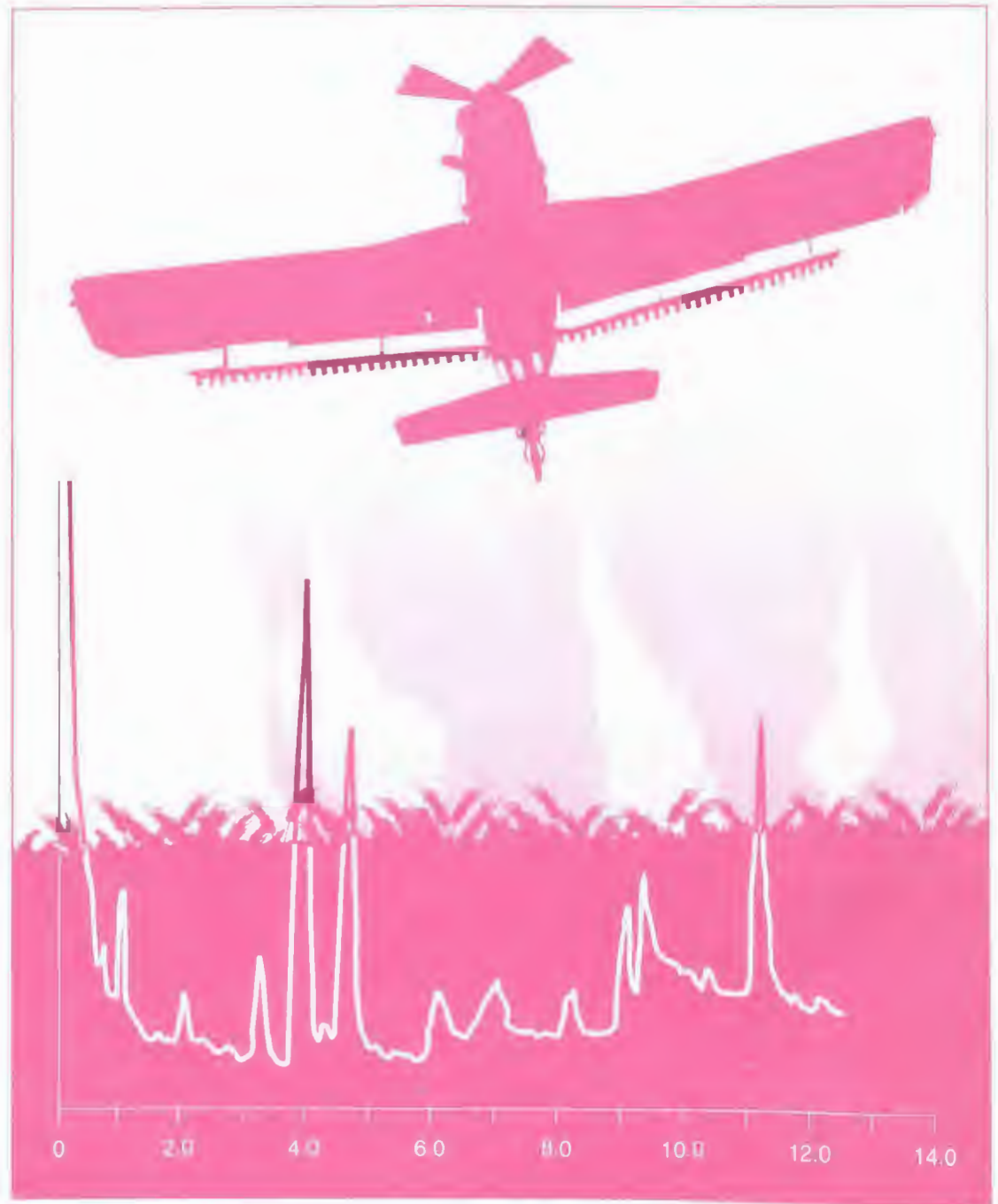




Methods For The Determination Of Nonconventional Pesticides In Municipal And Industrial Wastewater





Methods for the Determination of Nonconventional Pesticides in Municipal and Industrial Wastewater

Acknowledgements

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Disclaimer

This methods compendium has been reviewed by the Engineering and Analysis Division, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The Environmental Protection Agency (EPA) is proposing effluent limitations and guidelines at 40 *CFR* Part 455 to control the discharge of certain pesticide active ingredients into wastewater. This compendium of test procedures (analytical methods) supports this proposal.

This compendium includes many methods for determination of active ingredients in wastewater that have not previously been proposed or published, whether or not the active ingredient is included in the proposal. The purpose of including all of these methods is to create a single reference for analysts seeking to measure infrequently determined active ingredients.

These test procedures are proposed under the authority of Sections 304(h) and 501(a) of the Federal Water Pollution Control Act (FWPCA) (33 U.S.C. 1251 et. seq.). These sections of the FWPCA require EPA to promulgate guidelines establishing test procedures for the determination of pollutants in wastewater.

Many of the test procedures in this compendium were listed in Appendix E of EPA's original promulgation of rules for the pesticides category (50 *FR* 40708). These test procedures were withdrawn as a part of the remand of the pesticides rules in 1986 (51 *FR* 44911). Some of the test procedures that appeared in the original promulgation have been updated to include more analytes and/or include additional performance data.

Many of the test procedures in the original promulgation were also published by EPA's Effluent Guidelines Division in 1983 as publication EPA 440/1-83/079-C. This publication is now out of print. The publication included industry methods, EPA developed methods, and contractor methods.

The test procedures in this compendium are methods developed by EPA's Environmental Monitoring Systems Laboratory in Cincinnati, Ohio, (EMSL-Ci), methods developed by EPA's Engineering and Analysis Division (EAD; formerly the Industrial Technology Division and the Effluent Guidelines Division) within EPA's Office of Science and Technology (formerly the Office of Water Regulations and Standards), and an industry method for organo-tin compounds.

The EMSL-Ci methods were developed in the late 1970s and early 1980s. Some have been updated in the interim. These methods have three digit numbers beginning with 6 (e.g., 622). The methods written by EAD were developed to measure active ingredients in support of the pesticides rulemaking, and have therefore been applied to the specific wastewater for which they were intended. These methods have four digit numbers beginning with 16 (e.g., 1656). The industry methods for organo-tin are Methods EV-024 and EV-025.

This compendium comprises 41 different EPA methods and one industry method. A summary of all the analytes that may be detected using these methods is shown in the Cross Reference table. Some analytes can be detected by more than one method.

In addition to methods developed for today's proposed rule, EPA is investigating other methods and other analytical techniques to aid in the determination of non-conventional pesticides and other analytes of concern. EPA is interested in simplifying methods, where possible, and in reducing the potential pollution threat caused by the volumes of solvents used in some methods. An example of a simplification technique is the use of an immunoassay specific to a given analyte (such as a pesticide) or analyte group (such as the phenoxyacid herbicides) to allow EPA to screen rapidly for these analytes in discharges and in other environmental samples. EPA is also investigating the use of "solid phase extraction" (liquid-solid extraction) as a means of reducing the amount of solvent used in conventional

extraction procedures. Solid phase extraction (SPE) has been successfully applied to drinking water matrices, but initial tests with wastewaters containing high dissolved solids yielded low recoveries of the analytes of concern. More recent materials have yielded recoveries more consistent with conventional extraction techniques. EPA will continue to investigate these and other analytical techniques with the objective of producing lower cost, more rapid, and less environmentally damaging analytical methods.

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615	Chlorinated Herbicides	97
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617	Organohalide Pesticides and PCBs	137
618	Volatile Pesticides	169
619	Triazine Pesticides	187
620	Diphenylamine	209
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622.1	Thiophosphate Pesticides	247
627	Dinitroaniline Pesticides	269
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630	Dithiocarbamate Pesticides	303
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632	Carbamate and Urea Pesticides	347
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633	Organonitrogen Pesticides	379
633.1	Neutral Nitrogen-Containing Pesticides	397
634	Thiocarbamate Pesticides	417
635	Rotenone	437
636	Bensulide	455
637	MBTS and TCMTB	471
638	Oryzalin	489
639	Bendiocarb	507
640	Mercaptobenzothiazole	525
641	Thiabendazole	543
642	Biphenyl and Ortho-Phenylphenol	557
643	Bentazon	571
644	Picloram	585
645	Certain Amine Pesticides and Lethane	601
646	Dinitro Aromatic Pesticides	619
1656	Organohalide Pesticides	635
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1658	Phenoxy-Acid Herbicides	717
1659	Dazomet	753
1660	Pyrethrins and Pyrethroids	769
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Appendix

Methods EV-024 and EV-025:

Analytical Procedures for Determining Total Tin and Triorganot in Wastewater 805

Cross-Reference

<i>Parameter</i>	<i>CAS No.</i>	<i>Applicable Method(s)</i>
Acephate	30560-19-1	1656, 1657
Acifluorfen	50594-66-6	1656
Alachlor	15972-60-8	645
Aldrin	309-00-2	617, 1656
Allethrin (pynamin)	584-79-2	1660
Ametryn	834-12-8	619
Aminocarb	2032-59-9	632
Amobam	3566-10-7	630, 630.1
AOP	--	630
Aspon	3244-90-4	622.1
Atraton	1610-17-9	619
Atrazine	1912-24-9	619, 1656
Azinphos ethyl	2642-71-9	1657
Azinphos methyl	86-50-0	614, 622, 1657
Barban	101-27-9	632
Basalin (Fluchloralin)	33245-39-5	646
Bendiocarb	22781-23-3	639
Benfluralin	1861-40-1	627, 1656
Benomyl	17804-35-2	631
Bensulide	741-58-2	636
Bentazon (Basagran)	25057-89-0	643
α -BHC	319-84-6	617, 1656
β -BHC	319-85-7	617, 1656
γ -BHC	58-89-9	617, 1656
δ -BHC	319-86-8	617, 1656
Biphenyl	92-52-4	642, 1625
Bromacil	314-40-9	633, 1656
Bromoxynil octanoate	1689-99-2	1656
Bromoxynil	1689-84-5	1661, 1625
Busan 40	51026-28-9	630, 630.1
Busan 85	128-03-0	630, 630.1
Butachlor	23184-66-9	645, 1656
Butylate	2008-41-5	634
Captafol	2425-06-1	1656
Captan	133-06-2	617, 1656
Carbam-S	128-04-1	630, 630.1 ¹
Carbaryl	63-25-2	632

¹Carbam-S was not explicitly listed in these methods, but these methods are applicable to dithiocarbamate pesticides such as Carbam-S.

Cross-Reference (cont.)

<i>Parameter</i>	<i>CAS No.</i>	<i>Applicable Method(s)</i>
Carbendazim	10605-21-7	631
Carbofuran	1563-66-2	632
Carbophenothion	786-19-6	617, 1656
CDN	97-00-7	646
Chlordane	5103-74-2	617, 1656
Chlorfevinphos	470-90-6	1657
Chlorobenzilate	510-15-6	608.1, 1656
Chloroneb	2675-77-6	608.1, 1656
Chloropicrin	76-06-2	618
Chloropropylate	5836-10-2	608.1, 1656
Chlorothalonil	1897-45-6	608.2, 1656
Chlorpropham	101-21-3	632
Chlorpyrifos methyl	5598-13-0	622, 1657
Chlorpyrifos	2921-88-2	622, 1657
Coumaphos	56-72-4	622, 1657
Crotoxyphos	7700-17-6	1657
Cyanazine	21725-46-2	629
Cycloate	1134-23-2	634
Cycloprate	54460-46-7	616
Cyfluthrin (baythroid)	68359-37-5	1660
Dalapon	75-99-0	615, 1658
Dazomet	533-74-4	1659
2,4-D	94-75-7	615, 1658
2,4-DB	94-82-6	615, 1658
DBCP	96-12-8	1656
DCPA	1861-32-1	608.2
4,4'-DDD	72-54-8	617, 1656
4,4'-DDE	72-55-9	617, 1656
4,4'-DDT	50-29-3	617, 1656
Deet	134-62-3	633
DEF	78-48-8	1657
Demeton	8065-48-3	614, 622, 1657
Diallate	2303-16-4	1656
Diazinon	333-41-5	614, 622, 1657
Dibromochloropropane	96-12-8	608.1
Dicamba	1918-00-9	615, 1658
Dichlofenthion	97-17-6	622.1, 1657
Dichlone	117-80-6	1656
Dichloran	99-30-9	608.2, 617
Dichlorophen	97-23-4	604.1
Dichlorprop	120-36-5	615, 1658

Cross-Reference (cont.)

