for 30 seconds by using the vibrating mixer. Filter the sample through a 0.2 um acrodisc into an auto-sampler vial. *Analyze by liquid chromatograph.*

EQUIPMENT CONDITIONS:

- A. Liquid chromatography: Perkin Elmer Series 4.
Column: BECKMAN ODS, 5.0 um, 4.6 mm x 15.0 cm.
Guard column: BECKMAN ODS, 5.0 um, 4.6 mm x 4.5 cm.
Detector: Varian 2550 UV.
Flow rate: 1.0 mL/minute.
Mobile phase: 30% Solution A: Acetonitrile.
70% Solution B: 0.2% Acetic acid in water.
Wave length: 282 nm.
Retention time: ~ 4.70 minutes.

CALCULATIONS:

$$\text{PPB} = \frac{\text{Peak height of sample} \times \text{Amount of std (ng)} \times 1,000 \text{ uL}}{\text{Peak height of std} \times \text{volume injected} \times \text{sample weight(g)}}$$

DISCUSSION:

The following results were obtained by this method:

Chemical	Spike level (ppb)	Number of analysis (n)	Mean % Recovery	Standard deviation (+/-)
Benomyl	0.2	4	76.3	2.5
	0.4	4	94.4	10.1
	0.8	4	88.2	5.9
	10.0	4	83.8	6.16

During the method development, an experiment was conducted to see at what concentration Benomyl started breaking through a C18 Sep-Pak[®] as well as to see how polarity of spiking solvents affected the recovery of the chemical. The experiment indicated that acetone in spiking solution lowered recovery of the chemical and Benomyl started breaking through the Sep-Pak[®] at concentration of 10 ppb even when the spiking solution was methanol.

Therefore, to use this method, these procedures should be followed:

- a) The spiking solution for QC purposes should be made in methanol.
- b) The maximum Benomyl loading the Sep-Pak will handle is 5 ug and must not be exceed. Therefore, samples with apparent concentrations above 8 ppb should be re-analyzed using a 50 % smaller sample size.

REFERENCES:

Saini, Nirmal K. *Analysis of water, air tubes and patches for benomyl*, 1982 Environmental Monitoring Methods, California Department of Food and Agriculture.

WRITTEN BY: Duc Tran

Duc Tran

TITLE: Agricultural Chemist I

REVIEWED BY: Catherine Cooper

Catherine Cooper

TITLE: Agricultural Chemist III

APPROVED BY: S. Mark Lee

S. Mark Lee

TITLE: Principal Investigator

APPROVED BY: Terry Jackson

Terry Jackson

TITLE: Quality Assurance Officer

CALIFORNIA DEPT. OF FOOD & AGRIC.
CHEMISTRY LABORATORY SERVICES
ENVIRONMENTAL MONITORING SECTION
3292 Meadowview Road
Sacramento, CA 95832
(916)+427-4998/4999

Original Date:02/18/91
Supercedes: NEW
Current Date:10/23/91
Method #:

MOLINATE IN WATER BY GC.

SCOPE:

This method was developed to analyze Molinate in well water.

PRINCIPLE:

A C18 reverse phase Sep-Pak is used to trap Molinate from water samples. Methanol is then used to elute the chemical. Finally, the eluant is concentrated and analyzed for Molinate by GC.

REAGENTS AND EQUIPMENT:

Methanol, pesticide grade or equivalent.
Distilled water.
Working standards in Methanol (Dilute from stock standard.)
In house vacuum manifold.
360 mg C-18 reversed phase Sep-Pak, [®] Waters Division of Millipore.
Nylon acrodisc, 0.2 micron, Gelman Sciences.
VAC ELUT SPS 24, Analytical International.
Beakers, 600 mL.
Graduated test tubes, 15 mL.
Micro-Mate Syringes, 10 cc - Popper & Sons Inc.
N-EVAP - Meyers Organomation Associates Incorporated.
Vibrating mixer.
Sodium Sulfate, anhydrous, granular (ACS).

ANALYSIS:

1. For each sample, weigh 500 g of water sample into a 600 mL beaker.
2. Connect C18 reverse phase Sep-Pak to a 15 cm long glass tube that is attached to the in house vacuum manifold.
3. Place the Sep-Pak, while still attached as in two, into a beaker containing methanol. Turn vacuum on (at 10 psi) and allow methanol to elute through the Sep-pak for 1 minute. Remove Sep-Pak from methanol and repeat this operation with a beaker containing distilled water for 1 minute. The Sep-Pak is now conditioned and should *not* be allowed to dry during the extraction process.
4. Remove Sep-Pak from water and place it into the sample.
5. After all 500.0 grams of water sample has passed through the Sep-pak, remove it and place it in the VAC ELUT SPS 24. Turn the unit on in waste position for 10 minutes at 5 inches of Hg to dry Sep-Pak.
6. Turn vacuum off. Put the 15 mL test tube in place and set VAC ELUT SPS 24 to collect position.

ANALYSIS: continued.

- 7. Attach a 10 mL Luer tip syringe to the Sep-Pak and add 10 mL of methanol. Turn vacuum on and allow the sample to elute into the test tube.
- 8. Concentrate the eluent solvent to 1.0 mL using Nitrogen evaporator. Using a vibrating mixer, mix sample well for 30 seconds. Filter it through a 0.2 um acrodisc into a micro vial.
- 9. Analyze by gas chromatograph.

EQUIPMENT CONDITIONS:

PRIMARY ANALYSIS

Gas chromatograph: Varian 3700 with TSD.
 Column: HP-FFAP; 10m x 0.53 mm. Film thickness: 1.0 um.
 Temperature program: Initial: 150³C.
 Hold : 4 min.
 Rate : 25 ³C/min.
 Final : 220³C.
 Hold : 1 min.
 Injector: 220³C.
 Detector: 220³C.
 Carrier gas: Helium. Flow rate: 20 mL/min.
 Injection volume: 2 uL.
 Retention time: ~3.20 min p 0.1 minute.
 Linearity checked: 0.2 ng - 10 ng.

Gradient temperature operation eliminates matrix problems by baking out the column.

CONFIRMATION ANALYSIS

Gas chromatograph: Varian 6000 with TSD.
 Column: HP-5; 10m x 0.53 mm. Film thickness: 1.0 um.
 Temperature program: Initial: 130³C.
 Hold : 1 min.
 Rate : 10 ³C/min.
 Final : 200³C.
 Hold : 1 min.
 Injector: 220³C.
 Detector: 220³C.
 Carrier gas: Helium. Flow rate: 15 mL/min.
 Injection volume: 2 uL.
 Retention time: ~2.55 min p 0.1 minute.

PPB Molinate

$$\text{ppb in sample} = \frac{(\text{peak height sample})(\text{ng/uL std})(\text{uL injected std})(\text{final volume mLs})(1000)}{(\text{peak height std})(\text{uL injected sample})(\text{weight of sample g})}$$

FORTIFICATION:

Molinate was spiked into separate 500 mL volumes of water at the levels listed below.

RECOVERIES:

% Recoveries of Molinate:

<u>Levels</u> (ppb)	<u>Molinate</u> (mean % recovery)	<u>Standard Deviation</u> (p)
0.3 ppb (n-5)	87.5	3.68
1.0 ppb (n-5)	92.0	1.28

Recovery validation was done prior to samples. Recovery results were comparable to the results obtained using liquid-liquid extraction.² A weighed 1000 g of water sample was extracted three times with methylene chloride volumes: 100 mL one time and twice with 80 mL. Sample was concentrated to 1.0 mL, filtered through a 0.2 um acrodisc into a micro vial and analyzed by gas chromatography.

MINIMUM DETECTABLE LEVEL:

The minimum detectable level was 0.1 ppb (500 mL volume of sample used) at a signal to noise ratio of 4.

DISCUSSION:

Standards consisting of 0.1, 0.5, 1.0 ng/u were run at the beginning and the end of each set 2. The standards were also run after every 10-12 samples. A blank and a 0.3 ppb spike for molinate was run with each set of samples.

REFERENCE:

- 1) Tran, Duc, *Multipesticide Residue Analysis: Atrazine, Bromacil, Diuron, Prometon, and Simazine*, 1990, Environmental Monitoring, California Department of Food and Agriculture.
- 2) White Jane, *Diazinon, Chlorpyrifos, Parathion and Methidation in Fog Water*, 1989, Environmental Monitoring, California Department of Food and Agriculture.

WRITTEN BY: Jorge L. Hernandez

TITLE: Laboratory Technician

REVISED BY: Sylvia Richman

Sylvia Richman

TITLE: Agricultural Chemist II

REVIEWED BY: Catherine Cooper

Catherine Cooper

TITLE Agricultural Chemist III

APPROVED BY: Terry Jackson

Terry Jackson

TITLE: Quality Assurance Officer

APPROVED BY: S. Mark Lee

S. Mark Lee

TITLE: Research Agricultural Chemist

CALIFORNIA DEPT. OF FOOD & AGRIC.
CHEMISTRY LABORATORY SERVICES
ENVIRONMENTAL MONITORING SECTION
3292 Meadowview Road
Sacramento, CA 95832
(916)+427-4998/4999

Original Date: April 1, 1990.
Supercedes: NEW
Current Date: September 18, 1991
Method #:

DETERMINATION OF N-METHYL CARBAMATES IN RIVER WATER BY HPLC

SCOPE:

This method is for the determination of N-methyl carbamates in surface water samples from rice field drains. The sensitivity of this method is 0.05 ppb.

PRINCIPLE:

Residues in water are extracted with methylene chloride. After evaporating the solvent, the extracts are dissolved in water and separated by HPLC. The eluant is derivatized with OPA by post column reaction and detected with a fluorescence detector.

REAGENTS AND EQUIPMENT:

Solvents: methylene chloride (pesticide residue grade)
water, methanol and acetonitrile (HPLC grade)
Sodium sulfate (anhydrous)
Separatory funnels (500 mL)
Boiling flasks, flat bottomed, 24/40 joints (500 mL)
Glass stemmed funnels
Rotary evaporator (Büchi/Brinkmann, Model R 110)
Graduated test tubes (15 mL)
Nitrogen evaporator (Organomation, Model 12)
Vortex mixer (Thermolyne, Model 37600)

ANALYSIS:

- 1) Remove samples from refrigerated storage and allow them to come to room temperature. Samples consist of approximately 1 L and are stored in 1 L amber glass bottles to prevent photodegradation.
- 2) Shake samples and weigh out approximately 100 g by difference. Place this aliquot into 500 mL separatory funnel.
- 3) Extract samples by adding 100 mL of methylene chloride and shaking vigorously for one minute.
- 4) Allow layers to separate. Drain the organic layer into a 500 mL flask.
- 5) Repeat steps 3 and 4 two more times using 100 mL of methylene chloride.
- 6) Concentrate extract to 3-5 mL on a rotary evaporator at a vacuum of ca.

15 in Hg, using a 30-35°C water bath.

- 7) Add ca. 1 gram anhydrous sodium sulfate to remove any water droplets.
- 8) Filter the extract through a 0.45 micron Acrodisk disposable unit and transfer the extract to a precalibrated 15 mL conical centrifuge tube.
- 9) Rinse the flask and sodium sulfate two times with 2 mL methylene chloride each time. Filter through the same unit and collect in the same tube.
- 10) Place extract in a nitrogen evaporator with water bath set at 35°C and evaporate just to dryness under a gentle stream of nitrogen.
- 11) Add 0.1 mL methanol and mix contents by vortexing for about 15 seconds.
- 12) Immediately prior to HPLC analysis of each sample add 0.9 mL water and mix contents by vortexing for about 15 seconds.
- 13) Transfer the contents to a 2 mL autosampler vial for HPLC analysis.

EQUIPMENT CONDITIONS:

Chromatograph: Hewlett-Packard 1090 Liquid Chromatograph
 Hitachi F1000 Fluorescence Spectrometer
 Pickering Labs Post-Column Derivatization System (1951
 Colony St., Suite S, Mountain View, CA 94043)

Column: Pickling Laboratories "Carbamates Analysis" C18 4.6 mm x 25 cm x 5 um
 Flow: 1.0 mL/min

Injection volume: 250 uL

Gradient: Time (min)	% Water	% Acetonitrile
0.01	100.0	0
3.0	90.0	10.0
10.0	30.0	70.0
13.0	30.0	70.0
16.0	90.0	10.0
20.0	100.0	0

Fluorescence Detector: Excitation - 340 nm
 Emission - 450 nm
 Time constant - 0.3 sec

CALCULATIONS:

Parts per billion of pesticide:

$$PPB = \frac{(\text{peak area sample})(\text{ng std. injected})(\text{sample final vol.})(1000 \text{ ul/ml})}{(\text{peak area std})(\text{ul sample injected})(\text{g of sample})}$$

METHOD VALIDATION:

The minimum detection limit for N-methyl carbamates using this method is 0.05 ppb. Method validation recoveries for 9 carbamates are as follows:

Carbamates	Spike Level (ppb)	n	Recovery		SD	CV
			\bar{x} (ppb)	%		
Aldicarb SO	0.5	5	0.356	71	0.023	6.5
	1.0	"	0.678	68	0.062	9.2
	5.0	"	3.224	65	0.427	13.3
	10.0	"	7.198	72	0.158	2.2
Aldicarb SO ₂	0.5	5	0.530	106	0.010	1.9
	1.0	"	0.944	99.4	0.062	6.6
	5.0	"	4.870	97.4	0.169	3.5
	10.0	"	9.980	99.8	0.217	2.2
Vydate	0.5	5	0.520	104	0.031	5.9
	1.0	4	1.00	100	0.074	7.42
	5.0	5	4.764	95.3	0.779	16.4
	10.0	"	9.16	91.6	1.180	12.9
Lannate	0.5	5	0.458	91.6	0.068	14.8
	1.0	4	1.01	101.	0.063	6.17
	5.0	5	5.03	100.6	0.252	5.02
	10.0	"	9.51	95.1	0.631	6.63
3-OH-Carbofuran	0.5	5	0.458	91.6	0.068	14.8
	1.0	"	0.806	80.6	0.454	5.63
	5.0	"	5.03	100.6	0.252	5.02
	10.0	"	9.51	95.1	0.631	6.63

3-Keto-Carbofuran Chemical breakdown occurs in the autosampler vials.

Aldicarb	0.5	5	0.468	93.6	0.046	9.83
	1.0	"	0.916	91.6	0.099	10.8
	5.0	"	4.528	90.5	0.449	9.91
	10.0	"	9.46	94.6	0.598	6.32
Carbaryl	0.5	5	0.498	99.6	0.011	2.21
	1.0	"	0.960	96.0	0.064	6.66
	5.0	"	4.674	96.0	0.25	5.35
	10.0	"	10.98	110	0.740	6.74
Carbofuran	0.5	5	0.490	98.0	0.025	4.99
	1.0	"	0.996	99.6	0.073	7.33
	5.0	"	4.814	96.3	0.125	2.59
	10.0	"	10.12	101	0.342	3.42
1-Naphthol	0.5	5	0.272	54.4	0.018	6.58
	1.0	"	0.544	54.4	0.340	62.4
	5.0	"	3.172	63.4	0.850	26.8
	10.0	"	8.0	80.2	1.374	17.1

METHOD VALIDATION:continued

<u>Carbamates</u>	<u>Spike Level</u> (ppb)	<u>n</u>	<u>Recovery</u> \bar{x} (ppb)	<u>%</u>	<u>SD</u>	<u>CV</u>
Methiocarb	0.5	5	0.518	104	0.033	6.46
	1.0	"	0.936	93.6	0.077	8.23
	5.0	"	4.750	95.0	0.212	4.47
	10.0	"	10.06	101	0.666	6.62

DICUSSION:

It is our experience that excessive heat applied to carbamate samples during evaporation causes substantially lowered recoveries. To achieve acceptable recoveries prolonged heat must be avoided and the recommended temperature must be used.

The large ratio of methylene chloride to sample water is necessary to obtain high recovery of aldicarb sulfoxide which is equally soluble in water and methylene chloride.

There is a marked decrease in the amount of several carbamates upon sample storage in glass autosampler vials in aqueous solution. This is particularly true of methiocarb sulfone and 3-keto carbofuran where variability due to this factor was so marked they could not confidently be included in method validation. The dilution of each sample with water immediately before injection is a response to this behavior.

ACKNOWLEDGEMENT:

We thank Ms Jane White for her tireless help in performing method validation and Mr. James Echelberry for his help in data treatment.

REFERENCES:

Muth, G.L., Erro, F., "A Rapid Carbamate Multiresidue Procedure for Vegetable Crops", Bull. Environ. Contam. Toxicol., 1980, 24, 759-765.

Ting, K.C., "High Performance Liquid Chromatographic Method for Determination of Six N-Methylcarbamates in Vegetables and Fruits", Bull. Environ. Contam. Toxicol., 1984, 33, 538-547.

Krause, R.T., "Further Characterization and Refinement of an HPLC Post Column Fluorometric Labeling Technique for the Determination of Carbamate Insecticide", J. Chromatogr. Sci., 1978, 16, 281-288.

WRITTEN BY: Paul Lee and Sylvia Richman

Paul Lee & Sylvia Richman

TITLE: Agricultural Chemists III and II respectively

REVIEWED BY: Catherine Cooper

Catherine Cooper

TITLE: Agricultural Chemist III

APPROVED BY: Terry Jackson



TITLE: Quality Assurance Officer

APPROVED BY: S. Mark Lee



TITLE: Research Agricultural Chemist

CALIFORNIA DEPT. OF FOOD & AGRIC.
CHEMISTRY LABORATORY SERVICES
3292 Meadowview Road
Sacramento, CA 95832
(916)+427-4999

Original Date:12/10/1990
Supercedes: New
Current Date:12/10/1990
Method #:

OXADIAZON IN SURFACE WATER

SCOPE:

These methods are for the determination of oxadiazon in surface water. The detection limit of both methods is 0.1 ppb.