<i>Parameter</i>	<i>CAS No.</i>	<i>Applicable Method(s)</i>
Dichlorvos	62-73-7	622, 1657
Dicofol	115-32-2	617, 1656
Dicrotophos	141-66-2	1657
Dieldrin	60-57-1	617, 1656
Dimethoate	60-51-5	1657
Dinocap	39300-45-3	646
Dinoseb	88-85-7	615, 1658
Dioxathion	78-34-2	614.1, 1657
Diphenamid	957-51-7	645
Diphenylamine	122-39-4	620
Disulfoton	298-04-4	614, 622, 1657
Diuron	330-54-1	632
Endosulfan I	959-98-8	617, 1656
Endosulfan II	33213-65-9	617, 1656
Endosulfan sulfate	1031-07-8	617, 1656
Endrin aldehyde	7421-93-4	617, 1656
Endrin	72-20-8	617, 1656
Endrin ketone	53494-70-5	1656
EPN	2104-64-5	614.1, 1657
EPTC	759-94-4	634
Ethalfuralin	55283-68-6	627, 1656
Ethion	563-12-2	614, 614.1, 1657
Ethoprop	13194-48-4	622, 1657
Ethylene dibromide	106-93-4	618
Etridiazole	2593-15-9	608.1, 1656
EXD	502-55-6	630.1
Famphur	52-85-7	622.1, 1657
Fenarimol	60168-88-9	633.1, 1656
Fenitrothion	122-14-5	622.1
Fensulfothion	115-90-2	622, 1657
Fenthion	55-38-9	622, 1657
Fenuron	101-42-8	632
Fenuron-TCA	4482-55-7	632
Fenvalerate	51630-58-1	1660
Ferbam	14484-64-1	630, 630.1
Fluometuron	2164-17-2	632
Fluridone	59756-60-4	645
Fonophos	944-22-9	622.1
Heptachlor epoxide	1024-57-3	617, 1656
Heptachlor	76-44-8	617, 1656
Hexachlorophene	70-30-4	604.1

Cross-Reference (cont.)

<i>Parameter</i>	<i>CAS No.</i>	<i>Applicable Method(s)</i>
Hexamethyl- phosphoramidate	680-31-9	1657
Hexazinone	51235-04-2	633
Isodrin	465-73-6	617, 1656
Isopropalin	33820-53-0	627, 1656
Kepone	143-50-0	1656
Kinoprene	42588-37-4	616
KN Methyl	137-41-7	630, 630.1
Leptophos	21609-90-5	1657
Lethane	112-56-1	645
Linuron	330-55-2	632
Malathion	121-75-5	614, 1657
Mancozeb	8018-01-7	630
Maneb	12427-38-2	630
MBTS	120-78-5	637
MCPA	94-74-6	615, 1658
MCPP	7085-19-0	615, 1658
Mercaptobenzothiazole	149-30-4	640
Merphos	150-50-5	622, 1657
Metham	137-42-8	630, 630.1
Methamidophos	10265-92-6	1657
Methiocarb	2032-65-7	632
Methomyl	16752-77-5	632
Methoprene	40596-69-8	616
Methoxychlor	72-43-5	608.2, 617, 1656
Metribuzin	21087-64-9	633, 1656
Mevinphos	7786-34-7	622, 1657
Mexacarbate	315-18-4	632
MGK 264-A	113-48-4	633.1
MGK 264-B	113-48-4	633.1
MGK 326	136-45-8	633.1
Mirex	2385-85-5	617, 1656
Molinate	2212-67-1	634
Monocrotophos	6923-22-4	1657
Monuron	150-68-5	632
Monuron-TCA	140-41-0	632
Nabam	142-59-6	630, 630.1
Nabonate	138-93-2	630.1
Naled	300-76-5	622, 1657
Napropamide	15299-99-7	632.1
Neburon	555-37-3	632

Cross-Reference (cont.)

<i>Parameter</i>	<i>CAS No.</i>	<i>Applicable Method(s)</i>
Niacide	8011-66-3	630
Nitrofen (TOK)	1836-75-5	1656
Norflorazon	27314-13-2	645, 1656
Organo-tin	—	EV-024 and EV-025
Oryzalin	19044-88-3	638
Oxamyl	23135-22-0	632
Parathion ethyl	56-38-2	614, 1657
Parathion methyl	298-00-0	614, 622, 1657
PCB-1016	12674-11-2	617, 1656
PCB-1221	11104-28-2	617, 1656
PCB-1232	11141-16-5	617, 1656
PCB-1242	53469-21-9	617, 1656
PCB-1248	12672-29-6	617, 1656
PCB-1254	11097-69-1	617, 1656
PCB-1260	11096-82-5	617, 1656
PCNB	82-68-8	608.1, 617, 1656
Pebulate	1114-71-2	634
Pendimethalin	40487-42-1	1656
Permethrin	52645-53-1	608.2, 1656, 1660
Perthane	72-56-0	617, 1656
o-Phenylphenol	132-27-4	642
Phorate	298-02-2	622, 1657
Phosmet	732-11-6	622.1, 1657
Phosphamidon	13171-21-6	1657
Picloram	1918-02-1	644
Polyram	9006-42-2	630
Profluralin	26399-36-0	627
Prometon	1610-18-0	619
Prometryn	7287-19-6	619
Pronamide	23950-58-5	633.1
Propachlor	1918-16-7	608.1, 1656
Propanil	709-98-8	632.1
Propazine	139-40-2	619, 1656
Propham	122-42-9	632
Propoxur	114-26-1	632
Pyrethrin I	121-21-1	1660
Pyrethrin II	121-29-9	1660
Resmethrin	10453-86-8	616, 1660
Ronnel	299-84-3	1657
Ronnel	299-84-3	622, 1657
Rotenone	83-79-4	635

Cross-Reference (cont.)

<i>Parameter</i>	<i>CAS No.</i>	<i>Applicable Method(s)</i>
Secbumeton	26259-45-0	619
Siduron	1982-49-6	632
Simazine	122-34-9	619, 1656
Simetryn	1014-70-6	619
Sodium dimethyldithiocarbamate	128-04-1	630, 630.1
Stirofos (Tetrachlorvinphos)	961-11-5	622, 1657
Strobane	8001-50-1	617, 1656
Sulfotepp	3689-24-5	1657
Sulprofos (bolstar)	35400-43-2	622
Sumithrin (phenothrin)	26002-80-2	1660
Swep	1918-18-9	632
2,4,5-T	93-76-5	615, 1658
TCMTB	21564-17-0	637
TEPP	107-49-3	1657
Terbacil	5902-51-2	633, 1656
Terbufos	13071-79-9	614.1, 1657
Terbuthylazine	5915-41-3	619, 1656
Terbutryn	886-50-0	619
Tetramethrin	7696-12-0	1660
Thiabendazole	148-79-8	641
Thionazin	297-97-2	622.1
Thiram	137-26-8	630, 630.1
Tokuthion	34643-46-4	622, 1657
Toxaphene	8001-35-2	617, 1656
2,4,5-TP	93-72-1	615, 1658
Triadimefon	43121-43-3	633, 1656
Trichlorofon	52-68-6	1657
Trichloronate	327-98-0	622, 1657
Tricresylphosphate	78-30-8	1657
Tricyclazole	41814-78-2	633
Trifluralin	1582-09-8	617, 627, 1656
Trimethylphosphate	512-56-1	1657
Trithion methyl	953-17-3	1657
Vacor	53558-25-1	632.1
Vernolate	1929-77-7	634
ZAC	—	630
Zineb	12122-67-7	630, 630.1
Ziram	137-30-4	630, 630.1

Method 604.1
The Determination of
Hexachlorophene and
Dichlorophen in Municipal and
Industrial Wastewaters

Method 604.1

The Determination of Hexachlorophene and Dichlorophen in Municipal and Industrial Wastewater Method

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of certain phenolic pesticides. The following parameters can be determined by this method:

<i>Parameters</i>	<i>CAS No.</i>
Dichlorophen	97-23-4
Hexachlorophene	70-30-4

- 1.2 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3 The method detection limit (MDL, defined in Section 14) for each compound is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.5 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second liquid chromatographic column that can be used to confirm measurements made with the primary column.

2. SUMMARY OF METHOD

- 2.1 A measured volume of sample, approximately 1 L, is adjusted to pH 4 to 4.5 and extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to methanol during concentration to a volume of 5 mL or less. Liquid chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by HPLC using an ultraviolet detector (UVD).¹

3. INTERFERENCES

- 3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in liquid chromatograms. All reagents and apparatus must be routinely demonstrated to be free from

interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The acid/base extraction cleanup described in Section 10 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified³⁻⁵ for the information of the analyst.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with PTFE. Aluminum foil may be substituted for PTFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of

the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel: 2000-mL, with PTFE stopcock.

5.2.2 Drying column: Chromatographic column 400 mm long by 10 mm ID with coarse frit.

5.2.3 Chromatographic column: 400 mm long by 19 mm ID with 250-mL reservoir at the top and PTFE stopcock (Kontes K-420290 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish: 25-mL, graduated (Kontes K-570050-0250 or equivalent). Calibration must be checked at the volumes employed in the test. A ground-glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish: 1000-mL (Kontes K-570001-1000 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Snyder column, Kuderna-Danish: Two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.8 Vials: Amber glass, 5- to 10-mL capacity with PTFE-lined screw-cap.

5.3 Boiling chips: Approximately 10/40 mesh carborundum. Heat to 400°C for 4 hours or perform a Soxhlet extraction with methylene chloride.

5.4 Water bath: Heated, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.

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

5.6 Liquid chromatograph: Analytical system complete with liquid chromatograph and all required accessories including syringes, analytical columns, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Pump: Isocratic pumping system, constant flow.

5.6.2 Column 1: Reversed-phase column, 5 μ Spherisorb-ODS, 250 mm long by 4.6 mm or equivalent. This column was used to develop the method performance statements in Section 14. Alternative columns may be used in accordance with provisions described in Section 12.1.

5.6.3 Column 2: Reversed-phase column, 5 μ Lichrosorb RP-2, 250 mm long by 4.6 mm or equivalent.

5.6.4 Detector: Ultraviolet absorbance detector, 254 nm. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 14. Alternative detectors may be used in accordance with the provisions described in Section 12.1.

6. REAGENTS

6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Methylene chloride, methanol, acetonitrile: Distilled-in-glass quality or equivalent.

- 6.3 Sodium sulfate: ACS, granular, anhydrous. Heat in a muffle furnace at 400°C overnight.
- 6.4 Sodium phosphate, monobasic: ACS, crystal.
- 6.5 1N sodium hydroxide: Dissolve 4.0 grams of NaOH (ACS) in 100 mL of distilled water.
- 6.6 Phosphoric acid (85%).
- 6.7 Stock standard solutions (1.00 µg/µL): Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.
 - 6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in distilled-in-glass quality methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 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.
 - 6.7.2 Transfer the stock standard solutions into PTFE-sealed screw-cap bottles. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
 - 6.7.3 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1 Establish liquid chromatographic operating parameters equivalent to those indicated in Table 1. The liquid chromatographic system can be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2 External standard calibration procedure:
 - 7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with 50/50 methanol/water. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
 - 7.2.2 Using injections of 20 to 50 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.
 - 7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than ±10%, the test must be

repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

- 7.3** Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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.
- 7.3.1** Prepare calibration standards at a minimum of three concentration levels for each parameter 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 50/50 methanol/water. One of the standards should be a representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.
- 7.3.2** Using injections of 20 to 50 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, 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} against RF.

- 7.3.3** The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.
- 7.4** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.
- 8.1.1** Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2** In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
- 8.1.3** The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2** To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
- 8.2.1** Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol, 1000 times more concentrated than the selected concentrations.
- 8.2.2** Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
- 8.2.3** Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.
- 8.2.4** Using the appropriate data from Table 2, determine the recovery and single-operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.
- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
- 8.3.1** Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁶ that are useful in observing trends in performance.

- 8.3.2** The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁶
- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.
- 8.5** Before processing any samples, the analyst should demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.
- 8.6** 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 monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques, such as liquid chromatography with a dissimilar column, must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. SAMPLES COLLECTION, PRESERVATION, AND HANDLING

- 9.1** Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2** The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3** Adjust the pH of the sample to 6 to 8 with sodium hydroxide or sulfuric acid immediately after sampling.

10. SAMPLE EXTRACTION

- 10.1** Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel.
- 10.2** The analyst may solvent-wash the sample at basic pH as described in Sections 10.2.1 and 10.2.2 to remove potential method interferences. For relatively clean samples, the wash should be omitted and the extraction, beginning with Section 10.3, should be followed.

- 10.2.1** Adjust the pH of the sample to 12.0 with 1N sodium hydroxide.
- 10.2.2** Add 60 mL of methylene chloride to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Discard the methylene chloride extract. Perform a second and third extraction in the same manner.
- 10.3** Add 50 g of NaH_2PO_4 to the sample in the separatory funnel and shake to dissolve the solid. The sample pH should be between 4.0 and 4.5. If necessary, adjust the pH with phosphoric acid or sodium hydroxide. Add 200 mL of methylene chloride to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separations. Collect the methylene chloride extract in a 1-L Erlenmeyer flask .
- 10.4** Add a second 200 mL volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.
- 10.5** Assemble a Kuderna-Danish (K-D) concentrator by attaching a 25-mL concentrator tube to a 1000-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.6** Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with 30 to 40 mL of methylene chloride.
- 10.7** Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in approximately 60 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.8** Increase the temperature of the hot water bath to about 80°C. Momentarily remove the Snyder column, add 15 mL of methanol and a new boiling chip, and attach a micro-Snyder. Pour about 1 mL of methanol into the top of the micro-Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 minutes. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

- 10.9** Remove the micro-Snyder column and adjust the volume to 2.5 mL with methanol. Transfer the liquid to a 5-mL volumetric flask and dilute to the mark with reagent water. Mix thoroughly prior to analysis. If the extracts will not be analyzed immediately, they should be transferred to PTFE-sealed screw-cap vials and refrigerated. If the sample extract requires no further cleanup, proceed with liquid chromatographic analysis. If the sample requires additional cleanup, proceed to Section 11.
- 10.10** Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1** Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method, namely the acid/base extraction described in Section 10, has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of additional cleanup, the analyst must demonstrate that the recovery of each compound of interest is no less than 85%.

12. LIQUID CHROMATOGRAPHY

- 12.1** Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. An example of the separation achieved by Column 1 is shown in Figure 1. Examples of the separation achieved by Column 2 are shown in Figure 2. Other columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.
- 12.2** Calibrate the liquid chromatographic system daily as described in Section 7.
- 12.3** If an internal standard approach is being used, the analyst must not add the internal standard until immediately before injection into the instrument. Mix thoroughly.
- 12.4** Inject 20 to 50 μL of the sample extract by completely filling the sample valve loop. Record the resulting peak sizes in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.
- 12.5** The width of the retention-time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6** If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7** If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_i)(V_s)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_s = Volume of total extract, in μL

V_e = Volume of water extracted, in mL

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_u)(\text{RF})(V_e)}$$

where

A_s = Response for parameter to be measured

A_u = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_e = Volume of water extracted, in L

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

14. METHOD PERFORMANCE

14.1 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.⁸ The MDL concentrations listed in Table 1 were obtained using reagent water.¹ Similar results were achieved using representative wastewaters.

14.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from $10 \times \text{MDL}$ to $1000 \times \text{MDL}$.

- 14.3** In a single laboratory, Battelle's Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 2 were obtained. Seven replicates of each of two different wastewaters were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 2.¹

References

1. "Development of Methods for Pesticides in Wastewaters," EPA Contract Report 68-03-2956 (In preparation).
2. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.
3. "Carcinogens - Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August, 1977.
4. "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976).
5. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
6. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U. S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory - Cincinnati, Ohio 45268, March 1979.
7. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.
8. Glaser, J. A. et. al, "Trace Analysis for Wastewaters," *Environmental Science and Technology*, 15, 1426 (1981).

Table 1. Chromatographic Conditions and Method Detection Limits

<i>Parameter</i>	<i>Retention Time (min)</i>		<i>Method Detection Limit (µg/L)</i>
	<i>Column 1</i>	<i>Column 2</i>	
Dichlorophen	4.2	8.2	1.0
Hexachlorophene	9.7	14.4	1.2

Dichlorophen, Hexachlorophene

Column 1 conditions: Spherisorb-ODS, 5 µ, 250 mm long by 4.6 mm; 1 mL/min flow; 65/35 acetonitrile/water, 0.05% H₃PO₄. A UV detector was used with this column to determine the MDL.

Column 2 conditions: Lichrosorb RP-2, 5 µ, 250 mm long by 4.6 mm; 1 mL/min flow; 50/50 acetonitrile/water, 0.5 acetic acid.

Table 2. Single-Laboratory Accuracy and Precision(a)

<i>Parameter</i>	<i>Sample Type (b)</i>	<i>Background µg/L</i>	<i>Spike Level µg/L</i>	<i>Mean Recovery (%)</i>	<i>Standard Deviation (%)</i>	<i>Number of Replicates</i>
Dichlorophen	1	ND (c)	10	58	12.4	7
	1	ND	50	107	3.9	7
Hexachlorophene	1	ND	10	82	2.7	7
	1	ND	50	102	5.8	7

(a) Column 1 conditions were used.

(b) 1 = POTW secondary effluent

(c) ND = Not detected

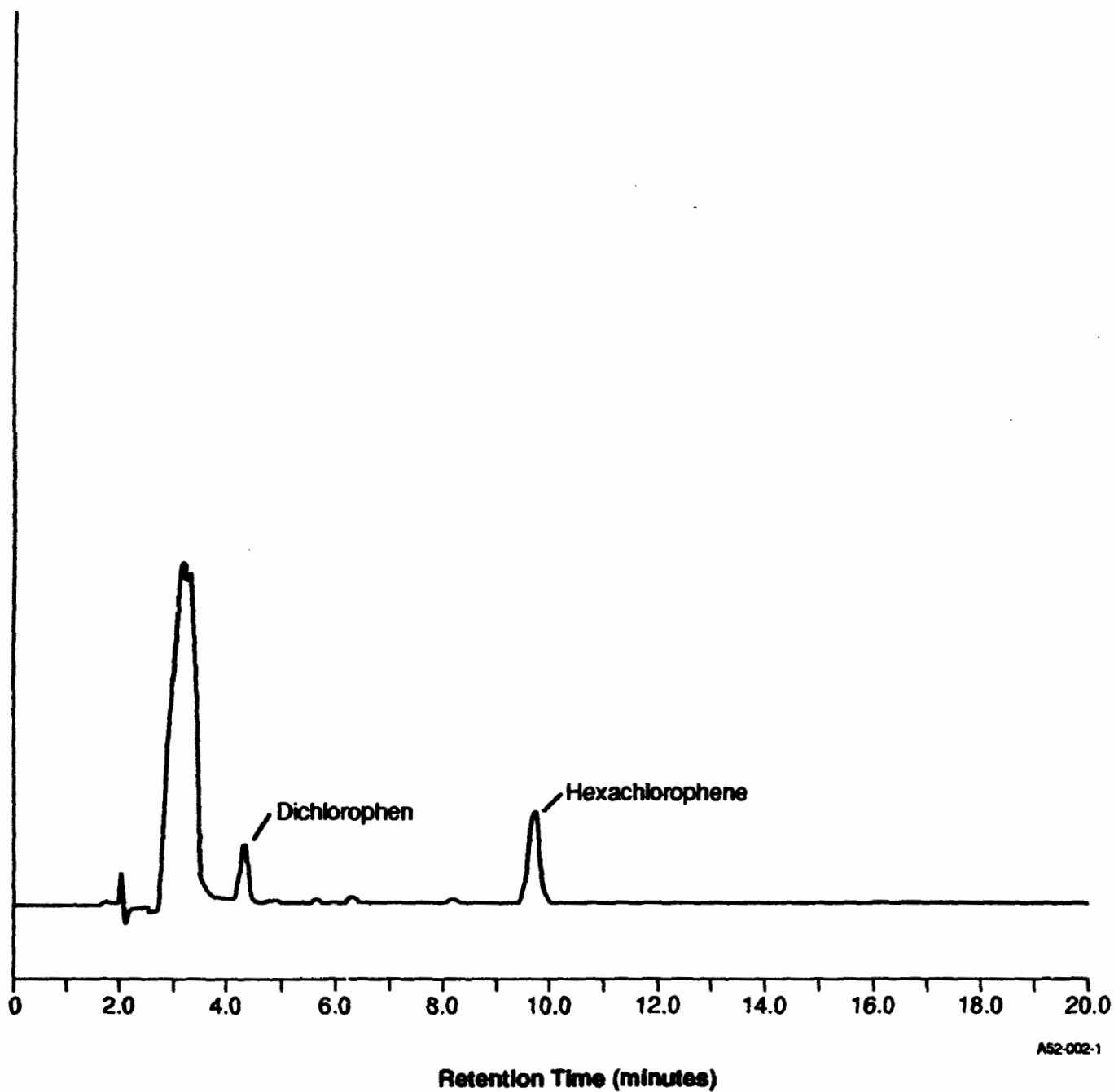
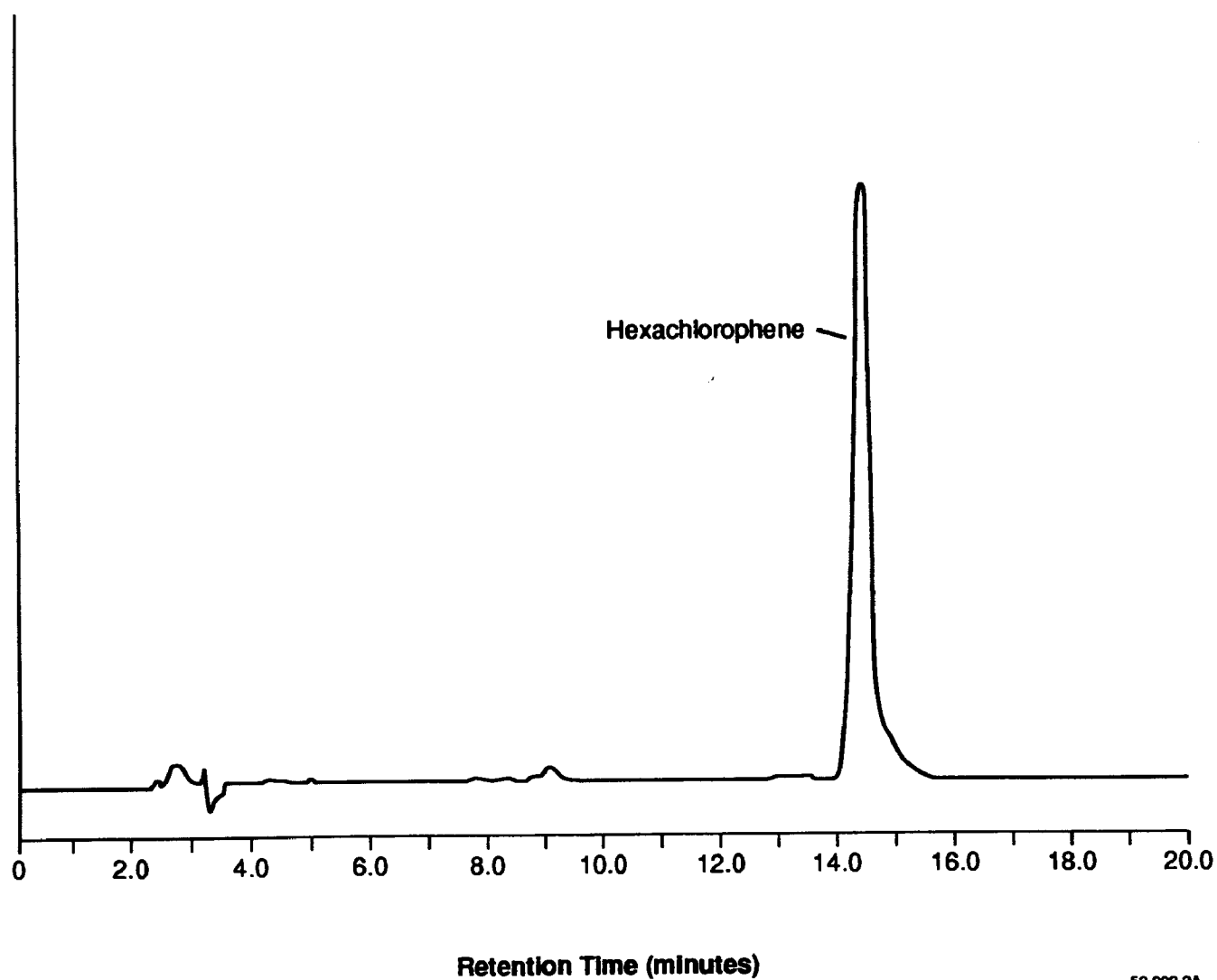


Figure 1. HPLC-UV Chromatogram of 10 ng Each of Hexachlorophene and Dichlorophen (Column 1).