PRINCIPLE:

Solid Phase Extraction:

The water sample is passed through a Sep-Pak™ C18 cartridge. The retained oxadiazon is eluted from the cartridge with ethyl acetate. The eluant is then concentrated and analyzed by gas chromatography with electron capture detection.

Liquid/Liquid Extraction:

The surface water sample is extracted with methylene chloride. The extract is filtered through sodium sulfate to remove residual water. The dried extract is evaporated to dryness on a rotary evaporator and brought to 1 mL final volume with acetone. The extract is then analyzed by gas chromatography with electron capture detection.

REAGENTS AND EQUIPMENT:

1. Solvents: methanol, methylene chloride, acetone, ethyl acetate, pesticide residue grade
2. Sodium sulfate, anhydrous, granular
3. Oxadiazon stock standard solutions (1mg/mL): Dissolve 50 mg of oxadiazon in acetone and dilute to 50 mL in a volumetric flask.
4. Sep-Pak™ C18 cartridge, Waters Division of Millipore
5. Vortex mixer, Thermolyne Maxi Mix II, Syborn
6. Centrifuge, Clay-Adams
7. Nitrogen evaporator, Organomation Model #12
8. Rotary evaporator, Buchi/Brinkmann, R110
9. In-house vacuum manifold: consists of a 4 L side arm filtering flask with a #13 rubber stopper. A vacuum source is connected to the side arm with an in-line flow controller. Eight 4 mm holes are distributed evenly throughout the stopper. Eight 4", 4 mm O.D. glass tubes are inserted into the holes. The glass tubes are then connected to 3 ft, 4 mm I.D. tygon tubing. Another 4" glass tube with tapered ends is connected to the tygon tubing. The Sep-Paks are connected to the tapered glass tubing.

ANALYSIS:

Solid Phase Extraction:

1. Weigh 1000 g of well-mixed water sample into a 1 L beaker.
2. Connect a Sep-Pak™ C18 cartridge to the in-house vacuum manifold described above.
3. Condition the cartridge by aspirating 5 mL of methanol followed by 10 mL of distilled water through the cartridge. Do not allow the cartridge to run dry. Adjust the flow rate to about 15 mL/minute.
4. Pass the 1000 g water sample through the cartridge by dipping the Sep-Pak™ into the 1 L beaker. After the sample has passed through, allow the vacuum to remain on for 3 minutes.
5. Remove the cartridge from the glass tubing and place in a centrifuge tube. Centrifuge for 3 minutes at 1200 rpm.
6. Elute the cartridge with 4 mL of ethyl acetate and collect the eluant in a test tube. A small amount of residual water in the eluant is removed by adding 3 g of anhydrous sodium sulfate. Vortex the tube for about 20 seconds.
7. Transfer the dry eluant into a 15 mL graduated test tube. Wash the sodium sulfate with 2 mL ethyl acetate and also transfer the solvent into the same test tube.
8. Concentrate the eluant to a final volume of 1 mL under a gentle stream of nitrogen with no heat applied.

Liquid/Liquid Extraction:

1. Weight 800 g of well-mixed surface water into a 1 L separatory funnel.
2. Extract the sample with 100 mL of methylene chloride three times.
3. Dry the methylene chloride by passing it through 20 g of sodium sulfate in a glass funnel as it is transferred to a 500 mL boiling flask.
4. Evaporate the solvent with a rotary evaporator to dryness at 35°C and approximately 20 mm Hg vacuum.
5. Add 5 mL of acetone and swirl to dissolve the residue in the flask.
6. Transfer the extract to a 15 mL graduate test tube. Rinse flask 2 times each with 2 mL of acetone and transfer each wash to the same test tube.
7. Evaporate the extract to a final volume of 1 mL under a gentle stream of nitrogen with no heat applied.

EQUIPMENT CONDITIONS:

PRIMARY ANALYSIS:

Instrument: Varian 3700 GC with Electron Capture Detector
Column: HP-1, methyl silicone gum, 10 m x 0.53 mm x 2.65 um
Injector temperature: 210°C
Detector temperature: 300°C
Temperature program: 190°C for 1 min, increase at 10°C/min, to 240°C for 6 min.
Carrier gas flow rate: Helium, 20 mL/min
Sample injected vol.: 2 uL
Retention time: 3.97 ± 0.05 min

CONFIRMATION ANALYSIS:

Instrument: Varian 3700 GC with Thermionic Specific Detector
 Column: HP-5, 5% phenyl methyl silicone, 10 m x 0.53 mm x 2.65 um
 Injector temperature: 210°C
 Detector temperature: 250°C
 Temperature program: 190°C for 1 min, increase at 10°C/min, to 220°C for 4 min.
 Carrier gas flow rate: Helium, 20 mL/min
 Sample inject vol.: 2 uL
 Retention time: 3.90 ± 0.06 min

CALCULATIONS:

$$\text{ppb} = \frac{(\text{sample peak height})(\text{ng standard injected})(\text{ml sample final volume})1000}{(\text{standard peak height})(\text{uL sample injected})(\text{sample weight})}$$

RECOVERIES:

Solid Phase:

<u>Spike Levels</u>	<u>% Recovery (mean)</u>	<u>Standard Deviation</u>	<u>n</u>
0.1 ppb	94.6	6.8	5
1.0 ppb	102	5.0	5
10.0 ppb	98.0	3.8	5

Liquid/Liquid:

<u>Spike Levels</u>	<u>% Recovery (mean)</u>	<u>Standard Deviation</u>	<u>n</u>
0.1 ppb	92.2	4.2	5
1.0 ppb	102	3.1	5
10.0 ppb	97.6	7.2	5

The spike recoveries for oxadiazon were obtained by running each spike on five consecutive days.

DISCUSSION:

In this study, excellent recoveries were obtained by both solid phase and liquid/liquid extraction methods. For future applications, the fast and economical solid phase extraction method can be used for the analysis of clean surface water while the liquid/liquid extraction method can be used for dirty surface water.

Methylene chloride must be evaporated completely with the liquid/liquid extraction process. Otherwise, a trace amount of methylene chloride will interfere with chromatography using electron capture detection.

REFERENCES:

Shibamoto, T. S. and C. R. Mourer, "Report of Method for Analysis of Antor in Water to 0.1 ppb," Trace Analytical Laboratory, Department of Environmental Toxicology, University of California, Davis, Jan. 1990.

WRITTEN BY: Jean Hsu

Jean Hsu
TITLE: Agricultural Chemist I

REVIEWED BY: Catherine Cooper

Catherine Cooper
TITLE: Agricultural Chemist III

APPROVED BY: S. Mark Lee

S Mark Lee
TITLE: Research Agricultural Chemist

APPROVED BY: Terry Jackson

Terry Jackson
TITLE: Quality Assurance Officer

STANDARD
OPERATING
PROCEDURE

Subject or Title:
ORGANOPHOSPHOROUS PESTICIDES IN WATER BY GC/MS

Page 1 of

SOP No.:
LM-CAL-

Revision No.:
ORIGINAL

Effective Date:

Supersedes: NONE

1. SCOPE AND APPLICATION

- 1.1 To determine the residual levels of 43 organophosphorous pesticides and selected oxygen analogs in water. The compounds are listed in Sec 1.4.
- 1.2 The standard range for the organophosphorous pesticides by gas chromatography/mass spectrometry (GC/MS) is 0.1 ug/mL to 1.0 ug/mL. The final concentration of the sample extract is 1000 mL/1.0 mL; this gives a detection limit of 0.1 ug/L (parts per billion) for most compounds.
- 1.3 Some of the organophosphorous compounds such as acephate, methamidophos, diazoxon, oxydemeton-methyl, and omethoate are either not recovered or not detected by this method.
- 1.4 Below are the list of the 43 organophosphorous pesticides, oxygen analogs, and the Chemical Abstract Service (CAS) number, if available.

Analyte	CAS No.
1 Azinphos-methyl (guthion)	86-50-1
2 Azinphos-methyl OA (guthion OA)	
3 Bensulide (prefar)	741-58-2
4 Bensulide OA	
5 Chorpyrifos (dursban)	2921-88-2
6 Chlorpyrifoxon	
7 DDVP (dichlorvos)	62-73-7
8 Demeton-O (systox-II)	126-75-0
9 Demeton-S (systox-I, disulfoton OA)	298-03-3
10 Demeton-S sulfone	
11 Demeton-S sulfoxide	

Prepared By:
Dennis E. Gall

Date:
January 2, 1991

Management Approval:

Date:

QA Officer Approval:

Date:

Subject or Title:
ORGANOPHOSPHOROUS PESTICIDES IN WATER BY GC/MS

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	<u>Analyte</u>	<u>CAS No.</u>
12	Diazinon	333-41-5
13	Dimethoate	60-51-5
14	Disulfoton (disyston)	298-04-4
15	Disulfoton sulfone	2497-06-5
16	Disulfoton sulfoxide	
17	Ethoprop (prophos, mocap)	13194-48-4
18	Fenamiphos (nemacur)	22224-92-6
19	Fenamiphos sulfoxide (nemacur sulfoxide)	
20	Fenamiphos sulfone (nemacur sulfone)	
21	Fensulfothion	115-90-2
22	Fensulfothion OA (fenson)	
23	Fonofos (dyfonate)	944-22-9
24	Fonofoxon	
25	Malathion	121-75-5
26	Malaoxon	1634-78-2
27	Methidathion (supracide)	950-37-8
28	Methyl Parathion	290-00-0
29	Methyl Paraoxon (methyl parathion OA)	
30	Mevinphos (phosdrin)	298-01-1
31	Naled (dibrom)	300-76-5
32	Ethyl Parathion (parathion)	56-38-2
33	Paraoxon (parathion OA)	311-45-5
34	Phorate (thimet)	298-02-2
35	Phorate sulfoxide	
36	Phorate sulfone	
37	Phoratoxon (phorate OA, thimet OA)	
39	Phoratoxon sulfone (phorate OA sulfone)	
40	Phosalone (zolone)	2310-17-0
41	Phosmet (imidan)	732-11-6
42	Phosmet OA	
43	Sulfotepp	3689-24-5
38	Phoratoxon sulfoxide (phorate OA sulfoxide)	

2. METHOD SUMMARY

Organophosphorous pesticides and some of the oxygen analogs are extracted from water with methylene chloride. Sodium chloride is added to increase the ionic strength of water. The methylene chloride extract is dried with anhydrous sodium sulfate, concentrated first by Kuderna-Danish apparatus and then under a stream of nitrogen to final volume and analyzed a by GC/MS.

3. COMMENTS

3.1 Extraction and analysis should be conducted by trained personnel.

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Effective Date:

Analyte	CAS No.
39 Phoratoxon sulfone (phorate OA sulfone)	
40 Phosalone (zolone)	2310-17-0
41 Phosmet (imidan)	732-11-6
42 Phosmet OA	
43 Sulfotepp	3689-24-5

2. METHOD SUMMARY

Organophosphorous pesticides and some of the oxygen analogs are extracted from water with methylene chloride. Sodium chloride is added to increase the ionic strength of water. The methylene chloride extract is dried with anhydrous sodium sulfate, concentrated first by Kuderna-Danish apparatus and then under a stream of nitrogen to final volume and analyzed a by GC/MS.

3. COMMENTS

- 3.1 Extraction and analysis should be conducted by trained personnel.
- 3.2 Personnel must be familiar with Enseco policies for documentation and record keeping, and general laboratory safety procedures.
- 3.3 All glassware must be rinsed with three solvents as usual (acetone, hexane, and methylene chloride).
- 3.4 The analyst is responsible for the following areas:
 - 3.4.1 Adhering to the method without deviation. Any modification to the method can only be done with the written approval of the supervisor and documented in logbook or on a benchsheet.
 - 3.4.2 Complete and accurate documentation for a particular project.
 - 3.4.3 Documentation of any anomalies that were encountered during the extraction procedure.

4. SAFETY ISSUES

DRAFT

STANDARD OPERATING PROCEDURE

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ORGANOPHOSPHOROUS PESTICIDES IN WATER BY GC/MS

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-
- 4.1 This method utilizes chemicals that are known carcinogens. Protective gear such as safety glasses, lab coats and gloves must be worn at all times when performing this analysis.
 - 4.2 Take extreme precautions when handling acids and bases, especially concentrated reagents.
 - 4.3 Personnel should be familiar with the location of acid, base and solvent spill kits and with the location and use of fire extinguishers.
 - 4.4 All extractions must be carried out in the hood.

5. APPARATUS

5.1 Equipment.

- 5.1.1 Electronic top-loading analytical balance, readable to 0.0001 grams; for preparation of analytical standards.
- 5.1.2 Electronic top-loading balance, readable to 0.1 grams and a capacity up to ~ 3500 grams; for weighing water samples and solid reagents.
- 5.1.3 N-Evaporator, Organomation Analytical Associates model 112 or equivalent; for reducing extracts to smaller volumes (~ 1 mL).
- 5.1.4 Water bath, temperature-controlled at 80-85°C ; for concentrating methylene chloride extracts by KD.

5.2 Glassware.

- 5.2.1 Filtering Funnel, 100-mm, glass, short-stem or stemless; for containing a pad of anhydrous sodium sulfate to dry methylene chloride extracts.
- 5.2.2 Kuderna-Danish Apparatus (KD), glass; for concentrating extracts to smaller volumes (~ 5 mL).
 - 5.2.2.1 Reservoir, 500-mL.
 - 5.2.2.2 Concentrator tube, 10-mL.

Subject or Title:
ORGANOPHOSPHOROUS PESTICIDES IN WATER BY GC/MS

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5.2.2.3 Macrosnyder 3-ball column.

5.2.3 Separatory Funnel, 2000-mL, glass, with glass or teflon stopper and with teflon stopcock.

6. REAGENTS AND STANDARDS

6.1 Reagents.

6.1.1 Acetone, pesticide quality.

6.1.2 Methylene chloride, pesticide quality; the extraction solvent.

6.1.3 Sodium chloride, granular, analytical reagent grade.

6.1.4 Sodium sulfate, anhydrous, granular, analytical reagent grade; pre-rinsed and dried before using.

6.2 Preparation of organophosphorous pesticide standards.

6.2.1 Preparation of stock solutions.

6.2.1.1 Obtain individual stock solutions of the organophosphorous pesticides at 0.10 to 10 mg/mL in acetone or toluene, either by preparing from neat or by ordering from suppliers such as Nanogen Analytical Standards. Store the solution in a refrigerator and replace the solution every twelve months or sooner from the date of preparation, if known, or from the date of receipt.

6.2.2 Preparation of fortification solutions and analytical GC/MS solutions.

NOTE: Due to the number of analytes (43) in the analysis, the analytes are divided into groups to achieve better separation and analysis. See Table 1, Appendix I

6.2.2.1 Prepare a 50 ug/mL mixed standard in acetone to a total volume of 25 to 50 mL from the appropriate individual stock solutions. Store the solution in a

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glass amber bottle and replace the solution every six months or sooner.

6.2.2.2 Using the mixed analyte solution from 6.2.2.1, prepare a fortification standard at 1.0 ug/mL in acetone to a total volume of 50 to 100 mL. Store the solution in a glass amber bottle and replace the solution every six months or sooner.

6.2.2.3 Using the mixed analyte solution from 6.2.2.1, prepare a five point calibration curve (0.1 ug/mL, 0.4 ug/mL, 1.0 ug/mL, 2.0 ug/mL, and 10 ug/mL) in methylene chloride. Replace the solution every six months or sooner.

6.2.3 Reference Standards

6.2.3.1 Prepare reference standards to verify the quality of the fortification solutions and analytical standards from 6.2.2.

6.2.3.2 Reference standard is any standard solution made from a source other than the stock standard. Reference standard may be from the EPA, the manufacturer, or from another reliable source.

6.2.3.3 If the secondary source is not available, a separate intermediate stock solution will be made from the same neat by another chemist or from a neat with a different lot number.

7. PROCEDURE

7.1 EXTRACTION SET UP

7.1.1 Label the Cal id with the suffix 'OP-GCMS'.

7.1.2 Measure the volume of the sample in the 1-L amber glass bottle into a 2-L separatory funnel.

7.1.3 If applicable, add the appropriate fortification solution to the sample prior to extraction to yield the appropriate fortification level:

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- 7.1.3.1 For a 1.0 ppb level, add 100 μ L of the 10 μ g/mL standard.
- 7.1.4 Add 100 g of sodium chloride and shake well to dissolve.
- 7.1.5 Extract the sample serially with three 60 mL portions of methylene chloride, shake 2 minutes each time and vent frequently. Collect the methylene chloride extracts into a 250-mL erlenmeyer flask or pass it through sodium sulfate as described below.
- 7.1.6 Pass the methylene chloride extract through approximately 30 grams of anhydrous sodium sulfate into the KD apparatus.
- 7.1.7 Rinse the sodium sulfate well with approximately 30 mL of methylene chloride and collect in with the extract.
- 7.1.8 Carefully concentrate the extract to approximately 5 mL using steam bath at 80-85°C.
- 7.1.9 Reduce the extract under nitrogen to approximately 1 mL.
- 7.1.10 Transfer the extract quantitatively with small amount of methylene chloride (e.g., two 0.5 mL portions) to a 2 mL vial.
- 7.1.11 Carefully, reduce the extract under nitrogen and adjust the volume to 1.0 mL with methylene chloride. Do not use the water bath, and do not allow it to go dry.
- 7.1.12 Final sample concentration; (1000) mL/1.0 mL. Sample is now ready for GC/MS analysis.
- 7.1.13 Organize the vials in a vial box, and store in the refrigerator at 2-6°C until analysis.