52-002-2A

Figure 2. HPLC-UV Chromatogram of 250 ng Each of Hexachlorophene (Column 2).

Method 608.1
The Determination of
Organochlorine Pesticides in
Municipal and Industrial
Wastewater

Method 608.1

The Determination of Organochlorine Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of certain organochlorine pesticides. The following parameters can be determined by this method:

<i>Parameter</i>	<i>STORET No.</i>	<i>CAS No.</i>
Chlorobenzilate	39460	510-15-6
Chloroneb	-	2675-77-6
Chloropropylate	-	5836-40-2
Dibromochloropropane	-	96-12-8
Etridiazole	-	2593-15-9
PCNB	-	82-68-8
Propachlor	-	1918-16-7

- 1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3 The estimated method detection limit (MDL, defined in Section 125) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4 This method presents an extension in scope of Method 608. Further, the sample extraction and concentration steps in this method are essentially the same as several others in the 600-series methods. Thus, a single sample may be extracted to measure the parameters included in the scope of each of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures. Under gas chromatography, the analyst is allowed the latitude to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters (see Section 12).
- 1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column for chlorobenzilate and chloropropylate that can be used to confirm measurements made with the

primary column. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. SUMMARY OF METHOD

- 2.1** A measured volume of sample, approximately 1 L, is extracted with 15% methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to hexane during concentration to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by electron capture (EC) gas chromatography.¹
- 2.2** This method provides an optional Florisil column cleanup procedure to aid in the elimination or reduction of interferences which may be encountered.

3. INTERFERENCES

- 3.1** Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.
 - 3.1.1** Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.
 - 3.1.2** The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.2** Interferences by phthalate esters can pose a major problem in pesticide analysis when the EC detector is used. These compounds generally appear in the chromatogram as large late-eluting peaks, especially in the 15 and 50% fractions from the Florisil column cleanup. Common flexible plastics contain varying amounts of phthalates. These phthalates are easily extracted or leached from such materials during laboratory operations. Cross-contamination of clean glassware occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Interferences from phthalates can be minimized by avoiding the use of plastics in the laboratory. Exhaustive cleanup of reagents and glassware may be required to eliminate background phthalate contamination.^{3,4} The interferences from phthalate esters can be avoided by using a microcoulometric or electrolytic conductivity detector.
- 3.3** Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure

in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

- 4.1** The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified for the information of the analyst.⁵⁻⁷
- 4.2** The following parameters covered by this method have been tentatively classified as known or suspected human or mammalian carcinogens: chlorobenzilate, dibromochloropropane, and PCNB. Primary standards of these toxic compounds should be prepared in a hood.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel: 2000-mL, with TFE-fluorocarbon stopcock, ground-glass or TFE stopper.

5.2.2 Drying column: Chromatographic column 400 mm long by 19 mm ID with coarse-fritted disc.

5.2.3 Chromatographic column: 400 mm long by 19 mm ID with coarse-fritted disc at bottom and TFE-fluorocarbon stopcock (Kontes K-420540-0224 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground-glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

- 5.2.6 Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).
- 5.2.7 Vials: Amber glass, 10- to 15-mL capacity with TFE-fluorocarbon-lined screw-cap.
- 5.3 Boiling chips: Approximately 10/40 mesh. Heat at 400°C for 30 minutes or perform a Soxhlet extraction with methylene chloride.
- 5.4 Water bath: Heated, with concentric ring cover, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.
- 5.5 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6 Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.
 - 5.6.1 Column 1: 180 cm long by 2 mm ID glass, packed with 1.5% SP-2250/1.95% SP-2401 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 12.1.
 - 5.6.2 Column 2: 180 cm long by 2 mm ID glass, packed with Ultrabond 20M (100/120 mesh) or equivalent.
 - 5.6.3 Detector: Electron capture. This detector has proven effective in the analysis of wastewater for the parameters listed in the scope and was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. REAGENTS

- 6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2 Acetone, hexane, isooctane, methylene chloride, methanol: Pesticide-quality or equivalent.
- 6.3 Ethyl ether: Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips, (available from Scientific Products Co., Cat. No. P1126-8, and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.
- 6.4 Sodium sulfate: ACS, granular, anhydrous. Condition heating in a shallow tray at 400°C for a minimum of 4 hours to remove phthalates and other interfering organic substances. Alternatively, heat 16 hours at 450 to 500°C in a shallow tray or perform a Soxhlet extraction with methylene chloride for 48 hours.
- 6.5 Florisil: PR grade (60/100 mesh). Purchase activated at 675°C and store in dark in glass container with ground-glass stopper or foil-lined screw-cap. Before use, activate each batch at least 16 hours at 130°C in a foil-covered glass container.
- 6.6 Stock standard solutions (1.00 $\mu\text{g}/\mu\text{L}$): Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.
 - 6.6.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in pesticide-quality isooctane and dilute to volume in a

10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

- 6.6.2 Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw-cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
- 6.6.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2 External standard calibration procedure:
 - 7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with isooctane. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
 - 7.2.2 Using injections of 1 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.
 - 7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.
- 7.3 Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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.
 - 7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter 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 isooctane. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

- 7.3.2** Using injections of 1 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} , against RF.

- 7.3.3** The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.
- 7.4** The cleanup procedure in Section 11 utilizes Florisil chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil which is used, the use of the lauric acid value is suggested. This procedure⁸ determines the adsorption from hexane solution of lauric acid, in milligram, per gram of Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.
- 7.5** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

- 8.1.1** Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2** In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
- 8.1.3** The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2** To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
- 8.2.1** Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone, 1000 times more concentrated than the selected concentrations.
- 8.2.2** Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
- 8.2.3** Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.
- 8.2.4** Using the appropriate data from Table 2, determine the recovery and single operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.
- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
- 8.3.1** Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁹ that are useful in observing trends in performance.

- 8.3.2** The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the

requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁹

- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.
- 8.5** Before processing any samples, the analyst must demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.
- 8.6** 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 monitor the precision of the sampling technique. 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 perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. *SAMPLE COLLECTION, PRESERVATION, AND HANDLING*

- 9.1** Grab samples must be collected in glass containers. Conventional sampling practices¹⁰ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2** The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3** All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

10. *SAMPLE EXTRACTION*

- 10.1** Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel.
- 10.2** Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

- 10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.
- 10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.
- 10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.7 Increase the temperature of the hot water bath to about 80°C. Momentarily remove the Snyder column, add 50 mL of hexane and a new boiling chip, and reattach the Snyder column. Pour about 1 mL of hexane into the top of the Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 minutes. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.8 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to TFE-fluorocarbon-sealed screw-cap vials. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis. If the sample requires cleanup, proceed to Section 11.
- 10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various industrial and municipal effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

- 11.2** The following Florisil column cleanup procedure has been demonstrated to be applicable to the four organochlorine pesticides listed in Table 3. It should also be applicable to the cleanup of extracts for PCNB.
- 11.2.1** Add a weight of Florisil (nominally 20 g) predetermined by calibration (Section 7.4 and 7.5) to a chromatographic column. Settle the Florisil by tapping the column. Add anhydrous sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatography column. Discard the eluate.
- 11.2.2** Adjust the sample extract volume to 10 mL with hexane and transfer it from the K-D concentrator tube to the Florisil column. Rinse the tube twice with 1 to 2 mL hexane, adding each rinse to the column.
- 11.2.3** Place a 500-mL K-D flask and clean concentrator tube under the chromatography column. Drain the column into the flask until the sodium sulfate layer is nearly exposed. Elute the column with 200 mL of 6% ethyl ether in hexane (V/V) (Fraction 1) using a drip rate of about 5 mL/min. Remove the K-D flask and set aside for later concentration. Elute the column again, using 200 mL of 15% ethyl ether in hexane (V/V) (Fraction 2), into a second K-D flask. Perform a third elution, using 200 mL of 50% ethyl ether in hexane (V/V) (Fraction 3), into a separate K-D flask. The elution patterns for four of the pesticides are shown in Table 3.
- 11.2.4** Concentrate the eluates by standard K-D techniques (Section 10.6), substituting hexane for the glassware rinses and using the water bath at about 85°C. Adjust final volume to 10 mL with hexane. Analyze by gas chromatography.

12. GAS CHROMATOGRAPHY

- 12.1** Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.
- 12.2** Calibrate the system daily as described in Section 7.
- 12.3** If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.
- 12.4** Inject 1 to 5 μL of the sample extract using the solvent-flush technique.¹¹ Record the volume injected to the nearest 0.05 μL , and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.
- 12.5** The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

- 12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

- 13.1 Determine the concentration of individual compounds in the sample.

- 13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_i)(V_s)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_i = Volume of total extract, in μL

V_s = Volume of water extracted, in mL

- 13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where

A_s = Response for parameter to be measured

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_o = Volume of water extracted, in L

- 13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. GC/MS CONFIRMATION

- 14.1** It is recommended that GC/MS techniques be judiciously employed to support qualitative compound identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 seconds per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.
- 14.2** Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.¹²
- 14.3** At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.¹³
- 14.4** To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
- 14.4.1** All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to $\pm 10\%$. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20% to 40%.
- 14.4.2** The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.
- 14.4.3** Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.
- 14.5** Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.
- 14.6** Should these MS procedures fail to provide satisfactory results, additional steps may be taken before re-analysis. These may include the use of alternative packed or capillary GC columns or additional cleanup (Section 11).

15. METHOD PERFORMANCE

- 15.1** 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.¹⁴ The MDL concentrations listed in Table 1 were estimated from the response of an electron capture detector to each compound. The estimate is based upon the amount of material required to yield a signal

five times the GC background noise, assuming a 5- μ L injection from a 10-mL final extract of a 1-L sample.

- 15.2** In a single laboratory (West Cost Technical Services, Inc.), using effluents from pesticide manufacturers and publicly owned treatment works (POTW), the average recoveries presented in Table 2 were obtained after Florisil cleanup.¹ The standard deviations of the percent recoveries of these measurements are also included in Table 2.

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Table 1. Chromatographic Conditions and Estimated Method Detection Limits

<i>Parameter</i>	<i>Column 1</i>		<i>Column 2</i>	<i>Estimated MDL (µg/L)</i>
	<i>Temperature °C</i>	<i>Retention Time (minutes)</i>	<i>Retention Time (minutes)</i>	
Dibromochloropropane	100	3.1	--	0.04
Etridiazole	140	1.3	--	0.04
Chloroneb	150	2.0	--	0.04
Propachlor	150	3.8	--	1.0
PCNB	160	2.4	--	0.06
Chloropropylate	215	3.6	8.4	0.2
Chlorobenzilate	215	3.8	10.7	0.2

Column 1 conditions: Supelcoport (100/120 mesh) coated with 1.5% SP-2250/1.95% SP-2401 packed in a 1.8 m long by 2 mm ID glass column with nitrogen carrier gas at a flow rate of 30 mL/min. Column temperatures are listed above. An electron capture detector was used with this column to estimate the MDL.