7.2 INSTRUMENT OPERATING CONDITIONS

- 7.2.1 Analysis is conducted using a gas chromatograph equipped with a mass spectrometer (GC/MS).
- 7.2.2 Analytical Conditions
 - 7.2.2.1 See Table 2, Appendix I

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7.2.3 Standard/Sample Analysis

7.2.3.1 Spike 1 mL of the sample extract or analytical standard with 5.0 uL of 1.0 mg/mL 2,2'-difluorobiphenyl (internal standard) prior to injection.

7.2.3.2 Inject a 2 uL aliquot of the standard or sample into the GC/MS and analyze by selected ion monitoring (SIM) and monitor all of the ions in Table 3, Appendix I.

8. QA/QC REQUIREMENTS

8.1 The method blank is mandatory and is performed for each type of matrix used and for every 20 samples, or as per protocol specifications.

8.2 The matrix spike and the matrix spike duplicate are optional and must be requested. They are performed for each matrix and for every 20 samples, or as per protocol specifications.

9. CALCULATIONS

9.1 A standard curve of the 0.1 ug/mL, 0.4 ug/mL, and 1.0 ug/mL calibration standards will be injected prior to the analysis of the field samples.

9.2 Calculate the Relative Response Factor (RRF) for each analyte at each level using the following equation:

$$RRF = \frac{(Area A) (Area IS)}{(Area IS) (Amt A)}$$

Where: Area A = Area of the target analyte
Area IS = Area of the Internal Standard (IS)
Amt A = Concentration of the target analyte
Amt IS = Concentration of the IS

9.3 If the percent relative standard deviation is less than 30% over the working range, then linearity can be assumed and the average response factor is used for the sample calculations.

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9.4 The concentration of each of the target analytes in a sample is calculated as follows:

$$\text{Concentration (ug/L)} = \frac{(\text{Area S}) (\text{Amt IS}) (V_x)}{(\text{Area IS}) (\text{RRF}_a) (V_s) (1000)}$$

Where: Area S = Area of the sample
Area IS = Area of the IS
Amt IS = Amount of IS added
RRF_a = Average Relative Response Factor
V_x = Final volume of the extract
V_s = Initial volume of the sample

10. REPORTING REQUIREMENTS

10.1 The detection limit and "dynamic range" for the analysis for each of the analytes is listed in Table 4, Appendix I. Report lower reporting limits if possible.

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Appendix I

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Table 1

**GROUP 1A - ALL PARENTS EXCEPT
DDVP AND SULFOTEP**

Mevinphos (phosdrin)
Naled (dibrom)
Phorate (thimet)
Dimethoate
Fonofos (dyfonate)
Disulfoton (disyston)
Methyl parathion
Chlorpyrifos
Methidathion (supracide)
Fensulfothion
Phosalone
Bensulide (prefar)

**GROUP 1B - ALL PARENTS EXCEPT
DDVP AND SULFOTEP**

Ethoprop (prophos, mocap)
Demeton-O (systox-II)
Demeton-S (systox-I)
Diazinon
Malathion
Ethyl parathion (parathion)
Fenamiphos (nema-cur)
Phosmet
Azinphos-methyl (guthion)

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Table 1 (cont)

GROUP 2A - OA AND SULFOXIDES

Fonofoxon
Methyl paraoxon
Phoratoxon sulfoxide
Phorate sulfoxide
Chlorpyrifoxon
Demeton-S sulfoxide
Phosmet OA
Guthion OA

GROUP 2B - OA AND SULFOXIDES

Phoratoxon
Malaaxon
Para-oxon
Fensulfothion OA (fenson)
Disulfoton sulfoxide
Nemacur sulfoxide
Bensulide OA

**GROUP 3 - DDVP, SULFOTEPP,
AND SULFONES**

DDVP (dichlorvos)
Sulfotepp
Phoratoxon sulfone
Phorate sulfone
Demeton-S sulfone
Disulfoton sulfone
Nemacur sulfone

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Table 2

GC/MS Operating Conditions

Column: DB-5 or equivalent

Column Dimensions: 60 m X 0.32 mm id

Film Thickness: 0.25 um

GC Temperature settings:

Injector: 260°C

Transfer line: 290°C

Column: (program)

Initial Temp: 100°C

Initial Hold: 4 minutes

Program Rate: 8°C/minute

Final Temp: 310°C

Final Hold: 2 minutes

Carrier gas: Helium

Mass Spectrometer Conditions:

Electron energy : 70eV

Scan rate: 1 scan/sec.

Source Temp: 150°C

Manifold Temp: 70°C

Note: If a change to the GC/MS conditions is necessary, it will be noted on the GC/MS chromatogram or data sheet.

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Table 3

Compound	Ion Monitored	Retention Time
DDVP (dichlorvos)	109	10:14
Disulfoton Sulfoxide	97	10:28
Mevinphos (phosdrin)	127	13:47
Demeton-S (systox-I)	88	17:01
Phoratoxon	81	17:07
Ethoprop (prophos, mocap)	158	17:22
Naled (dibrom)	109	17:48
Sulfotepp	322	18:10
Phoratoxon Sulfone	109	18:11
Phorate (thimet)	75	18:22
Fonofoxon	93	18:25
Phorate Sulfoxide	125	18:25
Demeton-O (systox-II)	60	18:49
Dimethoate	87	18:52
Fonofos (dyfonate)	109	19:44
Diazinon	137	19:55
Methyl Paraoxon	96	20:04
Disulfoton (disyston)	88	20:05
Phoratoxon Sulfoxide	81	NA
Phoratoxon Sulfone	109	21:09
Malaaxon	127	21:14
Methyl parathion	125	21:17
Paraoxon	81	21:37
Malathion	173	22:16
Chlorpyrifoxon	197	22:27
Phorate Sulfone	97	22:28
Chlorpyrifos	97	22:37
Ethyl Parathion (parathion)	109	22:38
Fenson	268	22:58
Demeton-S Sulfone	197	23:17
Demeton-S Sulfoxide	109	NA
Methidathion (supracide)	85	24:10
Disulfoton Sulfone	97	24:24
Fenamiphos (nemacur)	154	24:39
Fensulfothion	97	26:00
Phosmet OA	160	27:10
Nemacur Sulfoxide	122	27:59

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Table 3 (cont)

Compound	Ion Monitored	Retention Time
Guthion OA	132	28:09
Nemacur Sulfone	320	28:00
Phosmet	160	28:20
Phosalone	182	29:12
Azinphos-methyl (guthion)	160	29:15
Bensulide (prefar)	128	NA
Bensulide OA	77	NA

Internal Standard

2,2'-Difluorobiphenyl	190	12:25
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Table 4

Compound	Dynamic Range
DDVP (dichlorvos)	
Mevinphos (phosdrin)	
Demeton-S (systox-I)	
Phoratoxon	
Ethoprop (prophos, mocap)	
Naled (dibrom)	
Sulfotepp	
Phorate (thimet)	
Fonofoxon	
Demeton-O (systox-II)	
Dimethoate	
Fonofos (dyfonate)	
Diazinon	
Methyl Paraoxon	
Disulfoton (disyston)	
Phoratoxon Sulfone	
Phoratoxon Sulfoxide	
Malaaxon	
Methyl parathion	
Paraoxon	
Malathion	
Chlorpyrifoxon	
Phorate Sulfone	
Phorate Sulfoxide	
Ethyl Parathion (parathion)	
Chlorpyrifos	
Demeton-S Sulfone	
Demeton-S Sulfoxide	
Methidathion (supracide)	
Disulfoton Sulfone	
Disulfoton Sulfoxide	
Fenamiphos (nemacur)	
Fensulfothion	
Phosmet OA	
Nemacur Sulfoxide	
Guthion OA	
Nemacur Sulfone	

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Table 4 (cont)

Compound	Dynamic Range
Phosmet	
Phosalone	
Azinphos-methyl (guthion)	
Bensulide (prefar)	
Bensulide OA	
Fensulfothion OA (fenson)	

CALIFORNIA DEPT. OF FOOD & AGRIC.
CHEMISTRY LABORATORY SERVICES
ENVIRONMENTAL MONITORING SECTION
3292 Meadowview Road
Sacramento, CA 95832
(916)+427-4998/4999

Original Date: April 1, 1990.
Supercedes: NEW
Current Date: July 30, 1990
Method #:

DETERMINATION OF CARBOFURAN,
METHYL PARATHION AND MALATHION IN WATER

SCOPE:

This method is for the determination of carbofuran, methyl parathion and malathion in water samples from rice field drains. The sensitivity of this method is 0.05 ppb for carbofuran and 0.1 ppb for methyl parathion and malathion.

PRINCIPLE:

Residues in water are extracted with methylene chloride. After evaporating the solvent, the extracts are dissolved in acetone and analyzed by GC for methyl parathion and malathion, and by HPLC for carbofuran. The recoveries for all three compounds are above 90%.

REAGENTS AND EQUIPMENT:

Solvents: methylene chloride and acetone (pesticide residue grade)
 water and methanol (HPLC grade)
Sodium sulfate (anhydrous)
Separatory funnels (1 L)
Boiling flasks, flat bottomed, 24/40 joints (500 ml)
Glass stemmed funnels
Rotary evaporator (Büchi/Brinkmann, Model R 110)
Graduated test tubes (15 ml)
Nitrogen evaporator (Organomation, Model 12)
Vortex mixer (Thermolyne, Model 37600)
Analytical balance (Mettler, PC 4400)
Filter paper (Whatman #4, 15 cm)

ANALYSIS:

1) Remove samples from refrigerated storage and allow them to come to room temperature. Samples consist of approximately 1 L and are stored in 1 L amber glass bottles to prevent photodegradation.

2) Shake samples and weigh out approximately 800 g by difference. Place this aliquot into 1 L separatory funnel.

3) Extract samples by adding 100 ml of methylene chloride and shaking vigorously for one minute.

4) Allow layers to separate. Drain the organic layer into a 500 ml flask through a 9 cm funnel containing 25 g anhydrous sodium sulfate supported by filter paper.

5) Repeat steps 3 and 4 two more times using 80 ml of methylene chloride.

6) Rinse sodium sulfate with 20 ml additional methylene chloride and collect in the same 500 ml boiling flask.

7) Take extract just to dryness on a rotary evaporator at a vacuum of ca. 15 in Hg, using a 30-35°C water bath.

8) Dissolve the residue in about 2 mls of acetone and transfer to a precalibrated 15 ml conical centrifuge tube. Rinse the flask three times with 1-2 ml of acetone each time. Combine the extracts.

9) Place extract in a nitrogen evaporator with water bath set at 35°C and evaporate to a final volume of 1 ml under a gentle stream of nitrogen.

10) Stopper the graduated test tube and mix contents by vortexing for about 15 seconds. Submit extracts to GC analysis for malathion and methyl parathion, or to HPLC analysis for carbofuran.

EQUIPMENT CONDITIONS:

ANALYSIS FOR CARBOFURAN

PRIMARY ANALYSIS (HPLC)

Chromatograph: Hewlett-Packard 1090 Liquid Chromatograph
 Hewlett-Packard 1046A Programmable Fluorescence Detector
 Pickering Labs Post-Column Derivatization System (1951
 Colony St., Suite S * Mountain View, CA 94043)

Column: Beckman Ultrasphere ODS, 4.6 mm x 15 cm x 5 um

Flow: 1.0 ml/min

Injection volume: 25 ul

Gradient:	Time (min)	% Water	% Methanol
	0.2	95.0	5.0
	3.0	95.0	5.0
	15.0	1.0	99.0
	16.0	1.0	99.0
	20.0	95.0	5.0

Retention time of Carbofuran: 14.508 ±0.004 min (n = 10)

Fluorescence Detector: Excitation - 340 nm
 Emission - 450 nm
 PMT Gain - 12
 Lamp Frequency - 110 Hz
 Response Time - 1000 msec
 Gate - 0.03 msec

CALCULATIONS:

Parts per billion of pesticide:

$$\text{PPB} = \frac{(\text{peak area sample})(\text{ng std. injected})(\text{sample final vol.})(1000 \text{ ul/ml})}{(\text{peak area std})(\text{ul sample injected})(\text{g of sample})}$$

DISCUSSION:

The minimum detection limit for carbofuran using this method is 0.05 ppb. Method validation recoveries for carbofuran are as follows:

<u>Spike Level</u> ppb	<u>Carbofuran</u> % Recovery (X)	<u>SD</u>	<u>n</u>
0.1	96.0	5.48	5
0.5	92.8	3.03	5
2.0	103	5.50	5
10.0	104	5.48	5
Overall	99.0	6.70	20

The minimum detection limit for malathion and methyl parathion using this method is 0.1 ppb. Method validation recoveries for malathion and methyl parathion are listed below:

<u>Spike Level</u> ppb	<u>Methyl Parathion</u> % Recovery (X)	<u>SD</u>	<u>n</u>
0.1	108	8.37	5
0.5	94.0	4.90	5
2.0	92.9	3.85	5
10.0	98.0	2.90	5
Overall	98.2	7.87	20

<u>Spike Level</u> ppb	<u>Malathion</u> % Recovery (X)	<u>SD</u>	<u>n</u>
0.1	102	4.47	5
0.5	104	5.55	5
2.0	96.9	2.22	5
10.0	105	4.83	5
Overall	102	5.09	20

REFERENCES:

Muth, G.L., Erro, F., "A Rapid Carbamate Multiresidue Procedure for Vegetable Crops", Bull. Environ. Contam. Toxicol., 1980, 24, 759-765.

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"Method 8141, Organophosphorus Pesticides, Capillary Column", EPA Test Methods for Evaluating Solid Waste. Revised Methods, 1987.

WRITTEN BY: Karen Hefner and Sylvia Richman

Karen Hefner Sylvia Richman

TITLE: Agricultural Chemists II

REVIEWED BY: Catherine Cooper

Catherine Cooper

TITLE: Agricultural Chemist III

APPROVED BY: Terry Jackson

Terry Jackson

TITLE: Quality Assurance Officer

APPROVED BY: S. Mark Lee

Tom Joe (Acting)

TITLE: Research Agricultural Chemist

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STANDARD
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Subject or Title:
Triazines in Water by LC/MS/MS (Short Method)

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November 3, 1990

Supersedes: LM-CAL-3046, May 11, 1990

1. PRINCIPLE

The five components listed below are extracted from water using a C18 SPE device and analyzed by Liquid Chromatography Thermospray Mass Spectrometry/Mass Spectrometry (LC/MS/MS). The analytes are quantified by use of an internal standard (d_5 -Atrazine), which is added prior to analysis. The method detection limit with a 100 mL/1.0 mL concentration factor is 0.1 ug/L.

Figure 1

<u>Test Component</u>	<u>Class</u>	<u>CAS Number</u>
Simazine	Triazine	122-34-9
Atrazine	Triazine	912-24-9
Prometon	Triazine	1610-18-0
Bromacil	Urea	314-40-9
Diuron	Urea	330-54-1

2. STANDARDS

2.1 Internal Standards

2.1.1 Prepare a 1 mg/mL atrazine-ethylamine- d_5 stock solution in acetone. Replace once a year.

2.1.2 Prepare a 10 ug/mL atrazine-ethylamine- d_5 standard in acetone from the 1 mg/mL stock. Replace every six months.

Prepared By:
DENNIS E. GALL

Date:
9-17-90

Management Approval:

Calvin A. Smith

Date:
11/2/90

QA Officer Approval:

B. McNeill

Date:
11/6/90

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2.2 Fortification and Analytical Standards

- 2.2.1 Prepare a 1 mg/mL individual stock solution of each analyte in HPLC grade methanol. Replace stock solution once a year.
- 2.2.2 Using 1 mg/mL individual stock solutions from 2.2.1 prepare a 10 ug/mL standard mix in methanol. Replace mixed analyte solution every six months.
- 2.2.3 Using the 10 ug/mL mixed analytical standard solution from 2.2.2, prepare a 1.0 ug/mL and a 0.10 ug/mL spiking standard in methanol. Replace the spiking standard every six months.
- 2.2.4 Using the 10 ug/mL and 1.0 ug/mL mixed analytical standard solutions from 2.2.2 and 2.2.3., prepare standards at 5 ng/mL, 10 ng/mL, 25 ng/mL, 100 ng/mL, and 250 ng/mL in 1:1 methanol-water, and to each solution include atrazine ethylamine-d₅ at 50 ng/mL from 2.1.2. A wider range may be used for the curve providing linearity criteria are met. Replace calibration standards every six months.

2.3 Reference Standards

- 2.3.1 Prepare reference standards to verify the quality of the fortification solutions, analytical standards, and the internal standards above (2.1 and 2.2).
- 2.3.1.1 Reference standard is any standard solution made from a source other than the stock standard. Reference standard may be from the EPA, the manufacturer, or from another reliable source.
- 2.3.1.2 If the secondary source is not available, a separate intermediate stock solution will be made from the same neat by another chemist or from a neat with a different lot number.

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3. PROCEDURE

3.1 Extraction

- 3.1.1 Add 100 mL of sample to a 250 mL centrifuge bottle. If applicable, prepare fortification samples by adding fortification standard.
- 3.1.1.1 Add 100 μ L of the 0.10 μ g/mL fortification standard to yield 0.10 μ g/L (ppb).
- 3.1.1.2 Add 0.5 mL of the 0.1 μ g/mL fortification standard to yield 0.5 μ g/L (ppb).
- 3.1.1.3 Add 100 μ L of the 1.0 μ g/mL fortification standard to yield 1.0 μ g/L (ppb).
- 3.1.2 Centrifuge for 10 minutes at about 3800 rpm.
- 3.1.3 Set up the 3 mL C18 SPE columns and the manifold.
- 3.1.3.1 Rinse with two column volumes of methanol followed by two column volumes of distilled water using vacuum. **DO NOT ALLOW TO GO DRY AT THIS STAGE.**
- 3.1.3.2 Turn off vacuum. Load sample on column and attach the 75-mL reservoir. Load the sample in reservoir and turn on vacuum at 10 inches of Hg or less.
- 3.1.3.3 Add 10 mL of distilled water to the sample container and centrifuge for approximately 10 minutes at about 3800 rpm. Pour this rinse onto the column. Do not let the column go dry until this step is completed.
- 3.1.3.4 Allow the column to dry for 5 minutes using vacuum.
- 3.1.4 Elute the column with 2.0 mL of Methanol; collect the eluate in an 8-mL test tube using vacuum at or below 5 inches of Hg.
- 3.1.5 Filter the extract through a 0.45 μ m syringe filter into a 4 mL vial. Rinse the syringe filter by pushing 0.5 mL MeOH through the filter, collecting the rinse in the vial.

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- 3.1.6 Reduce the volume of the eluate under nitrogen to less than 0.5 mL. The nitrogen flow can be quite strong.
- 3.1.7 Add 5 uL of the 10 ug/mL atrazine-ethylamine-d₅ standard solution for internal quantitation to each extract.
- 3.1.8 Adjust the final volume to 1.0 mL with distilled water.
- 3.1.9 Organize the sample vials into the vial box, and store it in the refrigerator until analysis.

4. LIQUID CHROMATOGRAPH OPERATING PARAMETERS

- 4.1 The LC Chromatography system parameters have been optimized for these analytes as follows:

Column: 100mm X 2.1mm (5u) Hypersil or equivalent

Flow: 0.4 mL/min

Mobile Phase: Acetonitrile/Water (0.1M ammonium acetate)

Gradient: 35/65 Acetonitrile/Water
Isocratic
Variations on these conditions are allowed.

Post Column: Water (0.1M Ammonium acetate) at 0.8 mL/min

Approximate Retention Times:	Bromacil	1:47
	Simazine	2:00
	Atrazine	3:16
	Prometon	3:49
	Diuron	3:42

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Triazines in Water by LC/MS/MS (Short Method)

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November 3, 1990

5. TSP - MASS SPECTROMETER OPERATING PROCEDURES

5.1 Instrument Tuning

- 5.1.1 Generally, lens tuning with reagent ions or a background peak is sufficient. In any event, the instrument must be tuned and calibrated in the Q1MS, Q3MS, and MS/MS modes.
- 5.1.2 It has been determined that optimal transmission and product ion generation is achieved at collision energy = -13V and collision pressure = 2 mtorr
- 5.1.3 The instrument must be capable of detecting 100 pg of each analyte, (20 uL of 5 ng/mL) with a signal/noise (S/N) ratio of 10:1.

5.2 TSP-LCMS Operation Parameters

- 5.2.1 The following TSP-LCMS parameters must be optimized in order to achieve an optimum performance. Below are suggested operating parameters:

Vaporizer Control Point - 100 °C
Aerosol Temp - 250 °C
Repeller Voltage - 40 V

- 5.2.2 It has been determined that optimal sensitivity is achieved with filament on ionization mode, however, either mode is acceptable.

5.3 Selected Ion Monitoring

- 5.3.1 Samples and standards are analyzed by selected reaction monitoring (SRM) of product ions derived from the protonated molecular ion.
- 5.3.2 The appropriate masses to be monitored are listed below:

Analyte	Precursor (MH ⁺)	Product Ion	Dwell Time
Bromacil	m/z 261	m/z 205 ± 0.3 amu	0.2 seconds
Simazine	m/z 202	m/z 132 ± 0.3 amu	0.2 seconds
Atrazine	m/z 216	m/z 174 ± 0.3 amu	0.2 seconds
Prometon	m/z 226	m/z 184 ± 0.3 amu	0.2 seconds
Diuron	m/z 233	m/z 72 ± 0.3 amu	2.0 seconds

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5.4 Calibration and Sample Analysis

5.4.1 During the course of analysis of a batch of samples each of the calibration standards (see Section 2.1.4) is run once. Between 1 and 10 samples are analyzed between standards.

5.4.2 Calculate the response factor (RF) for each analyte at each level defined as follows:

$$RF = \frac{(\text{Area A}) (\text{Amt IS})}{(\text{Area IS}) (\text{Amt A})}$$

Where: Area A = Area response of analyte
Area IS = Area response of Internal Standard (I.S.)
Amt A = Concentration of analyte
Area IS = Concentration of I.S.

5.4.3 If the percent relative standard deviation is less than 20% over the working range, linearity can be assumed and the average response factor is used for sample calculations.

5.4.4 Inject 20-50 uL of standard/extract into the TSP-LCMS system for analysis.

6. CALCULATIONS

6.1 The concentration of each analyte in a sample is calculated as follows:

$$\text{Concentration (ug/L)} = \frac{(A_x) (\text{AmtIS}) (V_x)}{(AIS) (RFA) (V_s) (1000)}$$

RFA = Average Response Factor
A_x = Sample Total Area Counts
V_x = Sample Extract Final Volume (mL)
V_s = Initial Sample Volume (L)
AIS = Internal Standard Area Response
AmtIS = Internal Standard Amount (ug/mL)

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STANDARD
OPERATING
PROCEDURE

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Supersedes: January 24, 1990		

1. PRINCIPLE

Triazines (listed below) and Bromacil are extracted from water using a C18 SPE device and analyzed by Liquid Chromatography Thermospray Mass Spectrometry/Mass Spectrometry (LC/MS/MS). The analytes are quantified by use of an internal standard (d₅-Atrazine), which is added prior to analysis. The method detection limit with a 100 mL/1.0 mL concentration factor is 0.1 ug/L. The structures of the triazines are presented in Figure 1.

Figure 1

<u>Test Component</u>	<u>CAS Number</u>
Simazine	122-34-9
Cyanazine	21725-46-2
Metribuzin	21087-64-9
Atrazine	1912-24-9
Prometon	1610-18-0
Ametryn	834-12-8
Propazine	139-40-2
Prometryn	7287-19-6
Terbutryn	886-50-0
Bromacil	314-40-9

2. STANDARDS

2.1 Internal Standards

- 2.1.1 Prepare a 1 mg/mL atrazine-ethylamine-d₅ stock solution in acetone.
- 2.1.2 Prepare a 10 ug/mL atrazine-ethylamine-d₅ standard in acetone from the 1 mg/mL stock.

Prepared By: BOB BETHEM	Date: 1-08-90
Management Approval: <i>Walter A. Mills</i>	Date: May 11, 1990
QA Officer Approval: <i>B McNeil</i>	Date: May 11, 1990

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2.2 Fortification Standards

- 2.2.1 Prepare a 1 mg/mL individual stock solution of each analyte in HPLC grade methanol. Replace stock solution every six months.
- 2.2.2 Using 1 mg/mL individual stock solutions from 2.2.1 prepare a 10 ug/mL standard mix in methanol. Replace mixed analyte solution every six months.
- 2.2.3 Using the 10 ug/mL mixed analytical standard solution from 2.2.2, prepare a 0.10 ug/mL spiking standard in methanol. Replace the spiking standard every six months.
- 2.2.4 Using the 10 ug/mL standard solution from 2.2.2, prepare standards at 0.005 ug/mL, 0.025 ug/mL, 0.05 ug/mL, and 0.1 ug/mL in methanol. Replace calibration standards every six months.

3. PROCEDURE

3.1 Extraction

- 3.1.1 Add 100 mL of sample to a 250 mL centrifuge bottle. If applicable, prepare fortification samples by adding fortification standard.
 - 3.1.1.1 Add 100 uL of the 0.10 ug/mL fortification standard to yield 0.10 ug/L (ppb).
 - 3.1.1.2 Add 0.5 mL of the 0.1 ug/mL fortification standard to yield 0.5 ug/L (ppb).
 - 3.1.1.3 Add 100 uL of the 1.0 ug/mL fortification standard to yield 1.0 ug/L (ppb).
- 3.1.2 Centrifuge for 10 minutes at about 3800 rpm.
- 3.1.3 Set up the 3 mL C18 SPE columns and the manifold.

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- 3.1.3.1 Rinse with two column volumes of methanol followed by two column volumes of distilled water.
- 3.1.3.2 Allow the column to dry for approximately 5 minutes.
- 3.1.3.3 With the vacuum setting at 10 inches of Hg or lower, condition the column with one column volume of distilled water. Do not let the column go dry. Attach the 75-mL reservoir and load the sample.
- 3.1.3.4 Add 10 mL of distilled water to the sample container and centrifuge for approximately 10 minutes at about 3800 rpm. Pour this rinse onto the column. Do not let the column go dry until this step is completed.
- 3.1.3.5 Allow the column to dry for 5 minutes using vacuum.
- 3.1.4 Elute the column with 2.0 mL of Methanol collecting the elute in an 8-mL test tube.
- 3.1.5 Filter the extract through a 0.45 um syringe filter into an 8 mL test tube. Rinse the syringe filter by pushing 0.5 mL MeOH through the filter, collecting the rinse in the 8 mL test tube.
- 3.1.6 Reduce the volume of the eluate under nitrogen to less than 0.5 mL. The nitrogen flow can be quite strong.
- 3.1.7 Add 5 uL of the 10 ug/mL atrazine-ethylamine-d5 standard solution for internal quantitation to each extract.
- 3.1.8 Adjust the final volume to 1.0 mL with distilled H2O.
- 3.1.9 Transfer the extracts to 4 mL vials.
- 3.1.10 Organize the sample vials into the vial box, and store it in the refrigerator until analysis.

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4. LIQUID CHROMATOGRAPH OPERATING PARAMETERS

4.1 The LC Chromatography system parameters have been optimized for these analytes as follows:

Column: LC18 15 cm x 4.6 mm ID (3um) ODS with
LC18 Guard Column

Flow rate: 1.2 mL/min.

Mobile Phase: ACN/Water
(Water contains 0.1M ammonium acetate)

Gradient: 40/60 ACN/H₂O
Gradient to 55/45 at 2.0 minutes
Return to 40/60 at 9 minutes

Retention Times:	Bromacil	5.0	minutes (approx)
	Simazine	5.5	minutes (approx)
	Cyanazine	5.75	minutes (approx)
	Metribuzin	6	minutes (approx)
	Atrazine	7	minutes (approx)
	Prometon	7.75	minutes (approx)
	Ametryn	8.75	minutes (approx)
	Propazine	9	minutes (approx)
	Prometryn	11	minutes (approx)
	Terbutryn	11.5	minutes (approx)

4.2 As an alternative method, chromatography can be performed on a narrow diameter LC column with post column additional of additional buffer as follows:

Column: 100mm X 2.1mm (5u) Hypersil or equivalent

Flow: 0.4 mL/min

Mobile Phase: Acentonitrile/Water (0.1M ammonium acetate)

Gradient: 40/60 ACN/Water
Gradient to 80/20 in 2 minutes
Return to 40/60 at 3 minutes

Post Column: Water (0.1M NH₄OAC) at 0.8 mL/min

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Retention Times:	Bromacil	2:45
	Simazine	3:15
	Cyanazine	3:25
	Metribuzin	3:47
	Atrazine	4:57
	Prometon	5:19
	Ametryn	7:23
	Propazine	7:45
	Prometryn	9:31
	Terbutryn	9:51

5. TSP - MASS SPECTROMETER OPERATING PROCEDURES

5.1 Instrument Tuning

- 5.1.1 Generally, lens tuning with reagent ions or a background peak is sufficient. In any event, the instrument must be tuned and calibrated in the Q1MS, Q3MS, and MS/MS modes.
- 5.1.2 It has been determined that optimal transmission and product ion generation is achieved at collision energy = -13V and collision pressure = 2 mtorr
- 5.1.3 The instrument must be capable of detecting 100 pg of each analyte, (20 uL of 0.005 ug/mL) with a signal/noise (S/N) ratio of 10:1.

5.2 TSP-LCMS Operation Parameters

- 5.2.1 The following TSP-LCMS parameters must be optimized in order to achieve an optimum performance.

Vaporizer Control Point - 75 °C - 130 °C
 Aerosol Temp - 250 °C
 Repeller Voltage - 40 V

- 5.2.2 It has been determined that optimal sensitivity is achieved with filament on ionization mode, however, either mode is acceptable.

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5.3 Selected Ion Monitoring

5.3.1 Samples and standards are analyzed by selected reaction monitoring (SRM) of product ions derived from the protonated molecular ion.

5.3.2 The appropriate masses to be monitored are listed below:

Analyte	Precursor (MH ⁺)	Product Ion	Dwell Time
Bromacil	m/z 261	m/z 205 ± 0.3 amu	0.2 seconds
Simazine	m/z 202	m/z 132 ± 0.3 amu	0.2 seconds
Cyanazine	m/z 241	m/z 214 ± 0.3 amu	0.2 seconds
Metribuzin	m/z 215	m/z 187 ± 0.3 amu	0.2 seconds
Atrazine	m/z 216	m/z 174 ± 0.3 amu	0.2 seconds
Prometon	m/z 226	m/z 184 ± 0.3 amu	0.2 seconds
Ametryn	m/z 228	m/z 186 ± 0.3 amu	0.2 seconds
Propazine	m/z 230	m/z 188 ± 0.3 amu	0.2 seconds
Prometryn	m/z 242	m/z 158 ± 0.3 amu	0.2 seconds
Terbutryn	m/z 242	m/z 186 ± 0.3 amu	0.2 seconds

5.4 Calibration and Sample Analysis

5.4.1 During the course of analysis of a batch of samples each of the calibration standards (see Section 2.1.4) is run once. Between 1 and 10 samples are analyzed between these standards.

5.4.2 Calculate the response factor (RF) for each analyte at each level defined as follows:

$$RF = \frac{(\text{Area } A) (\text{Amt } IS)}{(\text{Area } IS) (\text{Amt } A)}$$

Where: Area A = Area response of analyte
 Area IS = Area response of Internal Standard (I.S.)
 Amt A = Concentration of analyte
 Area IS = Concentration of I.S.

5.4.3 If the percent relative standard deviation is less than 15% over the working range, linearity can be assumed and the average response factor is used for sample calculations.

5.4.4 Inject 20-50 uL of standard/extract into the TSP-LCMS system for analysis.

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6. CALCULATIONS

6.1 The concentration of each analyte in a sample is calculated as follows:

$$\text{Concentration (ug/L)} = \frac{(A_x) (Amt_{IS}) (V_x)}{(A_{IS}) (RFA) (V_s) (1000)}$$

RFA = Average Response Factor
 A_x = Sample Total Area Counts
 V_x = Sample Extract Final Volume (mL)
 V_s = Initial Sample Volume (L)
 A_{IS} = Internal Standard Area Response
 Amt_{IS} = Internal Standard Amount (ug/mL)

CALIFORNIA DEPT. OF FOOD & AGRIC.
CHEMISTRY LABORATORY SERVICES
3292 Meadowview Road
Sacramento, CA 95832
(916)427-4999

Original Date:10/02/1991
Supercedes: New
Current Date:10/02/1991
Method #:

ORGANOPHOSPHATE PESTICIDES IN SURFACE WATER BY GC

SCOPE:

This method is for the determination of organophosphate pesticides in surface water. The sensitivity of the method ranges from 0.05 ppb to 0.3 ppb for different pesticides.

PRINCIPLE:

The surface water sample is extracted with methylene chloride. The extract is filtered through sodium sulfate to remove residual water. The dried extract is evaporated to dryness on a rotary evaporator and brought to 0.5 mL final volume with acetone. The extract is then analyzed by gas chromatography with flame photometric detection (FPD).

REAGENTS AND EQUIPMENT:

1. Solvents: methylene chloride, acetone, pesticide residue grade
2. Sodium sulfate, anhydrous
3. Organophosphate pesticide stock standard solutions (1mg/mL): Dissolve 50 mg of stock standard in acetone and dilute to 50 mL in a volumetric flask.
4. Nitrogen evaporator, Organomation Model #12
5. Rotary evaporator, Büchi/Brinkmann, R110

ANALYSIS:

1. Remove water samples from refrigerator and allow them to come to room temperature.
2. Record weight of water by weighing sample bottle before and after water has been transferred into a 2 L separatory funnel.
3. Extract water by shaking with 100 mL of methylene chloride for 2 minutes.
4. Dry the methylene chloride by passing it through 20 g of sodium sulfate in a glass funnel as it is transferred to a 500 mL boiling flask.
5. Repeat steps 3 & 4 two more times using 80 mL of methylene chloride each time.
6. Evaporate the solvent with a rotary evaporator to dryness at 35°C and approximately 20 mm Hg vacuum.
7. Add 5 mL of acetone and swirl to dissolve the residue in the flask.
8. Transfer the extract to a 15 mL graduated test tube. Rinse flask 2 times each with 2 mL of acetone and transfer each wash to the same test tube.
9. Evaporate the extract to a final volume of 0.5 mL under a gentle stream of nitrogen with no heat applied.
10. Submit extract to GC analysis for organophosphate pesticides.