Column 2 conditions: Ultrabond 20M (100/120 mesh) packed in a 1.8 m long by 2 mm ID glass column with nitrogen carrier gas at a flow rate of 30 mL/min. Column temperature is 200°C.

Table 2. Single-Operator Accuracy and Precision

<i>Parameter</i>	<i>Sample Type</i>	<i>Background µg/L</i>	<i>Spike µg/L</i>	<i>Mean Recovery (%)</i>	<i>Standard Deviation (%)</i>	<i>Number of Replicates</i>
Chlorobenzilate	MW	ND	10.5	74	7.2	6
	MW	ND	52.5	97	3.2	7
Chloroneb	MW	ND	18.1	92	2.9	7
	MW	ND	181	93	7.7	7
	IW	0.84	6.1	53	38.*	2
	IW	110	484	97	18.*	2
Chloropropylate	MW	ND	10.0	78	8.6	6
	MW	ND	50.0	96	3.3	7
Dibromochloro- propane	MW	ND	1.9	83	12.4	7
	MW	ND	24	70	6.5	7
	IW	ND	1.9	61	—	1
	IW	ND	24	55	1.2*	2
Etridiazole	MW	ND	0.50	144	9.9	7
	MW	ND	9.9	91	1.7	7
PCNB	MW	ND	1.0	100	11.0	7
	MW	ND	20.0	91	3.1	7
Propachlor	IW	21.3	179	87	3.8	7
	MW	ND	895	83	3.8	7

ND = Not detected

MW = Municipal wastewater

IW = Industrial wastewater, pesticide manufacturing

* For duplicate analyses, range is listed.

Table 3. Distribution of Chlorinated Pesticides Into Florisil Column Fractions

<i>Parameter</i>	<i>Percent Recovery by Fraction</i>		
	<i>Fraction 1</i>	<i>Fraction 2</i>	<i>Fraction 3</i>
Chlorobenzilate	0	15	70
Chloroneb	93		
Chloropropylate	0	32	61
Etridiazole	100		

Eluant composition by fraction:

Fraction 1 - 200 mL of 6% ethyl ether in hexane

Fraction 2 - 200 mL of 15% ethyl ether in hexane

Fraction 3 - 200 mL of 50% ethyl ether in hexane

Method 608.2
The Determination of Certain
Organochlorine
Pesticides in Municipal and
Industrial Wastewater

Method 608.2

The Determination of Certain Organochlorine Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of certain organochlorine pesticides in industrial and municipal wastewater. The following parameters may be determined by this method.

<i>Parameter</i>	<i>Storet No.</i>	<i>CAS No.</i>
Chlorothalonil	—	1897-45-6
DCPA	39770	1861-32-1
Dichlorin	—	99-30-9
Methoxychlor	39480	72-43-5
Permethrin	—	5264553-1

- 1.2 The estimated detection limit (EDL) for each parameter is listed in Table 1. The EDL was calculated from the minimum detectable response of the electron capture detector equal to 5 times the detector background noise assuming a 10.0-mL final extract volume of a 1-L reagent water sample and a gas chromatographic (GC) injection volume of 5 μ L. The EDL for a specific wastewater may be different depending on the nature of interferences in the sample matrix.
- 1.3 This is a GC method applicable to the determination of the compounds listed above in municipal and industrial discharges. When this method is used to analyze unfamiliar samples for any or all of the compounds listed above, compound identifications should be supported by at least one additional qualitative technique. Section 13 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative confirmation of compound identifications.
- 1.4 This method is restricted to use by or under the supervision of analysts experienced in the operation of gas chromatographs and in the interpretation of chromatograms.

2. SUMMARY OF METHOD

- 2.1 Organochlorine pesticides are removed from the sample matrix by extraction with methylene chloride. The extract is dried, exchanged into hexane, and analyzed by gas chromatography. Column chromatography is used as necessary to eliminate interferences which may be encountered. Measurement of the pesticides is accomplished with an electron capture detector.
- 2.2 Confirmatory analysis by gas chromatography/mass spectrometry is recommended (Section 13) when a new or undefined sample type is being analyzed if the concentration is adequate for such determination.

3. INTERFERENCES

- 3.1** Solvent, reagents, glassware, and other sample-processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 9.1.
- 3.1.1** The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.1.2** Glassware must be scrupulously cleaned.¹ Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap water and reagent water. It should then be drained dry and heated in a muffle furnace at 400°C for 15 to 30 minutes. Solvent rinses with acetone and pesticide-quality hexane may be substituted for the heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store the glassware inverted or capped with aluminum foil.
- 3.2** Interferences coextracted from the samples will vary considerably from source to source, depending on the diversity of the industrial complex or municipality being sampled. While general cleanup procedures are provided as part of this method, unique samples may require additional cleanup approaches to achieve the detection limits listed in Table 1.

4. SAFETY

- 4.1** The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified^{2,4} for the information of the analyst.

5. APPARATUS AND EQUIPMENT

- 5.1** Sample containers: Narrow-mouth glass bottles, 1-L or 1-quart volume, equipped with polytetrafluoroethylene (PTFE)-lined screw-caps. Wide-mouth glass bottles, 1-quart volume, equipped with PTFE-lined screw-caps may also be used. Prior to use, wash bottles and cap liners with detergent and rinse with tap and distilled water. Allow the bottles and cap liners to air dry, then muffle the glass bottles at 400°C for 1 hour. After cooling, rinse the cap liners with hexane, seal the bottles with aluminum foil, and store in a dust-free environment.
- 5.1.1** Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed

by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Kuderna-Danish (K-D) glassware.

5.2.1 Synder column: Three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.2 Concentrator tube: 10-mL, graduated (Kontes K-570050-1025 or equivalent) with ground-glass stopper.

5.2.3 Evaporative flask: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.3 Gas chromatography system.

5.3.1 The gas chromatograph must be equipped with a glass-lined injection port compatible with the detector to be used. A data system is recommended for measuring peak areas.

5.3.1.1 Column 1: 180 cm long by 2 mm ID, glass, packed with 1.5% OV-17/1.95% OV-210 on Chromosorb W-HP (100/120 mesh) or equivalent.

5.3.1.2 Column 2: 180 cm long by 2 mm ID, glass, packed with 4-% SE-30/6-% SP-2401 on Supelcoport (100/120 mesh) or equivalent. Guidelines for the use of alternative column packings are provided in Section 10.3.1.

5.3.1.3 Detector: Electron capture. This detector has proven effective in the analysis of wastewaters for the parameters listed in Section 1.1 and was used to develop the method performance statements in Section 12. Guidelines for the use of alternative detectors are provided in Section 10.3.1.

5.4 Chromatographic column: 400 mm long by 19 mm ID Chromaflex, equipped with coarse-fritted bottom plate and PTFE stopcock. (Kontes K-420540-0224 or equivalent).

Chromatographic column: 300 mm long by 10 mm ID, equipped with coarse-fritted bottom plate and PTFE stopcock (Kontes K-430540-0213 or equivalent).

5.5 Drying column: Approximately 400 mm long by 20 mm ID borosilicate glass, equipped with coarse-fritted bottom plate.

5.6 Miscellaneous.

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

5.6.2 Separatory funnel: 2-L, equipped with PTFE stopcock.

5.6.3 Water bath: Heated with concentric ring cover, capable of temperature control ($\pm 2^{\circ}\text{C}$). The bath should be used in a hood.

5.6.4 Standard solution storage containers: 15-mL bottles with PTFE-lined screw-caps.

5.6.5 Boiling chips: Approximately 10/40 mesh. Heat to 400°C for 30 minutes, or perform a Soxhlet extraction overnight with methylene chloride.

6. REAGENTS AND CONSUMABLE MATERIALS

6.1 Reagents

- 6.1.1** Acetone, hexane, ethanol and methylene chloride: Demonstrated to be free of analytes.
- 6.1.2** Ethyl ether: Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Cat. No. P1126-8, and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.
- 6.1.3** Florisil: PR grade (60/100 mesh). Purchase activated at 675°C and store in dark in glass containers with glass stoppers or foil-lined screw-caps. Before use, activate each batch overnight at 130°C in foil-covered glass container.
- 6.1.4** Silica gel: Activate approximately 100 g of silica gel at 200°C for 16 hours in a tared 500-mL Erlenmeyer flask with ground-glass stopper. Allow to cool to room temperature, and determine the weight of activated silica gel. Deactivate by adding 3% by weight of distilled water. Restopper the flask, and shake on a wrist-action shaker for at least 1 hour. Allow to equilibrate for 3 or more hours at room temperature.
- 6.1.5** Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.1.6** Sodium hydroxide (NaOH) solution (10N): Dissolve 40 g NaOH in reagent water and dilute to 100 mL.
- 6.1.7** Sodium sulfate: Granular, anhydrous. Condition by heating at 400°C for 4 hours in a shallow tray.
- 6.1.8** Sulfuric acid (H₂SO₄) solution (1+1): Add a measured volume of concentrated H₂SO₄ to an equal volume of reagent water.

6.2 Standard stock solutions (1.00 µg/µL): These solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedures.

- 6.2.1** Prepare standard stock solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in hexane or other suitable solvent and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the standard stock.
- 6.2.2** Store standard stock solutions at 4°C in 15-mL bottles equipped with PTFE-lined screw-caps. Standard stock solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
- 6.2.3** Standard stock solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

- 7.1** Collect all samples in duplicate. Grab samples must be collected in glass containers. Conventional sampling practices⁵ should be followed, except that the bottle must not be prewashed with sample before collection.
- 7.2** The samples must be iced or refrigerated at 4°C from the time of collection until extraction. Chemical preservatives should not be used in the field unless more than 24 hours will elapse before extraction. If the samples will not be extracted within 48 hours of collection, the sample should be adjusted to a pH range of 6.0 to 8.0 with sodium hydroxide or sulfuric acid.
- 7.3** All samples must be extracted within 7 days of collection, and analyzed within 40 days of extraction.⁶

8. CALIBRATION AND STANDARDIZATION

8.1 Calibration.

- 8.1.1** A set of at least three calibration solutions containing the method analytes is needed. One calibration solution should contain each analyte at a concentration approaching but greater than the EDL (Table 1) for that compound; the other two solutions should contain analytes at concentrations that bracket the range expected in samples. For example, if the detection limit for a particular analyte is 0.2 µg/L, and a sample expected to contain approximately 5 µg/L is analyzed, standard solutions should be prepared at concentrations representing 0.3 µg/L, 5 µg/L, and 10 µg/L of the analytes.
- 8.1.2** To prepare a calibration solution, add an appropriate volume of a standard stock solution to a volumetric flask and dilute to volume with hexane.
- 8.1.3** Starting with the standard of lowest concentration, analyze each calibration standard according to Section 10.3.2 and tabulate peak height or area response versus the mass of analyte injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to concentration (calibration factor) is a constant over the working range (< 10% relative standard deviation), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.
- 8.1.4** The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any analyte varies from the predicted response by more than ± 10%, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a new calibration curve.

8.2 Florisil standardization.

- 8.2.1** Florisil from different batches or sources may vary in absorptive capacity. To standardize the amount of Florisil which may be used in the cleanup procedure (Section 10.2.2), use of the lauric acid value⁷ is suggested. The referenced procedure determines the adsorption from hexane solution of lauric acid in milligrams per gram of Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.

9. QUALITY CONTROL

9.1 Monitoring for interferences.

9.1.1 Analyze a laboratory reagent blank each time a set of samples is extracted. A laboratory reagent blank is a 1-L aliquot of reagent water. If the reagent blank contains a reportable level of any analyte, immediately check the entire analytical system to locate and correct for possible interferences and repeat the test.

9.2 Assessing accuracy.

9.2.1 After every ten samples, and preferably in the middle of each day, analyze a laboratory control standard. Calibration standards may not be used for accuracy assessments and the laboratory control standard may not be used for calibration of the analytical system.

9.2.1.1 Laboratory control standard concentrate: From stock standards prepared as described in Section 6.3, prepare a laboratory control standard concentrate that contains each analyte of interest at a concentration of 2 µg/mL in acetone or other suitable solvent.⁸

9.2.1.2 Laboratory control standard: Using a pipette, add 1.00 mL of the laboratory control standard concentrate to a 1-L aliquot of reagent water.

9.2.1.3 Analyze the laboratory control standard as described in Section 10. For each analyte in the laboratory control standard, calculate the percent recovery (P_i) with the equation:

Equation 1

$$P_i = \frac{100S_i}{T_i}$$

where

S_i = The analytical results from the laboratory control standard, in µg/L

T_i = The known concentration of the spike, in µg/L

9.2.2 At least annually, the laboratory should participate in formal performance evaluation studies, where solutions of unknown concentrations are analyzed and the performance of all participants is compared.

9.3 Assessing precision.

9.3.1 Precision assessments for this method are based upon the analysis of field duplicates (Section 7.1). Analyze both sample bottles for at least 10% of all samples. To the extent practical, the samples for duplication should contain reportable levels of most of the analytes.

9.3.2 For each analyte in each duplicate pair, calculate the relative range (RR_i) with the equation:

Equation 2

$$RR_i = \frac{100R_i}{X_i}$$

where

R_i = The absolute difference between the duplicate measurements X_1 and X_2 , in $\mu\text{g/L}$

X_i = The average concentration found $\left[\frac{X_1 + X_2}{2} \right]$, in $\mu\text{g/L}$

9.3.3 Individual relative range measurements are pooled to determine average relative range or to develop an expression of relative range as a function of concentration.

10. PROCEDURE

10.1 Sample extraction.

10.1.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel. Check the pH of the sample with wide-range pH paper and adjust to within the range of 5 to 9 with sodium hydroxide or sulfuric acid.

10.1.2 Add 60 mL of methylene chloride to the sample bottle and shake for 30 seconds to rinse the walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release vapor pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends on the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation. Collect the extract in a 250-mL Erlenmeyer flask.

10.1.3 Add an additional 60-mL volume of methylene chloride to the sample bottle and complete the extraction procedure a second time, combining the extracts in the Erlenmeyer flask.

10.1.4 Perform a third extraction in the same manner. Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in a 500-mL K-D flask equipped with a 10 mL concentrator tube. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.1.5 Add one or two clean boiling chips to the flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 to 65°C) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed in steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

When the apparent volume of liquid reaches about 3 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

- 10.1.6** Increase the temperature of the hot water bath to about 80 to 85°C. Momentarily remove the Snyder column, add 50 mL of hexane and a new boiling chip, and reattach the Snyder column. Pour about 1 mL of hexane into the top of the Snyder column, and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 minutes. When the apparent volume of liquid reaches about 3 mL, remove the K-D apparatus, and allow it to drain at least 10 minutes while cooling. Remove the Snyder column, rinse the flask and the lower joint into the concentrator tube with 1 to 2 mL of hexane, and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube, and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than 2 days, they should be transferred to PTFE-sealed screw-cap bottles. If the sample extract requires no cleanup, proceed with gas chromatographic analysis.
- 10.1.7** If the sample requires cleanup, the extract obtained must be divided into two fractions. One of the fractions is eluted through Florisil for the analysis of dicloran and DCPA. The other fraction is eluted through silica gel for the analysis of chlorothalonil, methoxychlor, and the permethrins.
- 10.1.8** Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1,000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

10.2 Cleanup and separation.

- 10.2.1** Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various clean waters and municipal effluents. The single-operator precision and accuracy data in Table 2 were gathered using the recommended cleanup procedures. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than that recorded in Table 2.

10.2.2 Florisil column cleanup.

- 10.2.2.1** Add a weighed amount of Florisil, about 21 g, to a chromatographic column. The exact weight should be determined by calibration.⁷ Tap the column to settle the Florisil. Add a 1 to 2 cm layer of sodium sulfate above the Florisil. Rinse the Florisil and sodium sulfate by adding 60 mL of hexane to the column. Just prior to exposure of the sodium sulfate to air, stop the draining of the hexane by closing the stopcock on the column. Discard the eluate.
- 10.2.2.2** Quantitatively, add the fraction of extract chosen for the analysis of dichloran and DCPA to the column. Drain the column into the flask, stopping just prior to exposure of the sodium sulfate layer.
- 10.2.2.3** Elute the column with 200 mL of 6% ethyl ether in hexane (Fraction 1) using a drip rate of about 5 mL/min. Remove and discard. Perform a second elution using 200 mL of 15% ethyl ether in hexane (Fraction 2), collecting the eluant in a 500-mL K-D flask equipped with a 10-mL concentrator tube.

10.2.2.4 Concentrate the eluate by standard K-D techniques (Section 10.1.5), substituting hexane for methylene chloride, and using the water bath at about 85°C. Adjust the final volumes to 10 mL with hexane. Analyze by gas chromatography.

10.2.3 Silica gel column cleanup.

10.2.3.1 Prepare silica gel columns using a 300 mm by 10 mm ID glass column. Rinse column with hexane. Add approximately 50 mL of hexane to the empty column. Add 3.5 g of 3% deactivated silica gel (Section 6.1.4). Pack by rotating slowly to release air bubbles. Top with 1.5 cm of Na₂SO₄. Drain hexane to the top of the Na₂SO₄.

10.2.3.2 Add the fraction of extract chosen for the analysis of chlorothalonil, methoxychlor, and the permethrins to the column. Open the stopcock and allow it to drain to the surface of the sodium sulfate. Elute with the following solutions:

Fraction 1: 25 mL of hexane

Fraction 2: 25 mL of 6% MeCl₂ in hexane (V/V)

Fraction 3: 25 mL of 50% MeCl₂ in hexane

10.2.3.3 Collect the Fraction³ in a 500-mL K-D flask equipped with a 10-mL concentrator tube, and add 50 mL of hexane. Concentrate on an 85°C water bath to 10.0 mL as described in Section 10.1.5.

10.2.4 The elution profiles obtained in these studies are listed in Tables 3 and 4 for the convenience of the analyst. The analyst must determine the elution profiles and demonstrate that the recovery of each compound of interest is no less than that reported in Table 2 before the analysis of any samples utilizing these cleanup procedures.

10.2.5 Proceed with gas chromatography.

10.3 Gas chromatographic analysis.

10.3.1 Recommended columns and detector for the gas chromatographic system are described in Section 5.3.1. Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and detection limits that can be achieved by this method. Examples of the separations achieved by Column 1 are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if data quality comparable to Table 2 are achieved. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and data quality comparable to Table 2 are achieved.

10.3.2 Inject 2 to 5 µL of the sample extract using the solvent-flush technique.⁹ Record the volume injected to the nearest 0.05 µL, the total extract volume, the fraction of total extract utilized in each cleanup scheme, and the resulting peak size in area or peak height units.

10.3.3 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of the day. Three times the standard deviation of a retention time for a compound can be used

to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

10.3.4 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

10.3.5 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

11. CALCULATIONS

11.1 Determine the concentration (C) of individual compounds in the sample in micrograms per liter with the equation:

Equation 3

$$C = \frac{(A)(V_i)(V_e)}{(V_t)(V_w)(V_f)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_w = Volume of water extracted, in mL

V_e = Volume of final extract after cleanup, in μL

V_f = Volume of extract utilized for cleanup scheme, in μL

11.2 Report the results for the unknown samples in μg/L. Round off the results to the nearest 0.1 μg/L or two significant figures.

12. METHOD PERFORMANCE

12.1 Estimated detection limits (EDL) and associated chromatographic conditions are listed in Table 1.¹⁰ The detection limits were calculated from the minimum detectable response of the EC detector equal to 5 times the background noise, assuming a 10.0-mL final extract volume of a 1-L sample and a GC injection of 5 μL.

12.2 Single-laboratory accuracy and precision studies were conducted by Environmental Science and Engineering, Inc.,⁶ using spiked industrial wastewater samples. The results of these studies are presented in Table 2.

13. GC/MS CONFIRMATION

13.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak, but not to exceed 7 scans per peak utilizing a 70 V (nominal) electron energy in the electron

impact ionization mode. A GC to MS interface constructed of all glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.

- 13.2** Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved. The calculation of tailing factors is illustrated in Method 625.¹¹
- 13.3** At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all DFTPP performance criteria are achieved.¹²
- 13.4** To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
 - 13.4.1** The molecular ion and other ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to $\pm 10\%$. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20 to 40%.
 - 13.4.2** The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.
 - 13.4.3** Compounds that have similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.
- 13.5** Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.
- 13.6** Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternative packed or capillary GC columns or additional cleanup.

References

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Table 1. Gas Chromatography of Organochlorine Pesticides

<i>Parameter</i>	<i>Retention Time (Min.)</i>		<i>Estimated Detection Limit (µg/L)</i>
	<i>Column 1*</i>	<i>Column 2**</i>	
Chlorothalonil	3.40	4.69	0.001
DCPA	4.19	5.44	0.003
Dicloran	2.23	2.62	0.002
Methoxychlor	22.35	10.85	0.04
cis-Permethrin***	18.52	16.04	0.2
trans-Permethrin***	20.02	17.53	0.2

* Column 1: 180 cm long by 2 mm ID, glass, packed with 1.5% OV-17/1.95% OV 210 on Chromosorb W-HP (100/120 mesh) or equivalent; 5% methane/95% Argon carrier gas at 30 mL/min flow rate. Column temperature is 200°C. Detector: electron capture.

** Column 2: 180 cm long by 2 mm ID, glass, packed with 4% SE-30/6% SP-2401 on Supelcoport (100/120 mesh) or equivalent; 5% methane/95% Argon carrier gas at 60 mL/min flow rate. Column temperature is 200°C. Detector: electron capture.

*** Column temperature is 220°C.

Table 2. Single-Laboratory Accuracy and Precision

<i>Parameter</i>	<i>Metric Type*</i>	<i>Spike Range (µg/L)</i>	<i>Number of Replicates</i>	<i>Average Percent Recovery</i>	<i>Standard Deviation (%)</i>
Chlorothalonil	1	37.8	7	84.1	16.4
	2	2,300	7	94.9	22.5
DCPA	1	16	7	77.6	25.7
	2	10,540	7	89.5	11.0
Dicloran	1	37.5	7	98.6	8.4
	2	21,200	7	90.8	20.3
Methoxychlor	1	24.5	7	102.4	12.4
	2	2,600	7	102.2	10.2
cis-Permethrin	1	6.3	7	99.5	18.8
	2	317	7	77.5	10.6
trans-Permethrin	1	5.7	7	78.8	16.1
	2	297	7	88.9	19.6

* 1 = Low-level industrial effluent
2 = High-level industrial effluent

Table 3. Elution Profiles for Florisil Cleanup

<i>Parameter</i>	<i>Percent Recover By Fraction*</i>		
	<i>1</i>	<i>2</i>	<i>3</i>
DCPA	0	99.3	0
Dicloran	0	96.3	0