EQUIPMENT CONDITIONS:

PRIMARY ANALYSIS:

Instrument: Varian 6000 GC with Flame Photometric Detector
 Column: HP-1, methyl silicone gum, 10 m x 0.53 mm x 2.65 um
 Injector temperature: 220°C
 Detector temperature: 250°C
 Temperature program: Ramp 1 Initial Temp: 150°C held for 1 min
 Rate: 10°C/min
 Ramp 2 Initial Temp: 200°C held for 2 min
 Rate: 20°C/min
 Final Temp: 250°C held for 5 min
 Carrier gas flow rate: Helium, 20 mL/min
 Sample injected vol.: 2 uL

CONFIRMATION ANALYSIS:

Instrument: Varian 3700 GC with Flame Photometric Detector
 Column: DB-210 (50% tri-fluoropropyl methyl polysiloxane) 15 m x 0.537 mm
 x 1.0 um
 Injector temperature: 220°C
 Detector temperature: 250°C
 Temperature program: 140°C for 2 min, increase at 10°C/min, to 250°C for
 6 min.
 Carrier gas flow rate: Helium, 20 mL/min
 Sample inject vol.: 2 uL

CALCULATIONS:

$$\text{ppb} = \frac{(\text{sample peak height})(\text{ng standard injected})(\text{ml sample final volume})1000}{(\text{standard peak height})(\text{uL sample injected})(\text{sample weight})}$$

RECOVERIES:

<u>Organophosphate Pesticides</u>	<u>Spike Level</u> (ppb)	<u>n</u>	<u>Recovery</u>		<u>SD</u>	<u>CV</u>
			\bar{x} (ppb)	%		
DDVP	0.1	3	0.098	98	0.004	4.2
	2.0	3	2.07	104	0.24	11.5
	5.0	3	4.65	93	0.27	5.9
Dimethoate	0.1	3	0.101	101	0.004	3.5
	2.0	3	1.96	98	0.15	7.4
	5.0	3	4.76	95	0.42	8.8
Diazinon	0.1	3	0.10	100	0.005	5.3
	2.0	3	1.80	91	0.27	14.8
	5.0	3	4.79	96	0.28	5.8
Methyl Parathion	0.1	3	0.095	95	0.004	4.2
	2.0	3	2.00	100	0.17	8.5
	5.0	3	4.89	98	0.38	7.7

RECOVERIES: continued

<u>Organophosphate Pesticides</u>	<u>Spike level</u> (ppb)	<u>n</u>	<u>Recovery</u>		<u>SD</u>	<u>CV</u>
			\bar{x} (ppb)	%		
Malathion	0.1	3	0.095	95	0.003	3.0
	2.0	3	1.98	99	0.16	7.9
	5.0	3	4.95	99	0.28	5.7
Ethyl parathion	0.1	3	0.097	97	0.002	1.6
	2.0	3	1.89	98	0.06	3.3
	5.0	3	4.92	98	0.27	5.5
Chlorpyrifos	0.1	3	0.093	93	0.003	3.4
	2.0	3	1.99	99	0.11	5.4
	5.0	3	4.79	96	0.57	11.6
Methidathion	0.1	3	0.094	94	0.007	7.4
	2.0	3	2.09	105	0.15	7.1
	5.0	3	5.03	101	0.41	8.1
Phosmet	0.5	3	0.52	104	0.02	3.3
	2.0	3	2.05	103	0.10	4.7
	5.0	3	5.27	105	0.29	5.6
Azinphos-Methyl	0.5	3	0.51	101	0.01	2.3
	2.0	3	2.01	101	0.15	7.5
	5.0	3	4.81	96	0.40	8.3
Phosalone	0.5	3	0.53	107	0.05	8.9
	2.0	3	1.99	99	0.18	9.1
	5.0	3	4.92	98	0.03	1.0
Diazinon OA	0.5	3	0.46	91	0.02	4.6
	2.0	3	1.99	99	0.15	7.3
	5.0	3	5.07	101	0.34	6.7
Methyl Paraoxon	0.5	3	0.43	87	0.01	1.3
	2.0	3	2.0	100	0.12	6.2
	5.0	3	4.97	95	0.36	7.3
Malaaxon	0.5	3	0.50	100	0.03	6.0
	2.0	3	1.96	98	0.17	8.8
	5.0	3	5.41	108	0.18	3.2
Ethyl Paraoxon	0.5	3	0.45	91	0.03	7.0
	2.0	3	1.94	97	0.08	4.4
	5.0	3	4.89	98	0.32	6.6
Chlorpyrifos OA	0.5	3	0.45	90	0.04	8.9
	2.0	3	1.97	98	0.14	6.9
	5.0	3	5.0	100	0.60	12.1

RECOVERIES: continued

Organophosphate

<u>Pesticides</u>	<u>Spike level</u> (ppb)	<u>n</u>	<u>Recovery</u>		<u>SD</u>	<u>CV</u>
			\bar{x} (ppb)	%		
Methidathion OA	0.5	3	0.47	94	0.04	7.7
	2.0	3	2.04	102	0.03	1.5
	5.0	3	4.88	98	0.41	8.3
Phosmet OA	0.5	3	0.51	98	0.05	9.0
	2.0	3	1.81	96	0.17	9.4
	5.0	3	4.91	98	0.47	9.5
Azinphos-Methyl OA	0.5	3	0.49	98	0.04	7.8
	2.0	3	1.92	98	0.02	1.1
	5.0	3	4.79	96	0.45	9.3
Phosalone OA	0.5	3	0.48	96	0.01	1.2
	2.0	3	2.09	102	0.25	11.9
	5.0	3	5.32	106	0.33	6.3

DISCUSSION

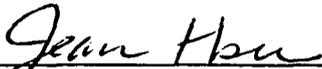
Several interference peaks first appeared in the chromatograms of the blank and samples which had the same retention times as those of Phosmet, Phosalone and Azinphos-Methyl. These interferences may have been caused by impurities in sodium sulfate. The interference peaks did not appear in later chromatograms after the sodium sulfate used in extraction had been washed three times with an equal amount of methylene chloride. To avoid these interferences wash sodium sulfate prior to use.

REFERENCE

"Method 8141, Organophosphorus Pesticides, Capillary Column". EPA Test Methods for Evaluating Solid Waste, Revised Methods, 1987.

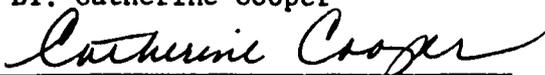
"EPA Method 507, Pesticides, Capillary column" EPA Test Method for Drinking water and raw source water, 1987

WRITTEN BY: Jean Hsu



TITLE: Agricultural Chemist I

REVIEWED BY: Catherine Cooper



TITLE: Agricultural Chemist III

APPROVED BY: S. Mark Lee

S. Mark Lee

TITLE: Research Agricultural Chemist

APPROVED BY: Terry Jackson

Terry Jackson

TITLE: Quality Assurance Officer

APPL UREA SCREEN METHOD

DETERMINATION OF UREA PESTICIDES
BY LIQUID CHROMATOGRAPHY / MASS SPECTROMETRY
(LC/MS)

1.0 SCOPE AND APPLICATION

1.1 This method is applicable to the determination, extraction, and detection of the following compounds in water matrices:

Analyte	CAS Registry No. *
Chloroxuron	
Diuron	330-54-1
Fenuron	101-42-8
Fluometuron	264-17-2
Linuron	330-55-2
Monolinuron	
Monuron	150-68-5
Neburon	555-37-3
Siduron	1982-49-2
Tebuthiuron	1918-18-9

* Chemical Abstract Services Registry Number.

2.0 SUMMARY OF METHOD

2.1 This method provides liquid chromatography (LC) and thermospray (TSP) mass spectrometry (MS) conditions for the detection and quantitation of the preceding list of analytes.

2.2 One liter aqueous samples are methylene chloride extracted, concentrated and solvent exchanged with methanol.

3.0 INTERFERENCES

3.1 Refer to Method 8000.

4.0 APPARATUS AND MATERIALS

4.1 Microsyringes: 500- μ l, 100- μ l, 10- μ l (Hamilton 710 N or equivalent).

4.2 High Performance Liquid Chromatograph (HPLC) - An analytical system with solvent programmable pumping system for at least a binary solvent system and all required accessories, including an injection capability of delivering 100- μ l volumes, analytical columns, purging gases, etc. Must be capable of interfacing with an MS.

4.2.1 HPLC Syringe Pump - Analysts desiring to use acetonitrile / water gradients will need to provide a system for post column addition of ammonium acetate and make appropriate chromatographic adjustments.

4.2.2 HPLC Columns - Two columns are necessary for analysis. One is the analytical column and the other is the guard column.

4.2.2.1 Column 1 - Zorbax ODS 250 mm X 4.5 mm i.d., 7- μ m particle size, or equivalent.

4.2.2.2 Column 2 - Guard Column - C18 reversed phase media, 37 - 53 μ m particle size, 20 mm X 4.6 mm i.d., 2 μ m frit, or equivalent.

4.2.3 HPLC Interface(s)

4.2.3.1 Micromixer - 10- μ L, interfaces HPLC column system with HPLC post-column addition solvent system. Necessary only for acetonitrile / water gradients.

4.2.3 HPLC Interface(s)

4.2.3.2 HPLC/MS - Thermospray ionization interface that will give acceptable calibration response for each analyte of interest at the concentration required.

4.3 Mass spectrometer system: This method is designed for single stage mass spectrometry.

4.3.1 Data System: A computer system that allows continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic run must be interfaced to the mass spectrometer. The computer must have software that allows searching any MS data file for ions of a specified mass and plotting such ion abundances versus time or scan number. This type of plot is defined as an Extracted Ion Profile (EIP) or Extracted Ion Current Profile (EICP). Software must also be available that allows integrating the abundances in any EIP between specified time or scan-number intervals.

4.4 Separatory funnel: 2-liter, with Teflon stopcock.

4.5 Kuderna-Danish (K-D) apparatus:

4.5.1 Concentrator tube: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Ground-glass stopper is used to prevent evaporation of extracts.

4.5.2 Evaporation flasks: 500-mL (Kontes K-570001-500 or equivalent). Attach to concentrator tube with springs.

4.5.3 Snyder column: Three ball macro (Kontes K-503000-0121 or equivalent).

4.5.4 Snyder Two ball micro (Kontes K569001-0219 or equivalent).

4.6 Boiling chips: Solvent extracted, approximately 10/40 mesh (silicon carbide or equivalent).

4.7 Water bath: Heated, with concentric ring cover, capable of temperature control ($\pm 5^{\circ}\text{C}$). The bath should be used in a hood.

4.8 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.

4.9 Concentrator adaptor (optional). See figure 1.

4.10 Rotary evaporator device (optional).

4.11 Vials: 10 - 15 mL, amber glass, with Teflon lined

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. unless otherwise indicated, it intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 Reagent water: Reagent water is defined as water in which an interferent is not observed at method detection limit of the compounds of interest.

5.3 Extraction/exchange solvent: Methylene chloride, methanol (pesticide quality or equivalent).

5.4 Ammonium acetate solution (0.1M) in HPLC - grade water. Filtering is optional.

5.5 Stock standard solutions: Can be prepared from pure standard materials or can be purchased as certified solutions. Commercially prepared stock standards can be used if they are verified against EPA standards. If EPA standards are not available for verification, then standards certified by the manufacturer and verified against a standard made from pure material is acceptable.

5.5.1 Prepare stock standard solutions by accurately weighing 0.0100 g of pure material. Dissolve the material in methanol and dilute to known volume in a volumetric flask. If compound purity is certified as 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

5.5.2 Transfer the stock standard solutions into bottles with Teflon lined screw-caps. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibrations standards from them.

5.6 Calibration standards: A minimum of five concentration levels for each parameter of interest should be prepared through dilution of the stock standards with methanol. One of the concentration levels should be at the MDL. The remaining concentration levels should correspond to the expected range of concentrations found in real samples or should define the working range of the LC/MS. Calibration standards should be replaced after one or two month, or sooner if comparison with check standards indicates a problem.

5.7 Surrogate standards: The analyst should monitor the performance of the extraction, cleanup (when used), and analytical system and the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard, and blank with one or two surrogates recommended to encompass the range of the gradient elution program. For this method a relatively non-volatile organophosphorus compound or a triazine herbicide will work suitably. (Warning - Terbutylazine is unsuitable.)

5.8 Internal standards: It is not an absolute requirement that analysts use internal standards. It is very strongly recommended that they be used in order to establish the integrity of each chromatographic run, even if they are not used for quantitation purposes. Deuterated analytes would be ideal. Monocrotophos, Metalaxyl, and Thiobencarb have been used extensively and are acceptable in that they are not found in real world samples very often. It is up to the analyst to determine what compounds they want to use but again the use of internal standards is extremely important.

5.9 HPLC/MS Tuning standard: There are two options.

5.9.1 Polyethylene glycol 400 (PEG-400). Dilute to 10 percent (v/v) in methanol.

5.9.2 Polypropylene glycol (Hewlett Packard Part No. 8500-4634) MW 425 (average) & MW 725 (average). Approximately 0.05 µg/ml in 25 percent methanol and 75 percent water containing 0.1M ammonium acetate.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 Refer to introductory material to Chapter 4 (SW - 846), Organic Analytes, section 4.1 Sampling Considerations. The analytes listed in this method do not require any special pH adjustments or preservation considerations.

6.2 Extracts are to be refrigerated at 4°C and analyzed within 40 days of extraction.

7.0 PROCEDURE

7.1 Preparation of aqueous samples:

7.1.1 One liter quantities are methylene chloride extracted using Method 3510. Sixty milliliters of methylene chloride are added to the sample water matrix in a 2 L separatory funnel. The mixture is shaken vigorously (with appropriate venting) for 1 - 2 minutes. The funnel is then allowed to sit for 10 minutes, whereupon the methylene chloride layer is collected into either a boiling flask or a KD. This process is repeated two more times. (No pH adjustments are necessary.)

7.1.2 Concentration steps can be achieved using a rotary evaporator or KD, to 5-10 ml volumes.

7.1.3 Final concentration and exchange to 1ml final volume of methanol, containing internal standards at low detection levels (@ 1 µg/ml) can be done preferably using an adapter similiar to Figure 1 on a rotary evaporator. If an adapter is unavailable the final concentration can be achieved using a gentle stream of air or nitrogen in a hood.

7.1.4 Sample cleanup is generally not necessary. Extremely dirty samples may be run through a C-18 sep pac cartridge for cleanup purposes. The specific mechanics are left up to the analyst to work out.

7.3 HPLC Conditions:

7.3.1 Water / Methanol gradient

7.3.1.1 Water and Methanol solvent systems contain 0.1M ammonium acetate and 1% glacial acetic acid (v/v) each. Further reference to water / methanol gradients should be understood that additives are included.

7.3.1.2 Flow rate is 1 ml / minute. A basic usable gradient is 5 percent methanol / water initial that ramps to 100 percent methanol over 15 minutes. Methanol at 100 percent is held for 5 minutes, then a 5 minute ramp back to 5 percent, after which there is a 5 minute hold before the next injection. Total runtime is 30 minutes.

7.3.2 Water / Acetonitrile gradient

7.3.2.1 If water acetonitrile gradients are desired one must go to post column addition and make the appropriate chromatographic adjustments.

7.3.3 Columns:

7.3.3.1 The columns recommended are not an absolute requirement, they were used in the preliminary methodology development. The analyst is left up to their preference and resourcefulness in choosing the best column and gradient

7.3.4 Injector loops:

7.3.4.1 The performance parameters tabulated used relatively low sensitivity source parameters and an on column injection volume of 100- μ l. Different systems may enable the analyst to go to lower injection volumes if they are able to hit appropriate detection levels.

7.4 Calibration

7.4.1 Recommended LC/TSP/MS operating conditions

7.4.1.1 Positive Ionization Mode

Note: Source parameters will vary: These are typical parameters used on a Hewlett Packard 5988 mass spectrometer equipped with a standard thermospray source.

Emission (μ A)	150	El Energy (eV)	200	AMU Gain	174
Repeller (V)	6.5	Ent Lens (mV)	110	AMU Offset	201
Ion Focus (V)	60	X - Ray (V)	80	Mass Gain	-57
Draw Out (V)	30.0	EM Volts (V)	2800	Mass Offset	46

Source temperature @ 276°C

7.4.1.2 Thermospray temperatures: Again probe parameters will vary from probe to probe. By performing probe surveys at appropriate solvent compositions (matching tuning solution composition) and taking a temperature 95 percent of an observed 'break point' will usually give the appropriate temperature in order to achieve a good calibration. The parameters are typical to those observed on the HP 5988.

Probe temp = 105°C (will according to survey)
Tip temp = 190°C to 210°C

7.4.2 Thermospray/MS system: The system needs to be hardware tuned for accurate mass assignment, sensitivity, and resolution. There are two options for tuning. One is use of polyethylene glycol (PEG) 400. The other is tuning to polypropylene glycol MW 425 & MW 725 (average).

7.4.2.1 Calibration using PEG 400: PEG 400 is diluted to 10 percent (v/v) in methanol and is introduced via the thermospray interface, circumventing the HPLC. Data acquisition parameters are as follows.