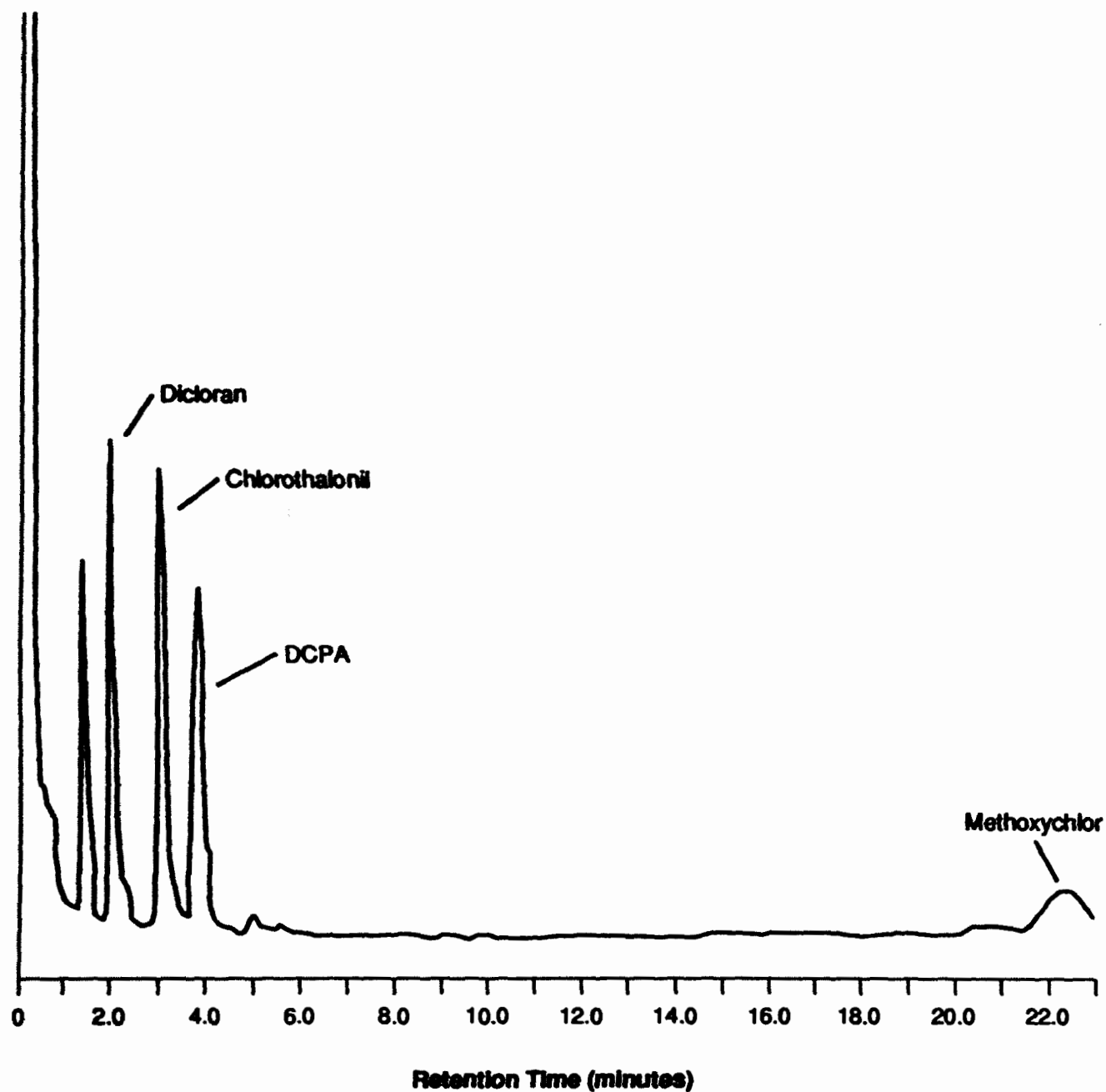
* Eluting solvent composition for each fraction given in Section 10.2.2.3.

Table 4. Elution Profiles for Silica Gel* Cleanup

<i>Parameter</i>	<i>Percent Recovery By Fraction**</i>		
	<i>1</i>	<i>2</i>	<i>3</i>
Chlorothalonil	0	0	93.8
Methoxychlor	0	0	93.8
cis-Permethrin	0	0	107.2
trans-Permethrin	0	0	92.5

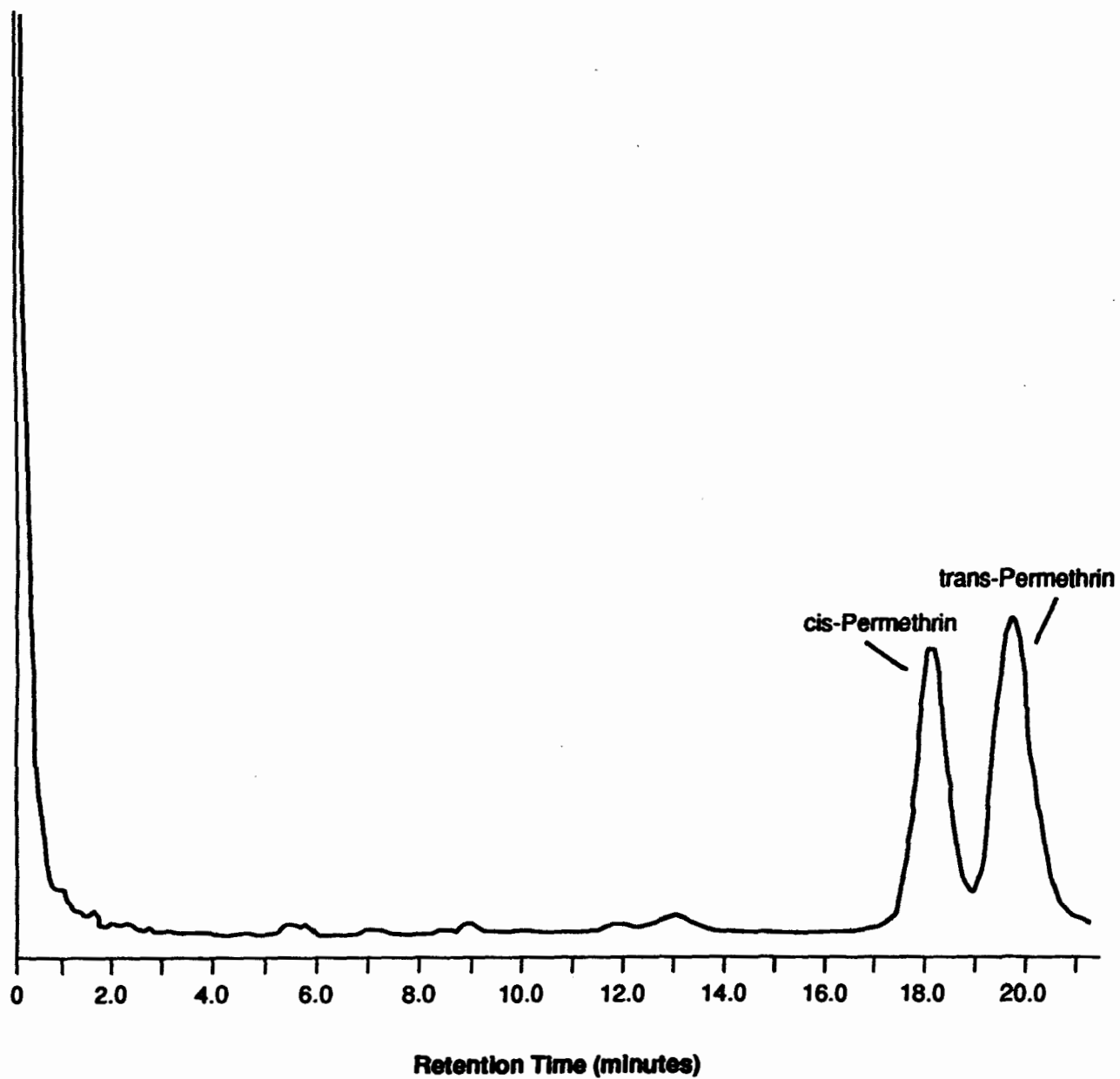
* 3% deactivated

** Eluting solvent composition for each fraction given in Sections 10.2.3.2 and 10.2.3.3.



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Figure 1. Gas Chromatogram of Chlorothalonil, DCPA, Dicloran, and Methoxychlor in a Wastewater Extract (Column 1).



AS2-002-13

Figure 2. Gas Chromatogram of Permethrin Sample (Column 1).

Method 614
The Determination of
Organophosphorus Pesticides in
Municipal and Industrial
Wastewater

Method 614

The Determination of Organophosphorus Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of certain organophosphorus pesticides. The following parameters can be determined by this method:

<i>Parameter</i>	<i>STORET No.</i>	<i>CAS No.</i>
Azinphos methyl	39580	86-50-0
Demeton	39560	8065-48-3
Diazinon	39570	333-41-5
Disulfoton	39010	298-04-4
Ethion	—	563-12-2
Malathion	39530	121-75-5
Parathion ethyl	39540	56-38-2
Parathion methyl	39600	298-00-0

- 1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3 The method detection limit (MDL, defined in Section 15) for several parameters are listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4 The sample extraction and concentration steps in this method are essentially the same as in Method 617. Thus, a single sample may be extracted to measure the parameters included in the scope of both of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures. Under gas chromatography, the analyst is allowed the latitude to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters (see Section 12).
- 1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Section 14 provides gas

chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. SUMMARY OF METHOD

- 2.1 A measured volume of sample, approximately 1 L, is extracted with 15% methylene chloride in hexane using a separatory funnel. The extract is dried and concentrated to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by flame photometric or thermionic bead gas chromatography.
- 2.2 Method 614 represents an editorial revision of a previously promulgated U.S. EPA method for organophosphorus pesticides.¹ While complete method validation data is not presented herein, the method has been in widespread use since its promulgation, and represents the state of the art for the analysis of such materials.
- 2.3 This method provides selected cleanup procedures to aid in the elimination of interferences which may be encountered.

3. INTERFERENCES

- 3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.
 - 3.1.1 Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.
 - 3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this

viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified³⁻⁵ for the information of the analyst.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel: 125-mL, 1000-mL, and 2000-mL, with TFE-fluorocarbon stopcock, ground-glass or TFE stopper.

5.2.2 Drying column: Chromatographic column 400 mm long by 19 mm ID with coarse-fritted disc.

5.2.3 Chromatographic column: 400 mm long by 19 mm ID with coarse-fritted disc at bottom and TFE-fluorocarbon stopcock (Kontes K-420540-0224 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground-glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Snyder column, Kuderna-Danish: Two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.8 Pipette, disposable: 140 mm long by 5 mm ID.

5.2.9 Vials: Amber glass, 10- to 15-mL capacity with TFE-fluorocarbon-lined screw-cap.

5.3 Boiling chips: Approximately 10/40 mesh. Heat at 400°C for 30 minutes or perform a Soxhlet extraction with methylene chloride.

- 5.4 Water bath: Heated, with concentric ring cover, capable of temperature control ($\pm 2^{\circ}\text{C}$). The bath should be used in a hood.
- 5.5 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6 Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.
 - 5.6.1 Column 1: 180 cm long by 4 mm ID glass, packed with 3% OV-1 on Gas Chrom Q (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 12.1.
 - 5.6.2 Column 2: 180 cm long by 4 mm ID glass, packed with 1.5% OV-17/1.95% QF-1 on Gas Chrom Q (100/120 mesh) or equivalent.
 - 5.6.3 Detector: Phosphorus-specific; flame photometric (FPD) (526 nm filter) or thermionic bead detector in the nitrogen mode. These detectors have proven effective in the analysis of wastewaters for the parameters listed in the scope. The FPD was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. REAGENTS

- 6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2 Acetone, hexane, isooctane, methylene chloride: Pesticide-quality or equivalent.
- 6.3 Ethyl ether: Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Cat. No. P1126-8, and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.
- 6.4 Acetonitrile, hexane-saturated: Mix pesticide-quality acetonitrile with an excess of hexane until equilibrium is established.
- 6.5 Sodium sulfate: ACS, granular, anhydrous. Condition by heating in a shallow tray at 400°C for a minimum of 4 hours to remove phthalates and other interfering organic substances. Alternatively, heat 16 hours at 450 to 500°C in a shallow tray or perform a Soxhlet extraction with methylene chloride for 48 hours.
- 6.6 Sodium chloride solution, saturated: Prepare saturated solution of NaCl in reagent water and extract with hexane to remove impurities.
- 6.7 Alumina: Woelm, neutral; deactivate by pipetting 1 mL of distilled water into a 125-mL ground-glass stoppered Erlenmeyer flask. Rotate flask to distribute water over surface of glass. Immediately add 19.0 g fresh alumina through small powder funnel. Shake flask containing mixture for 2 hours on a mechanical shaker.
- 6.8 Florisil: PR grade (60/100 mesh). Purchase activated at 675°C and store in dark in glass container with ground-glass stopper or foil-lined screw-cap. Before use, activate each batch at least 16 hours at 130°C in a foil-covered glass container.

6.9 Stock standard solutions (1.00 $\mu\text{g}/\mu\text{L}$): Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.

6.9.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in pesticide-quality isooctane or acetone and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.9.2 Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw-cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.9.3 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure.

7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with isooctane or other suitable solvent. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Using injections of 1 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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.

- 7.3.1** Prepare calibration standards at a minimum of three concentration levels for each parameter 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 isooctane or other suitable solvent. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.
- 7.3.2** Using injections of 1 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_i)}{(A_i)(C_s)}$$

where

A_s = Response for the parameter to be measured

A_i = Response for the internal standard

C_i = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_i , against RF.