Positive Ion Mode
Mass Range: 15 to 765 amu.
Scan Time : 5.00 sec/scan.

Approximately 100 scans should be acquired, with 2 to 3 injections made. The scan with the best fit to accurate mass table (see Table X). will be used as the calibration table.

7.4.2.2 Calibration using polypropylene glycol MW 375 & MW 725 (average). A 0.05 $\mu\text{g/ml}$ solution of polypropylene glycol is prepared in 25 percent methanol and 75 percent water containing 0.1M ammonium acetate. This solution is then introduced via the HPLC into the TSP/MS interface. The probe temperature is determined by performing probe surveys. Mass axis calibrations are to 268.2, 442.3, and 558.4. Adjust source parameters to achieve peak resolution values for full width half maximum values of 0.5, usually varies between 0.45 - 0.55. Electron multiplier voltages are run high (2800 - 3000 volts). Scan range is 100 - 1000 amu. Samples / A.D. @ 16, integration @ 150, and threshold @ 20. A spectrum scan should yield a total ion abundance of 5,000,000 to 10,000,000 counts. Achieving these abundances should enable appropriate analyte sensitivities.

7.5 Liquid chromatographic calibration:

7.5.1 Prepare calibration standards as outlined in Section 5.6 and 5.8.

7.5.2 Maintaining a calibration is extremely difficult. The various parameters that come into play doing LC/MS work introduce many variables that effect instrument response and analyte behavior. Assuming the cold trap capacity limits autosequence runtimes to around 18 - 20 hours, it is necessary to bracket samples with calibration standards at the beginning and end of each sequence. By doing five point calibrations at the beginning and end of sequences, it helps to identify the inevitable 'outliers' that will be encountered. Refer to Table 3 for reference on primary quantitation ions that extracted ion profiles are performed on for each analyte of interest. (Note: Analysts may use different primary ions for quantitation, depending on source parameters, different instruments etc.) Usually the primary molecular ion is the N+1 or the N+18 molecular adduct. After obtaining extracted ion profiles on each standard set there are two options for quantitation. They are linear regression analysis or response factor calculations using internal standards.

7.5.2.1 Calibration by linear regression:

Refer to Table 1 for a list of primary ions used for EIPS. After obtaining extracted ion profiles it is necessary to run linear regression analysis using analyte concentration versus area counts. Using bracketing calibrations, best results are achieved taking area counts from initial and final injections and averaging them for each concentration.

$$\text{Area Count Initial Conc.} + \text{Area Count Final Conc.} / 2$$

From these average values it is necessary to run a linear regression analysis on the injections made. Correlation coefficients obtained will not always yield values of 0.99 or better for all analytes. Low correlation coefficients should alert the analyst that the quantitation is questionable. The analyst does have the option on confirmed spectral identifications to quantitate by alternate means, i.e. post-column derivatization with fluorescence detection for carbamates or uv/vis for ureas, etc. Refer to EPA Method 531 and EPA Method 632 for further information.

A typical linear regression calculation: $\text{Analyte Level} = (\text{Response Analyte} - \text{Intercept of Line}) / \text{Slope of Line} * (\text{Final Volume Extract} / \text{Initial Volume or Weight Extracted})$

Refer to Method 8000 Section 7.8 for further information on calculations.

7.5.2.2 Calibration by response factor calculations using internal standards:

Assuming use of three internal standards that elute early, middle, and late it is necessary to perform response factor calculations for each analyte and the internal standard that has the closest elution time.

$$\text{R.F.} = \text{A.R.} * \text{I.C.} / \text{I.R.} * \text{A.C.}$$

R.F. = Response Factor
A.R. = Analyte Response (area counts or peak heights)
I.C. = Internal Standard Concentration ($\mu\text{g/ml}$)
I.R. = Internal Standard Response (area or peak heights)
A.C. = Analyte Concentration ($\mu\text{g/ml}$)

By calculating the response factor for each calibration injection it is necessary to calculate the average response factor.

$$\text{Average Response Factor} = \Sigma \text{R.F.} / \text{Number of R.F.'s}$$

Quantitation of analytes found in samples is calculated by:

$$C.A. = ((R.A. * I.C.) / (I.R. * A.R.F)) * D.F.$$

C.A. = Concentration Analyte
R.A. = Response Analyte
I.C. = Internal Standard Concentration
I.R. = Internal Standard Response
A.R.F = Average Response Factor
D.F. = Dilution Factor (refer to Method 8000)

It is necessary to generate statistical information along with average response factor calculations, i.e. percent relative standard deviation in order to have an indicator to how precise the calibration and ultimate quantitation may be.

7.6 Sample Analysis:

7.6.1 As previously stated, it necessary to bracket sample analyses with five point calibrations at the beginning and end of sequences.

7.6.2 After the intial calibration standards it is necessary to run a reagent blank (methanol) in order to determine any residual contamination of the Thermospray/HPLC/MS system.

7.6.3 Sample extracts are injected (100 μ l volumes) on column using the same LC and MS parameters as those for calibration injections. Potential analyte hits are identified by observation of extracted ion profile peaks that match retention times of standards. The ratio of the retention time of the sample analyte to the standard analyte should be 1.0 ± 0.1 , or if using scan rates listed in Section 7.4.2.2 sample analyte peak maxima should be ± 1 scan of those observed for standards. Confirmed identifications are obtained by comparison of spectral information. Background subtracted spectra are important in analyzing complicated samples. Secondary and tertiary ions should be present also.

7.6.4 Confirmed analytes are quantified using area counts obtained from extracted ion profiles for corresponding primary ions and either linear regression (Section 7.5.2.1) or response factor calculations using internal standards (Section 7.5.2.2).

8.0 QUALITY CONTROL

8.1 Refer to Chapter One for specific quality control procedures as outlined in Method 8000, Section 8.0.

8.2 A reagent blank, replicate, or matrix spike replicate must be analyzed for each analytical batch. These samples must be carried through all stages of sample preparation and measurement steps. The spike results will be used to assess analytical bias.

8.2.1 It may not be possible to determine the background concentrations prior to spiking. If this is the case, the spiking concentrations should be (1) the regulatory concentration limit, or, if none, (2) five times higher than the detection limit.

8.2.2 Analyze one unspiked and one spiked sample aliquot to determine percent recovery of the spiked analyte.

8.2.3 Tables 4 and 5 indicate single operator accuracy and precision for this method. Compare the results obtained with the results in the tables to determine if the data quality is acceptable.

8.2.4 If recovery is not within limits, check the following:

- o Check to be sure there are no errors in calculations. Also, check instrument performance. It is useful to quantitate extracts by alternate detection methods, i.e. post-column derivatization and fluorescence detection or uv-vis detection in order to determine whether it is an extraction or instrument problem.
- o Recalibrate the data and/or reanalyze the extract if any of the above checks reveal a problem.
- o Re-extract and reanalyze the sample if none of the above are a problem or flag the data as "estimated concentration".

8.3 Before processing any samples, demonstrate through the analysis of a reagent blank that all glassware and reagents are interference free. Each time a set of samples is extracted or there is a change in reagents, a reagent blank should be processed as a safeguard against chronic laboratory contamination.

8.4 Field blanks should be analyzed to assess possible contamination of samples in the field, and to monitor possible interferences.

8.5 Instrument performance - Check the performance of the entire analytical system daily using data gathered from analyses of blanks, standards, and replicate samples.

8.5.1 Typically early eluters will reveal problems in generating good correlation coefficients from linear regression analysis. For the most part the later eluters should yield correlation coefficients of 0.99 or better. Typically, when using response factor calculations, the %RSD should be under 25% for at least half of the target analytes.

8.5.2 The use of internal standards at minimum detectable levels are extremely important in verifying the integrity of each run. Along with verifying a minimum detectable level, they also give the analyst an indication of how the system is performing, plus the degree of variability that is encountered in thermospray work. Internal standards are also excellent indicators to the experienced eye that probe temperatures are incorrect or need adjustment in particular portion of the chromatographic run. Finally internal standards will give a verification that the chromatography of the system is within control.

8.5.3 See Section 7.6.3 regarding retention time window QC limits.

8.5.4 If any of the chromatographic QC limits are not met, the analyst should examine the LC system for:

- o Leaks,
- o Proper pressure delivery,
- o A dirty guard column; may need replacing or repacking, and
- o Possible partial thermospray plugging.

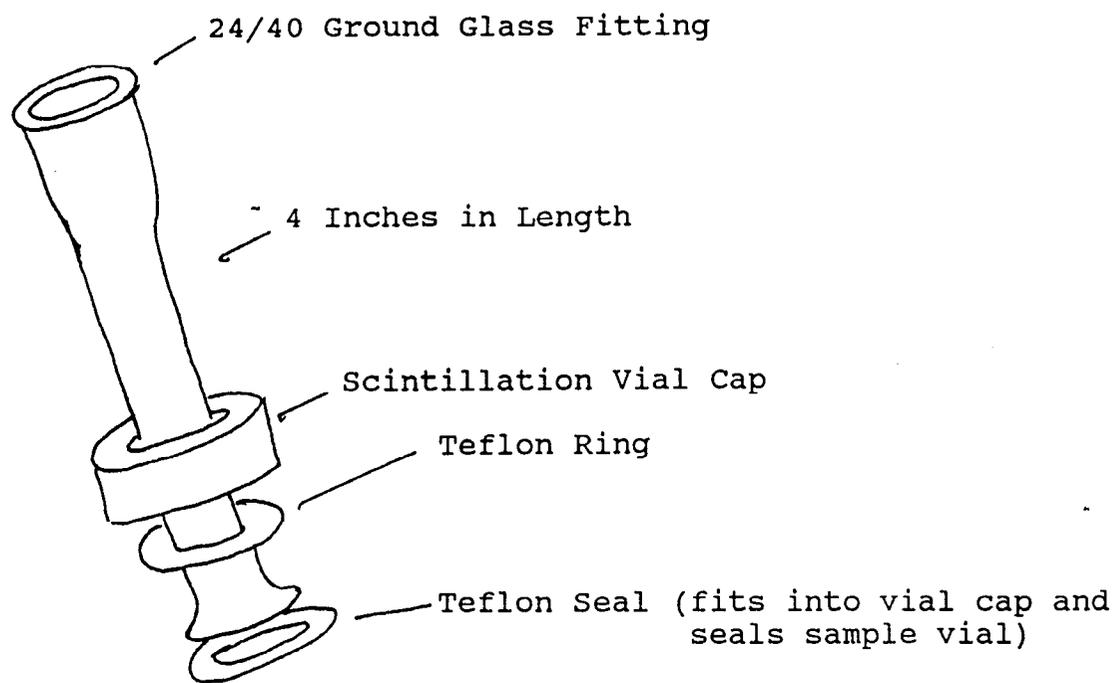
TABLE 1
ELUTION ORDER, RETENTION TIMES, AND
PRIMARY QUANTITATION ION FOR TARGET ANALYTES

ANALYTE	RETENTION TIME	QUANTITATION ION
Fenuron	11.89	165
Monuron	14.18	199
Tebuthiuron	14.52	229
Fluometuron	14.98	233
Monolinuron	15.33	232
Diuron	15.78	233
Siduron	16.23	233
Chloroxuron	16.69	291
Neburon	17.26	275

* See Section 7.3.1.2 for chromatographic conditions. Column used was a Zorbax 7 micron ODS , 250 mm X 4.5 mm. Note: Some analytes will flip in elution depending on column and gradient / solvent system used.

** The primary quantitation ion can vary, depending on source and probe parameters. It is possible to skew fragmentation patterns to either the high or low end, which can alter the base peak obtained, etc.

FIGURE 1
ROTARY EVAPORATOR CONCENTRATION ADAPTOR



Volumetric Flask Neck

GAS CHROMATOGRAPHY

1.0 SCOPE AND APPLICATION

1.1 Gas chromatography is a quantitative analytical technique useful for organic compounds capable of being volatilized without being decomposed or chemically rearranged. Gas chromatography (GC), also known as vapor phase chromatography (VPC), has two subcategories distinguished by: gas-solid chromatography (GSC), and gas-liquid chromatography (GLC) or gas-liquid partition chromatography (GLPC). This last group is the most commonly used, distinguished by type of column adsorbent or packing.

1.2 The gas chromatographic methods are recommended for use only by, or under the close supervision of, experienced residue analysts.

2.0 SUMMARY OF METHOD

2.1 Each organic analytical method that follows provides a recommended technique for extraction, cleanup, and occasionally, derivatization of the samples to be analyzed. Before the prepared sample is introduced into the GC, a procedure for standardization must be followed to determine the recovery and the limits of detection for the analytes of interest. Following sample introduction into the GC, analysis proceeds with a comparison of sample values with standard values. Quantitative analysis is achieved through integration of peak area or measurement of peak height.

3.0 INTERFERENCES

3.1 Contamination by carryover can occur whenever high-level and low-level samples are sequentially analyzed. To reduce carryover, the sample syringe or purging device must be rinsed out between samples with reagent water or solvent. Whenever an unusually concentrated sample is encountered, it should be followed by an analysis of a solvent blank or of reagent water to check for cross contamination. For volatile samples containing large amounts of water-soluble materials, suspended solids, high boiling compounds or high organohalide levels, it may be necessary to wash out the syringe or purging device with a detergent solution, rinse it with distilled water, and then dry it in a 105°C oven between analyses.

4.0 APPARATUS AND MATERIALS

4.1 Gas chromatograph: analytical system complete with gas chromatograph suitable for on-column injections and all required accessories, including detectors, column supplies, recorder, gases, and syringes. A data system for measuring peak height and/or peak areas is recommended.

4.2 Gas chromatographic columns: See the specific determinative method. Other packed or capillary (open-tubular) columns may be used if the requirements of Section 8.6 are met.

5.0 REAGENTS

5.1 See the specific determinative method for the reagents needed.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to this chapter, Organic Analytes, Section 4.1.

7.0 PROCEDURE

7.1 Extraction: Adhere to those procedures specified in the referring determinative method.

7.2 Cleanup and separation: Adhere to those procedures specified in the referring determinative method.

7.3 The recommended gas chromatographic columns and operating conditions for the instrument are specified in the referring determinative method.

7.4 Calibration:

7.4.1 Establish gas chromatographic operating parameters equivalent to those indicated in Section 7.0 of the determinative method of interest. Prepare calibration standards using the procedures indicated in Section 5.0 of the determinative method of interest. Calibrate the chromatographic system using either the external standard technique (Section 7.4.2) or the internal standard technique (Section 7.4.3).

7.4.2 External standard calibration procedure:

7.4.2.1 For each analyte of interest, prepare calibration standards at a minimum of five concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with an appropriate solvent. One of the external standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.4.2.2 Inject each calibration standard using the technique that will be used to introduce the actual samples into the gas chromatograph (e.g., 2- to 5- μ L injections, purge-and-trap, etc.). Tabulate peak height or area responses against the mass injected.

The results can be used to prepare a calibration curve for each analyte. Alternatively, for samples that are introduced into the gas chromatograph using a syringe, the ratio of the response to the amount injected, defined as the calibration factor (CF), can be calculated for each analyte at each standard concentration. If the percent relative standard deviation (%RSD) of the calibration factor is less than 20% over the working range, linearity through the origin can be assumed, and the average calibration factor can be used in place of a calibration curve.

$$\text{Calibration factor} = \frac{\text{Total Area of Peak}^*}{\text{Mass injected (in nanograms)}}$$

*For multiresponse pesticides/PCBs use the total area of all peaks used for quantitation.

7.4.2.3 The working calibration curve or calibration factor must be verified on each working day by the injection of one or more calibration standards. The frequency of verification is dependent on the detector. Detectors, such as the electron capture detector, that operate in the sub-nanogram range are more susceptible to changes in detector response caused by GC column and sample effects. Therefore, more frequent verification of calibration is necessary. The flame ionization detector is much less sensitive and requires less frequent verification. If the response for any analyte varies from the predicted response by more than $\pm 15\%$, a new calibration curve must be prepared for that analyte.

$$\text{Percent Difference} = \frac{R_1 - R_2}{R_1} \times 100$$

where:

R_1 = Calibration Factor from first analysis.

R_2 = Calibration Factor from succeeding analyses.

7.4.3 Internal standard calibration procedure:

7.4.3.1 To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.4.3.2 Prepare calibration standards at a minimum of five concentration levels for each analyte of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards and dilute to volume with an appropriate solvent.

One of the standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.4.3.3 Inject each calibration standard using the same introduction technique that will be applied to the actual samples (e.g., 2- to 5- μ L injection, purge-and-trap, etc.). Tabulate the peak height or area responses against the concentration of each compound and internal standard. Calculate response factors (RF) for each compound as follows:

$$RF = (A_S C_{IS}) / (A_{IS} C_S)$$

where:

A_S = Response for the analyte to be measured.

A_{IS} = Response for the internal standard.

C_{IS} = Concentration of the internal standard, μ g/L.

C_S = Concentration of the analyte to be measured, μ g/L.

If the RF value over the working range is constant ($\ll 20\%$ RSD), the RF can be assumed to be invariant, and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_S/A_{IS} versus RF.

7.4.3.4 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. The frequency of verification is dependent on the detector. Detectors, such as the electron capture detector, that operate in the sub-nanogram range are more susceptible to changes in detector response caused by GC column and sample effects. Therefore, more frequent verification of calibration is necessary. The flame ionization detector is much less sensitive and requires less frequent verification. If the response for any analyte varies from the predicted response by more than $\pm 15\%$, a new calibration curve must be prepared for that compound.

7.5 Retention time windows:

7.5.1 Before establishing windows, make sure the GC system is within optimum operating conditions. Make three injections of all single component standard mixtures and multiresponse products (i.e., PCBs) throughout the course of a 72-hr period. Serial injections over less than a 72-hr period result in retention time windows that are too tight.

7.5.2 Calculate the standard deviation of the three absolute retention times for each single component standard. For multiresponse products, choose one major peak from the envelope and calculate the

standard deviation of the three retention times for that peak. The peak chosen should be fairly immune to losses due to degradation and weathering in samples.

7.5.2.1 Plus or minus three times the standard deviation of the absolute retention times for each standard will be used to define the retention time window; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms. For multiresponse products (i.e., PCBs), the analyst should use the retention time window but should primarily rely on pattern recognition.

7.5.2.2 In those cases where the standard deviation for a particular standard is zero, the laboratory must substitute the standard deviation of a close eluting, similar compound to develop a valid retention time window.

7.5.3 The laboratory must calculate retention time windows for each standard on each GC column and whenever a new GC column is installed. The data must be retained by the laboratory.

7.6 Gas chromatographic analysis:

7.6.1 Introduction of organic compounds into the gas chromatograph varies depending on the volatility of the compound. Volatile organics are primarily introduced by purge-and-trap (Method 5030). However, there are limited applications where direct injection is acceptable. Use of Method 3810 or 3820 as a screening technique for volatile organic analysis may be valuable with some sample matrices to prevent overloading and contamination of the GC systems. Semivolatile organics are introduced by direct injection.

7.6.2 The appropriate detector(s) is given in the specific method.

7.6.3 Samples are analyzed in a set referred to as an analysis sequence. The sequence begins with instrument calibration followed by sample extracts interspersed with multilevel calibration standards. The sequence ends when the set of samples has been injected or when qualitative and/or quantitative QC criteria are exceeded.

7.6.4 **Direct Injection:** Inject 2-5 uL of the sample extract using the solvent flush technique. Smaller (1.0-uL) volumes can be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 uL and the resulting peak size in area units or peak height.

7.6.5 If the responses exceed the linear range of the system, dilute the extract and reanalyze. It is recommended that extracts be diluted so that all peaks are on scale. Overlapping peaks are not always evident when peaks are off scale. Computer reproduction of chromatograms, manipulated to ensure all peaks are on scale over a 100-fold range, are acceptable if linearity is demonstrated. Peak height measurements are recommended over peak area integration when overlapping peaks cause errors in area integration.

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7.6.6 If peak detection is prevented by the presence of interferences, further cleanup is required.

7.6.7 Examples of chromatograms for the compounds of interest are frequently available in the referring analytical method.

7.6.8 Calibrate the system immediately prior to conducting any analyses (see Paragraph 7.4). A midlevel standard must also be injected at intervals specified in the method and at the end of the analysis sequence. The calibration factor for each analyte to be quantitated, must not exceed a 15% difference when compared to the initial standard of the analysis sequence. When this criteria is exceeded, inspect the GC system to determine the cause and perform whatever maintenance is necessary (see Section 7.7) before recalibrating and proceeding with sample analysis. All samples that were injected after the sample exceeding the criteria must be reinjected.

7.6.9 Establish daily retention time windows for each analyte. Use the absolute retention time for each analyte from Section 7.6.8 as the midpoint of the window for that day. The daily retention time window equals the midpoint \pm three times the standard deviation determined in Section 7.5.

7.6.9.1 Tentative identification of an analyte occurs when a peak from a sample extract falls within the daily retention time window. Normally, confirmation is required: on a second GC column; by GC/MS if concentration permits; or by other recognized confirmation techniques. Confirmation may not be necessary if the composition of the sample matrix is well established by prior analyses.

7.6.9.2 Validation of GC system qualitative performance: Use the midlevel standards interspersed throughout the analysis sequence (Paragraph 7.6.8) to evaluate this criterion. If any of the standards fall outside their daily retention time window, the system is out of control. Determine the cause of the problem and correct it (see Section 7.7).

7.7 Suggested chromatography system maintenance: Corrective measures may require any one or more of the following remedial actions.

7.7.1 Packed columns: For instruments with injection port traps, replace the demister trap, clean, and deactivate the glass injection port insert or replace with a cleaned and deactivated insert. Inspect the injection end of the column and remove any foreign material (broken glass from the rim of the column or pieces of septa). Replace the glass wool with fresh deactivated glass wool. Also, it may be necessary to remove the first few millimeters of the packing material if any discoloration is noted, also swab out the inside walls of the column if any residue is noted. If these procedures fail to eliminate the degradation problem, it may be necessary to deactivate the metal injector body (described in Section 7.7.3) and/or repack/replace the column.

7.7.2 **Capillary columns:** Clean and deactivate the glass injection port insert or replace with a cleaned and deactivated insert. Break off the first few inches, up to one foot, of the injection port side of the column. Remove the column and solvent backflush according to the manufacturer's instructions. If these procedures fail to eliminate the degradation problem, it may be necessary to deactivate the metal injector body and/or replace the column.

7.7.3 **Metal injector body:** Turn off the oven and remove the analytical column when oven has cooled. Remove the glass injection port insert (instruments with off-column injection or Grob). Lower the injection port temperature to room temperature. Inspect the injection port and remove any noticeable foreign material.

7.7.3.1 Place a beaker beneath the injector port inside the GC oven. Using a wash bottle, serially rinse the entire inside of the injector port with acetone and then toluene; catching the rinsate in the beaker.

7.7.3.2 Prepare a solution of deactivating agent (Sylon-CT or equivalent) following manufacturer's directions. After all metal surfaces inside the injector body have been thoroughly coated with the deactivation solution, serially rinse the injector body with toluene, methanol, acetone, and hexane. Reassemble the injector and replace the GC column.

7.8 Calculations:

7.8.1 **External standard calibration:** The concentration of each analyte in the sample may be determined by calculating the amount of standard purged or injected, from the peak response, using the calibration curve or the calibration factor determined in Paragraph 7.4.2. The concentration of a specific analyte is calculated as follows:

Aqueous samples:

$$\text{Concentration (ug/L)} = [(A_x)(A)(V_t)(D)] / [(A_s)(V_i)(V_s)]$$

where:

A_x = Response for the analyte in the sample, units may be in area counts or peak height.

A = Amount of standard injected or purged, ng.

A_s = Response for the external standard, units same as for A_x .

V_i = Volume of extract injected, uL. For purge-and-trap analysis, V_i is not applicable and therefore = 1.

D = Dilution factor, if dilution was made on the sample prior to analysis. If no dilution was made, $D = 1$, dimensionless.

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V_t = Volume of total extract, uL. For purge-and-trap analysis, V_t is not applicable and therefore = 1.

V_s = Volume of sample extracted or purged, mL.

Nonaqueous samples:

$$\text{Concentration (ng/g)} = [(A_x)(A)(V_t)(D)] / [(A_s)(V_i)(W)]$$

where:

W = Weight of sample extracted or purged, g. The wet weight or dry weight may be used, depending upon the specific applications of the data.

A_x , A_s , A, V_t , D, and V_i have the same definition as for aqueous samples.

7.8.2 Internal standard calibration: For each analyte of interest, the concentration of that analyte in the sample is calculated as follows:

Aqueous samples:

$$\text{Concentration (ug/L)} = [(A_x)(C_{iS})(D)] / [(A_{iS})(RF)(V_s)]$$

where:

A_x = Response of the analyte being measured, units may be in area counts or peak height.

C_{iS} = Amount of internal standard added to extract or volume purged, ng.

D = Dilution factor, if a dilution was made on the sample prior to analysis. If no dilution was made, D = 1, dimensionless.

A_{iS} = Response of the internal standard, units same as A_x .

RF = Response factor for analyte, as determined in Paragraph 7.4.3.3.

V_s = Volume of water extracted or purged, mL.

Nonaqueous samples:

$$\text{Concentration (ug/kg)} = [(A_s)(C_{iS})(D)] / [(A_{iS})(RF)(W_s)]$$

where:

W_s = Weight of sample extracted, g. Either a dry weight or wet weight may be used, depending upon the specific application of the data.

A_s, C_{is}, D, A_{is}, and RF have the same definition as for aqueous samples.

8.0 QUALITY CONTROL

8.1 Each laboratory that uses these methods is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and an ongoing analysis of spiked samples to evaluate and document quality data. The laboratory must maintain records to document the quality of the data generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method. When results of sample spikes indicate atypical method performance, a quality control check standard must be analyzed to confirm that the measurements were performed in an in-control mode of operation.

8.2 Before processing any samples, the analyst should demonstrate, through the analysis of a reagent water blank, that interferences from the analytical system, glassware, and reagents are under control. Each time a set of samples is extracted or there is a change in reagents, a reagent water blank should be processed as a safeguard against chronic laboratory contamination. The blank samples should be carried through all stages of the sample preparation and measurement steps.

8.3 For each analytical batch (up to 20 samples), a reagent blank, matrix spike and matrix spike duplicate/duplicate must be analyzed (the frequency of the spikes may be different for different monitoring programs). The blank and spiked samples must be carried through all stages of the sample preparation and measurement steps.

8.4 The experience of the analyst performing gas chromatography is invaluable to the success of the methods. Each day that analysis is performed, the daily calibration sample should be evaluated to determine if the chromatographic system is operating properly. Questions that should be asked are: Do the peaks look normal?; Is the response obtained comparable to the response from previous calibrations? Careful examination of the standard chromatogram can indicate whether the column is still good, the injector is leaking, the injector septum needs replacing, etc. If any changes are made to the system (e.g, column changed), recalibration of the system must take place.

8.5 Required instrument QC:

8.5.1 Section 7.4 requires that the %RSD vary by <20% when comparing calibration factors to determine if a five point calibration curve is linear.

8.5.2 Section 7.4 sets a limit of +15% difference when comparing daily response of a given analyte versus the initial response. If the limit is exceeded, a new standard curve must be prepared.

8.5.3 Section 7.5 requires the establishment of retention time windows.

8.5.4 Paragraph 7.6.8 sets a limit of $\pm 15\%$ difference when comparing the initial response of a given analyte versus any succeeding standards analyzed during an analysis sequence.

8.5.5 Paragraph 7.6.9.2 requires that all succeeding standards in an analysis sequence must fall within the daily retention time window established by the first standard of the sequence.

8.6 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.6.1 A quality (QC) check sample concentrate is required containing each analyte of interest. The QC check sample concentrate may be prepared from pure standard materials or purchased as certified solutions. If prepared by the laboratory, the QC check sample concentrate must be made using stock standards prepared independently from those used for calibration.

8.6.1.1 The concentration of the QC check sample concentrate is highly dependent upon the analytes being investigated. Therefore, refer to Method 3500, Section 8.0 for the required concentration of the QC check sample concentrate.

8.6.2 Preparation of QC check samples:

8.6.2.1 Volatile organic analytes (Methods 8010, 8020, and 8030): The QC check sample is prepared by adding 200 μL of the QC check sample concentrate (Section 8.6.1) to 100 mL of reagent water.

8.6.2.2 Semivolatile organic analytes (Methods 8040, 8060, 8080, 8090, 8100, and 8120): The QC check sample is prepared by adding 1.0 mL of the QC check sample concentrate (8.6.1) to each of four 1-L aliquots of reagent water.

8.6.3 Four aliquots of the well-mixed QC check sample are analyzed by the same procedures used to analyze actual samples (Section 7.0 of each of the methods). For volatile organics, the preparation/analysis process is purge-and-trap/gas chromatography. For semivolatile organics, the QC check samples must undergo solvent extraction (see Method 3500) prior to chromatographic analysis.

8.6.4 Calculate the average recovery (\bar{x}) in $\mu\text{g/L}$, and the standard deviation of the recovery (s) in $\mu\text{g/L}$, for each analyte of interest using the four results.

8.6.5 For each analyte compare s and \bar{x} with the corresponding acceptance criteria for precision and accuracy, respectively, given the QC Acceptance Criteria Table at the end of each of the determinative methods. If s and \bar{x} for all analytes of interest meet the acceptance

criteria, the system performance is acceptable and analysis of actual samples can begin. If any individual s exceeds the precision limit or any individual \bar{X} falls outside the range for accuracy, then the system performance is unacceptable for that analyte.

NOTE: The large number of analytes in each of the QC Acceptance Criteria Tables present a substantial probability that one or more will fail at least one of the acceptance criteria when all analytes of a given method are determined.

8.6.6 When one or more of the analytes tested fail at least one of the acceptance criteria, the analyst must proceed according to Section 8.6.6.1 or 8.6.6.2.

8.6.6.1 Locate and correct the source of the problem and repeat the test for all analytes of interest beginning with Section 8.6.2.

8.6.6.2 Beginning with Section 8.6.2, repeat the test only for those analytes that failed to meet criteria. Repeated failure, however, will confirm a general problem with the measurement system. If this occurs, locate and correct the source of the problem and repeat the test for all compounds of interest beginning with Section 8.6.2.

8.7 The laboratory must, on an ongoing basis, spike at least one sample per analytical batch (maximum of 20 samples per batch) to assess accuracy. For laboratories analyzing one to ten samples per month, at least one spiked sample per month is required.

8.7.1 The concentration of the spike in the sample should be determined as follows:

8.7.1.1 If, as in compliance monitoring, the concentration of a specific analyte in the sample is being checked against a regulatory concentration limit, the spike should be at that limit or 1 to 5 times higher than the background concentration determined in Section 8.7.2, whichever concentration would be larger.

8.7.1.2 If the concentration of a specific analyte in the sample is not being checked against a limit specific to that analyte, the spike should be at the same concentration as the QC check sample (8.6.2) or 1 to 5 times higher than the background concentration determined in Section 8.7.2, whichever concentration would be larger.

8.7.1.3 For semivolatile organics, it may not be possible to determine the background concentration levels prior to spiking (e.g., maximum holding times will be exceeded). If this is the case, the spike concentration should be (1) the regulatory concentration limit, if any; or, if none (2) the larger of either 5 times higher than the expected background concentration or the QC check sample concentration (Section 8.6.2).

8.7.2 Analyze one unspiked and one spiked sample aliquot to determine percent recovery of each of the spiked compounds.

8.7.2.1 Volatile organics: Analyze one 5-mL sample aliquot to determine the background concentration (B) of each analyte. If necessary, prepare a new QC check sample concentrate (Section 8.6.1) appropriate for the background concentration in the sample. Spike a second 5-mL sample aliquot with 10 uL of the QC check sample concentrate and analyze it to determine the concentration after spiking (A) of each analyte. Calculate each percent recovery (p) as $100(A - B)/T$, where T is the known true value of the spike.

8.7.2.2 Semivolatile organics: Analyze one sample aliquot (extract of 1-L sample) to determine the background concentration (B) of each analyte. If necessary, prepare a new QC check sample concentrate (Section 8.6.1) appropriate for the background concentration in the sample. Spike a second 1-L sample aliquot with 1.0 mL of the QC check sample concentrate and analyze it to determine the concentration after spiking (A) of each analyte. Calculate each percent recovery (p) as $100(A - B)/T$, where T is the known true value of the spike.

8.7.3 Compare the percent recovery (p) for each analyte with the corresponding criteria presented in the QC Acceptance Criteria Table found at the end of each of the determinative methods. These acceptance criteria were calculated to include an allowance for error in measurement of both the background and spike concentrations, assuming a spike to background ratio of 5:1. This error will be accounted for to the extent that the analyst's spike to background ratio approaches 5:1. If spiking was performed at a concentration lower than the QC check sample concentration (8.6.2), the analyst must use either the QC acceptance criteria presented in the Tables, or optional QC acceptance criteria calculated for the specific spike concentration. To calculate optional acceptance criteria for the recovery of an analyte: (1) Calculate accuracy (x') using the equation found in the Method Accuracy and Precision as a Function of Concentration Table (appears at the end of each determinative method), substituting the spike concentration (T) for C; (2) calculate overall precision (S') using the equation in the same Table, substituting x' for X; (3) calculate the range for recovery at the spike concentration as $(100x'/T) \pm 2.44(100S'/T)\%$.

8.7.4 If any individual p falls outside the designated range for recovery, that analyte has failed the acceptance criteria. A check standard containing each analyte that failed the criteria must be analyzed as described in Section 8.8.

8.8 If any analyte fails the acceptance criteria for recovery in Section 8.7, a QC check standard containing each analyte that failed must be prepared and analyzed.

NOTE: The frequency for the required analysis of a QC check standard will depend upon the number of analytes being simultaneously tested, the

complexity of the sample matrix, and the performance of the laboratory. If the entire list of analytes given in a method must be measured in the sample in Section 8.7, the probability that the analysis of a QC check standard will be required is high. In this case the QC check standard should be routinely analyzed with the spiked sample.

8.8.1 Preparation of the QC check standard: For volatile organics, add 10 μ L of the QC check sample concentrate (Section 8.6.1 or 8.7.2) to 5 mL of reagent water. For semivolatile organics, add 1.0 mL of the QC check sample concentrate (Section 8.6.1 or 8.7.2) to 1 L of reagent water. The QC check standard needs only to contain the analytes that failed criteria in the test in Section 8.7. Prepare the QC check standard for analysis following the guidelines given in Method 3500 (e.g., purge-and-trap, extraction, etc.).

8.8.2 Analyzed the QC check standard to determine the concentration measured (A) of each analyte. Calculate each percent recovery (p_s) as $100 (A/T)\%$, where T is the true value of the standard concentration.

8.8.3 Compare the percent recovery (p_s) for each analyte with the corresponding QC acceptance criteria found in the appropriate Table in each of the methods. Only analytes that failed the test in Section 8.7 need to be compared with these criteria. If the recovery of any such analyte falls outside the designated range, the laboratory performance for that analyte is judged to be out of control, and the problem must be immediately identified and corrected. The result for that analyte in the unspiked sample is suspect and may not be reported for regulatory compliance purposes.

8.9 As part of the QC program for the laboratory, method accuracy for each matrix studied must be assessed and records must be maintained. After the analysis of five spiked samples (of the same matrix type) as in Section 8.7, calculate the average percent recovery (\bar{p}) and the standard deviation of the percent recovery (s_p). Express the accuracy assessment as a percent recovery interval from $\bar{p} - 2s_p$ to $\bar{p} + 2s_p$. If $\bar{p} = 90\%$ and $s_p = 10\%$, for example, the accuracy interval is expressed as 70-110%. Update the accuracy assessment for each analyte on a regular basis (e.g. after each five to ten new accuracy measurements).

8.10 To determine acceptable accuracy and precision limits for surrogate standards the following procedure should be performed.

8.10.1 For each sample analyzed, calculate the percent recovery of each surrogate in the sample.

8.10.2 Once a minimum of thirty samples of the same matrix have been analyzed, calculate the average percent recovery (\bar{p}) and standard deviation of the percent recovery (s) for each of the surrogates.

8.10.3 For a given matrix, calculate the upper and lower control limit for method performance for each surrogate standard. This should be done as follows:

$$\begin{aligned}\text{Upper Control Limit (UCL)} &= p + 3s \\ \text{Lower Control Limit (LCL)} &= p - 3s\end{aligned}$$

8.10.4 For aqueous and soil matrices, these laboratory established surrogate control limits should, if applicable, be compared with the control limits listed in Tables A and B of Methods 8240 and 8270, respectively. The limits given in these methods are multi-laboratory performance based limits for soil and aqueous samples, and therefore, the single-laboratory limits established in Paragraph 8.10.3 must fall within those given in Tables A and B for these matrices.

8.10.5 If recovery is not within limits, the following is required.

- Check to be sure there are no errors in calculations, surrogate solutions and internal standards. Also, check instrument performance.
- Recalculate the data and/or reanalyze the extract if any of the above checks reveal a problem.
- Reextract and reanalyze the sample if none of the above are a problem or flag the data as "estimated concentration."

8.10.6 At a minimum, each laboratory should update surrogate recovery limits on a matrix-by-matrix basis, annually.

8.11 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to assess the precision of the environmental measurements. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.0 METHOD PERFORMANCE

9.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The MDL concentrations listed in the referring analytical methods were obtained using reagent water. Similar results were achieved using representative wastewaters. The MDL actually achieved in a given analysis will vary depending on instrument sensitivity and matrix effects.

9.2 Refer to the determinative method for specific method performance information.

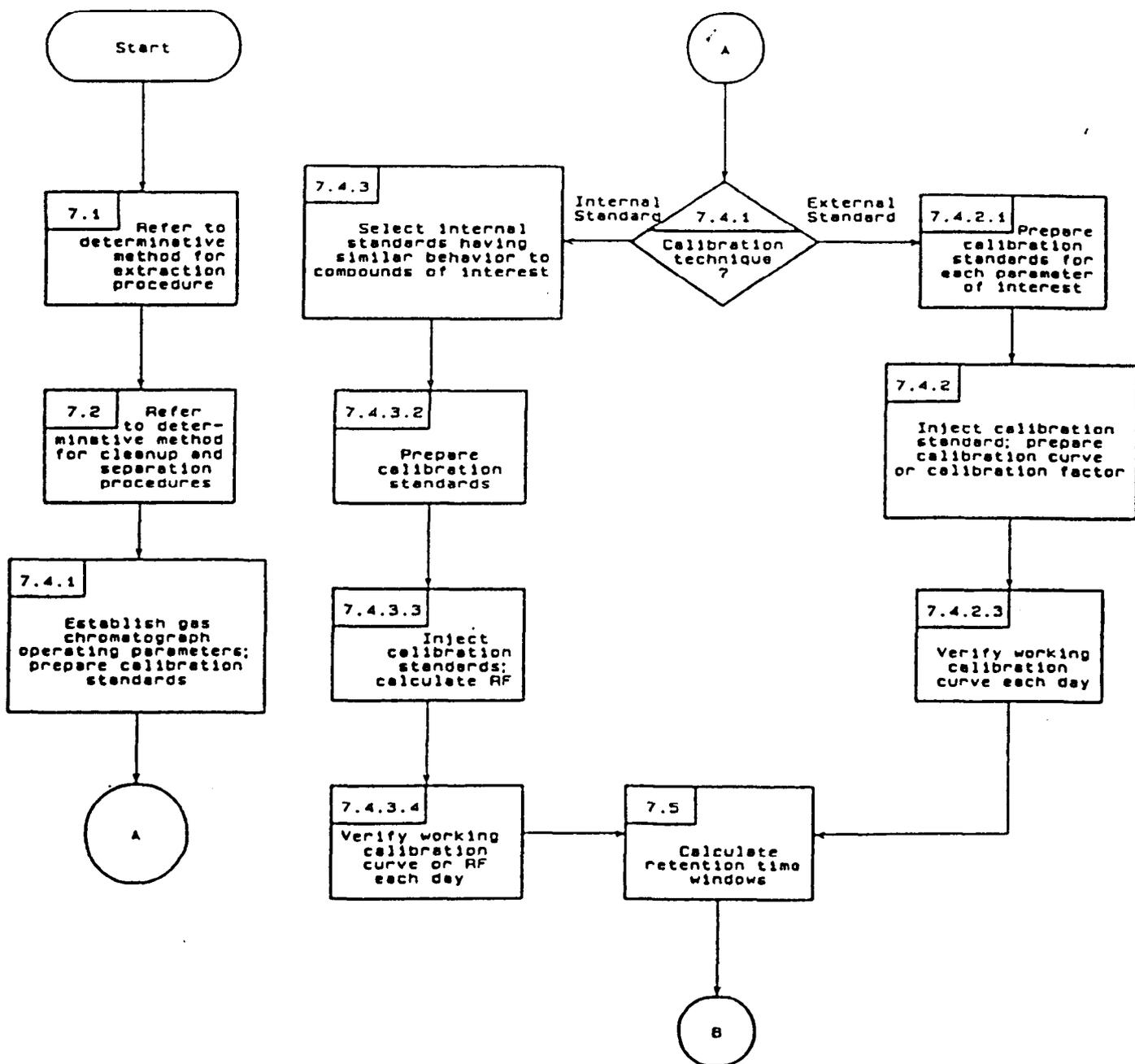
10.0 REFERENCES

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2. U.S. EPA 40 CFR Part 136, Appendix B. "Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act; Final Rule and Interim Final Rule and Proposed Rule," October 26, 1984.
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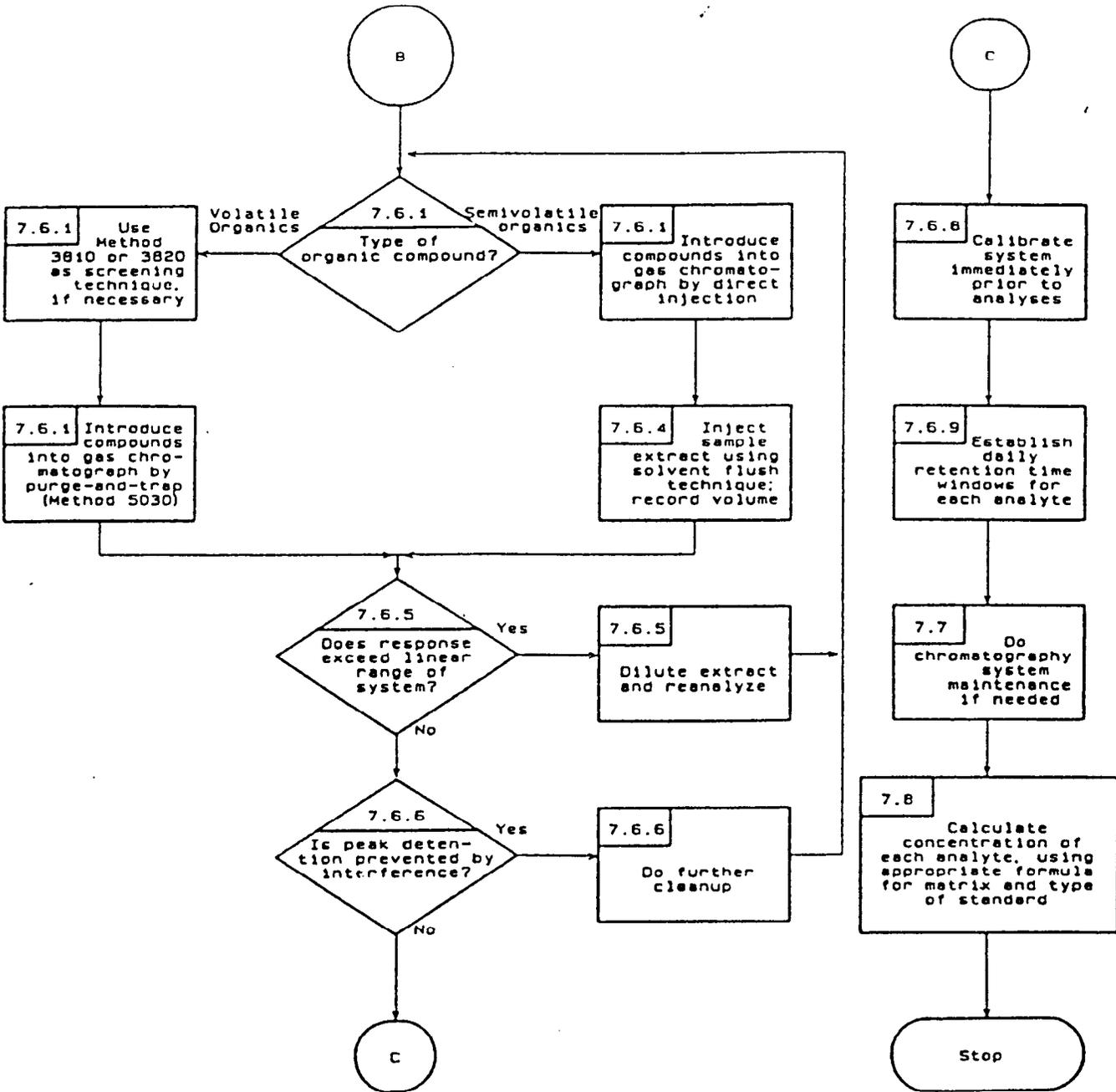
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METHOD 8000
GAS CHROMATOGRAPHY



METHOD 8000
 GAS CHROMATOGRAPH
 (Continued)



CALIFORNIA DEPT. OF FOOD & AGRIC.
CHEMISTRY LABORATORY SERVICES
ENVIRONMENTAL MONITORING SECTION
3292 Meadowview Road
Sacramento, CA 95832
(916) 427-4998/4999

Original Date: October 16, 1991.
Supercedes: NEW
Current Date: October 16, 1991.
Method #:

DETERMINATION OF 2,4-D IN WELL WATER USING MS DETECTION

SCOPE:

This method is for the determination of 2,4-D in well water and is applicable to water samples whose background is too high to use electron capture detection. The minimum detectable level achieved by this method is 0.1 ppb

PRINCIPLE:

The water samples are acidified with sulfuric acid (18 N) to a pH of less than 2. The 2,4-D is extracted from the acidified water with methylene chloride. The extract is treated with diazomethane and the resulting 2,4-D methyl ester derivative is then analyzed by gas chromatography with mass spectral detection. Recoveries from this method average 85-90%.

REAGENTS AND EQUIPMENT:

Solvents: methylene chloride (residue grade)
ethyl ether (Reagent, A.C.S., redistilled)
Sulfuric acid, 18 N (conc. sulfuric acid / water 1:1)
Sodium sulfate, anhydrous (Reagent, A.C.S)

Diazomethane in ethyl ether (prepared with Diazald[®] Kit

according to technical bulletin AL-131, Aldrich Chemical Co.

Note: Diazomethane is highly toxic and carcinogenic and has been known to explode both as a gas and in solution. All reactions involving use of diazomethane should be carried out in an efficient hood and behind a safety shield

Funnels, glass stem, 3 inch diameter

Filter Paper (Whatman #1 12.5 cm)

Separatory funnels (1 L)

Boiling flasks, flat-bottomed (500 mL)

Rotary evaporator (Büchi/Brinkmann, Model R110)

Graduated conical centrifuge tubes (15 mL)

Nitrogen evaporator (Myers N-EVAP, Organomation Associates Inc.)

ANALYSIS:

1. Allow sample to come to room temperature.
2. Mix sample by inverting the sample bottle 5 or 6 times and weigh 800 g of water sample into a 1 L separatory funnel.
3. Acidify the water sample with ~2 mL of 18 N sulfuric acid, Check with pH paper to ensure that pH value is less than 2.

4. Extract the acidified sample with 100 mL of methylene chloride three times
5. Dry the methylene chloride extract by passing it through 20 g of sodium sulfate supported by filter paper in a glass funnel, and collect the dry extract in a 500 mL boiling flask. Wash the sodium sulfate with 50 mL of methylene chloride.
6. Evaporate the solvent in a rotary evaporator to about 3-5 mL at 35°C. and approximately 20 mm Hg vacuum.
7. Add 5 mL of isooctane to the flask and continue to evaporate for 5 more minutes.
8. Remove the remaining methylene chloride using a nitrogen sweep evaporator at ambient temperature to a volume of 1-2 mL.
9. Methylate by adding about 3 mL of diazomethane solution, and stoppering for 15 min. (Note: Yellow color should persist throughout this time. If reaction mixture becomes colorless, add more diazomethane.)
10. Evaporate the remaining diazomethane at ambient temperature with a gentle stream of nitrogen.
11. Transfer the sample quantitatively to a 15 mL conical centrifuge tube with diethyl ether, and adjust the volume to 5 ml.

EQUIPMENT CONDITIONS:

Determination of 2,4-D was performed on a Hewlett Packard Model 5890 gas chromatograph equipped with a Model 5970 mass selective detector, a Model 9000-340 computer system, and a Model 7673A autosampler.

Column: HP-1 (methyl silicone gum) 22 m x 0.2 mm x 0.33 um film
Carrier: Helium
Column Head Pressure: 6 psi
Oven Temperature: Initial 60°C 1 min
Program rate 20°C/min
Final 270°C 10 min
Injector temperature: 250°C
Detector temperature: 250°C
Ions monitored (m/z): 175.0, 199.0, 201.0, 234.0, and 236.0.
Retention time 2,4-D-OMe: 9.20 ± .01 min (n=16)
Autosampler injection volume: 2 ul

CALCULATIONS:

$$PPB = \frac{(PA1)(FV)(SC)(1000)}{(PA2)(W)}$$

Where PA1 - peak area of analyte from sample injection
PA2 - peak area of analyte standard
FV - final volume of sample extract (ml)
W - sample weight (g)
SC - standard concentration (in ng/ul)

The parent ion at mass 234 was used for quantification, since its ion chromatogram showed the fewest background peaks. Any peak appearing at the expected retention time ± .05 min in the mass 233.4-236.5 chromatogram was scanned to show the relative abundances of the five ions listed above. For

all samples reported as positive, agreement between the ratios of at least three of these ions in the sample and standard was good (within 5%). (The base peak, 199.0, was enriched by a coeluting contaminant and could not be used for quantification. Attempts to eliminate this interference by subtracting ion scans from shoulder regions of the main peak were unsuccessful).

DISCUSSION:

The percent recoveries for three 1.0 ppb spikes and three 0.3 ppb spikes are given below.

<u>Spike Level</u> (ppb)	<u>Recovery</u> (%)	<u>Standard Deviation</u> (of recoveries)	<u>n</u>
0.3	85	8.2	3
1.0	90	6.4	3

Recovery is affected by the by the volatility of 2,4-D methyl ester. Care must be taken at steps 10 and 11 to ensure that the methyl ester does not evaporate--which is why ethyl ether was chosen as the transfer solvent and ambient temperature was used for the evaporation. Ethyl ether is not a completely satisfactory solvent for introducing samples into the GC because of its high volatility, and because it contains a relatively large number of impurities visible by MS. Distilling the ether on the rotary evaporator alleviated the latter problem and carrying out analyses promptly mitigated the former. Even though sample extracts had too many background peaks to be reliably analyzed using EC detection, additional cleanup was avoided by the use of mass selective detection in the selected ion monitoring mode.

ACKNOWLEDGEMENTS:

I wish to thank Paul Lee who suggested use of the GC/MSD for my samples, generously walked me through the procedure for using this instrument, and helped me out of trouble.

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WRITTEN BY: Sylvia Richman

Sylvia Richman

TITLE: Agricultural Chemist II

REVIEWED BY: Catherine Cooper

Catherine Cooper

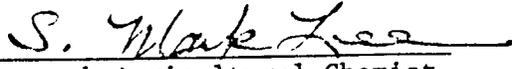
TITLE: Agricultural Chemist III

APPROVED BY: Terry Jackson



TITLE: Quality Assurance Officer

APPROVED BY: S. Mark Lee



TITLE: Research Agricultural Chemist