- 7.3.3** The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.
- 7.4** The cleanup procedure in Section 11 utilizes Florisil chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil which is used, the use of the lauric acid value is suggested. This procedure⁶ determines the adsorption from hexane solution of lauric acid, in milligrams, per gram of Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.
- 7.5** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.
- 8.1.1** Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2** In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
- 8.1.3** The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2** To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
- 8.2.1** Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone, 1000 times more concentrated than the selected concentrations.
- 8.2.2** Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
- 8.2.3** Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and calculations are performed.
- 8.2.4** Table 2 provides single-operator recovery and precision for diazinon, parathion methyl, and parathion ethyl. Similar results should be expected from reagent water for all organophosphorus compounds listed in this method. Compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.
- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
- 8.3.1** Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and S are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁷ that are useful in observing trends in performance.

- 8.3.2** The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁷
- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.
- 8.5** Before processing any samples, the analyst must demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.
- 8.6** 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 monitor the precision of the sampling technique. 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 perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

- 9.1** Grab samples must be collected in glass containers. Conventional sampling practices⁸ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2** The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3** All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

10. SAMPLE EXTRACTION

- 10.1** Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel.
- 10.2** Add 60 mL 15% methylene chloride in hexane (V:V) to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure.

Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Drain the aqueous phase into a 1000-mL Erlenmeyer flask and collect the extract in a 250-mL Erlenmeyer flask. Return the aqueous phase to the separatory funnel.

- 10.3 Add a second 60-mL volume of 15% methylene chloride in hexane to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the 250-mL Erlenmeyer flask. Perform a third extraction in the same manner.
- 10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of hexane to complete the quantitative transfer.
- 10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 80 to 85°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.7 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to PTFE-sealed screw-cap bottles. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis. If the sample requires cleanup, proceed to Section 11.
- 10.8 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various industrial and municipal effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

- 11.2 Acetonitrile partition:** The following acetonitrile partitioning procedure may be used to isolate fats and oils from the sample extracts. The applicability of this procedure to organophosphorus pesticides is indicated in Table 3.
- 11.2.1** Quantitatively transfer the previously concentrated extract to a 125-mL separatory funnel with enough hexane to bring the final volume to 15 mL. Extract the sample four times by shaking vigorously for 1 minute with 30-mL portions of hexanesaturated acetonitrile.
- 11.2.2** Combine and transfer the acetonitrile phases to a 1-L separatory funnel and add 650 mL of reagent water and 40 mL of saturated sodium chloride solution. Mix thoroughly for 30 to 45 seconds. Extract with two 100-mL portions of hexane by vigorously shaking for 15 seconds.
- 11.2.3** Combine the hexane extracts in a 1-L separatory funnel and wash with two 100-mL portions of reagent water. Discard the water layer and pour the hexane layer through a drying column containing 7 to 10 cm of anhydrous sodium sulfate into a 500-mL K-D flask equipped with a 10-mL concentrator tube. Rinse the separatory funnel and column with three 10-mL portions of hexane.
- 11.2.4** Concentrate the extracts to 6 to 10 mL in the K-D as directed in Section 10.6. Adjust the extract volume to 10 mL with hexane.
- 11.2.5** Analyze by gas chromatography unless a need for further cleanup is indicated.
- 11.3 Florisil column cleanup:** The following Florisil column cleanup procedure has been demonstrated to be applicable to the seven organophosphorus pesticides listed in Table 3. It should also be applicable to the cleanup of extracts for ethion.
- 11.3.1** Add a weight of Florisil (nominally 20 g) predetermined by calibration (Section 7.4 and 7.5) to a chromatographic column. Settle the Florisil by tapping the column. Add anhydrous sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatography column. Discard the eluate.
- 11.3.2** Adjust the sample extract volume to 10 mL with hexane and transfer it from the K-D concentrator tube to the Florisil column. Rinse the tube twice with 1 to 2 mL hexane, adding each rinse to the column.
- 11.3.3** Place a 500-mL K-D flask and clean concentrator tube under the chromatography column. Drain the column into the flask until the sodium sulfate layer is nearly exposed. Elute the column with 200 mL of 6% ethyl ether in hexane (V/V) (Fraction 1) using a drip rate of about 5 mL/min. Remove the K-D flask and set aside for later concentration. Elute the column again, using 200 mL of 15% ethyl ether in hexane (V/V) (Fraction 2) into a second K-D flask. Perform a third elution using 200 mL of 50% ethyl ether in hexane (V/V) (Fraction 3) and a final elution with 200 mL of 100% ethyl ether (Fraction 4) into separate K-D flasks. The elution patterns for seven of the pesticides are shown in Table 3.
- 11.3.4** Concentrate the eluates by standard K-D techniques (Section 10.6), using the water bath at about 85°C (75°C for Fraction 4). Adjust final volume to 10 mL with hexane. Analyze by gas chromatography.

- 11.4 Removal of sulfur:**⁹ Elemental sulfur will elute in Fraction 1 of the Florisil cleanup procedure. If a large amount of sulfur is present in the extract, it may elute in all fractions. If so, each fraction must be further treated to remove the sulfur.
- 11.4.1** Add one or two boiling chips to the 10-mL hexane solution contained in a concentrator tube. Attach a micro-Snyder column and concentrate the extract to about 0.2 mL in a hot water bath at 85°C. Remove the micro K-D from the bath, cool, and adjust the volume to 0.5 mL with hexane.
- 11.4.2** Plug a disposable pipette with a small quantity of glass wool. Add enough alumina to produce a 3-cm column after settling. Top the alumina with a 0.5-cm layer of anhydrous sodium sulfate.
- 11.4.3** Quantitatively transfer the concentrated extract to the alumina microcolumn using a 100- μ L syringe. Rinse the ampul with 200 μ L of hexane and add to the microcolumn.
- 11.4.4** Elute the microcolumn with 3 mL of hexane and discard the eluate.
- 11.4.5** Elute the column with 5 mL of 10% hexane in methylene chloride, and collect the eluate in a 10-mL concentrator tube. Adjust final volume to 10 mL with hexane. Analyze by gas chromatography.

12. GAS CHROMATOGRAPHY

- 12.1** Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention-times and method detection limits that can be achieved by this method. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.
- 12.2** Calibrate the system daily as described in Section 7.
- 12.3** If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.
- 12.4** Inject 1 to 5 μ L of the sample extract using the solvent-flush technique.¹⁰ Record the volume injected to the nearest 0.05 μ L, and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.
- 12.5** The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6** If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7** If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_s)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_s = Volume of water extracted, in mL

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where

A_s = Response for parameter to be measured

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_o = Volume of water extracted, in L

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. GC/MS CONFIRMATION

14.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative compound identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 seconds per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all glass or glass-lined materials is recommended. A computer system should be interfaced to the mass

spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.

- 14.2** Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.¹¹
- 14.3** At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.¹²
- 14.4** To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
 - 14.4.1** All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to plus or minus 10%. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20% to 40%.
 - 14.4.2** The retention-time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.
 - 14.4.3** Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention-time data.
- 14.5** Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.
- 14.6** Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. METHOD PERFORMANCE

- 15.1** 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.¹³ The MDL concentrations listed in Table 1 were obtained using reagent water.¹⁴
- 15.2** In a single laboratory, Susquehanna University, using spiked tap water samples, the average recoveries presented in Table 3 were obtained. The standard deviation of the percent recovery is also included in Table 3.¹⁴

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Table 1. Chromatographic Conditions and Method Detection Limits

<i>Parameter</i>	<i>Retention Time (minutes)</i>		<i>Method Detection Limit (µg/L)</i>
	<i>Column 1</i>	<i>Column 2</i>	
Diazinon	1.8	1.8	0.012
Disulfoton	1.9	2.1	ND
Demeton	2.3	2.1	ND
Parathion	2.5	3.7	0.012
methyl			
Malathion	2.9	3.9	ND
Parathion ethyl	3.1	4.5	0.012
Ethion	6.8	9.1	ND
Azinphos	14.5	29.9	ND
methyl			

ND = Not determined

Column 1 conditions: Gas-Chrom Q (100/120 mesh) coated with 3% OV-1 packed in a 1.8 m long by 4 mm ID glass column with nitrogen carrier gas at a flow rate of 60 mL/min. Column temperature, isothermal at 200°C. A flame photometric detector was used with this column to determine the MDL.

Column 2 conditions: Gas Chrom Q (100/120 mesh) coated with 1.5% OV-17+1.95% QF-1 packed in a 1.8 m long by 4 mm ID glass column with nitrogen carrier gas at 70 mL/min. flow rate. Column temperature, isothermal at 212°C.

Table 2. Single-Operator Accuracy and Precision

<i>Parameter</i>	<i>Average Percent Recovery</i>	<i>Standard Deviation (%)</i>	<i>Spike Range (µg/L)</i>	<i>Number of Analyses</i>	<i>Matrix Types</i>
Diazinon	94	5.2	0.04-40	27	4
Parathion methyl	95	3.2	0.06-60	27	4
Parathion ethyl	102	4.1	0.07-70	27	4

Table 3. Florisil Fractionation Patterns and Acetonitrile Partition Applicability

<i>Parameter</i>	<i>Percent Recovery by Fraction</i>				<i>Acetonitrile Partition Applicability</i>
	<i>No. 1</i>	<i>No. 2</i>	<i>No. 3</i>	<i>No. 4</i>	
Demeton	100				ND
Disulfoton	100				ND
Diazinon		100			Yes
Malathion		5	95		Yes
Parathion ethyl		100			Yes
Parathion methyl		100			Yes
Azinphos methyl			20	80	ND
Ethion	ND	ND	ND	ND	Yes

ND = Not determined

Florisil eluate composition by fraction

- Fraction 1 - 200 mL of 6% ethyl ether in hexane
- Fraction 2 - 200 mL of 15% ethyl ether in hexane
- Fraction 3 - 200 mL of 50% ethyl ether in hexane
- Fraction 4 - 200 mL of ethyl ether

Method 614.1
The Determination of
Organophosphorus Pesticides in
Municipal and Industrial
Wastewater

Method 614.1

The Determination of Organophosphorus Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of certain organophosphorus pesticides in municipal and industrial wastewater. The following parameters may be determined by this method.

<i>Parameter</i>	<i>STORET No.</i>	<i>CAS No.</i>
Dioxathion		78-34-2
EPN		2104-64-5
Ethion	39398	563-12-2
Terbufos		13071-79-9

- 1.2 The estimated detection limit (EDL) for each parameter is listed in Table 1. The EDL was calculated from the minimum detectable response of the nitrogen/phosphorus detector equal to 5 times the gas chromatographic (GC) background noise assuming a 1.0-mL final extract volume of a 1-L reagent water sample and an injection of 5 μ L. The EDL for a specific wastewater may be different depending on the nature of interferences in the sample matrix.
- 1.3 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges. When this method is used to analyze unfamiliar samples for any or all of the compounds listed above, compound identifications should be supported by at least one additional qualitative technique. Section 13 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative confirmation of compound identifications.
- 1.4 This method is restricted to use by or under the supervision of analysts experienced in the operation of gas chromatographs and in the interpretation of chromatograms.

2. SUMMARY OF METHOD

- 2.1 Organophosphorus pesticides are removed from the sample matrix by extraction with 15% methylene chloride in hexane. The extract is dried, exchanged into hexane, and analyzed by gas chromatography. Column chromatography is used as necessary to eliminate interferences which may be encountered. Measurement of the pesticides is accomplished with a nitrogen/phosphorus-specific detector.
- 2.2 Confirmatory analysis by GC/MS is recommended when a new or undefined sample type is being analyzed if the concentration is adequate for such determination.

3. INTERFERENCES

- 3.1 Solvent, reagents, glassware, and other sample-processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials

must be demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 9.1.

- 3.1.1 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.1.2 Glassware must be scrupulously cleaned.¹ Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap water and reagent water. It should then be drained dry and heated in a muffle furnace at 400°C for 15 to 30 minutes. Solvent rinses with acetone and pesticide-quality hexane may be substituted for the heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store the glassware inverted or capped with aluminum foil.
- 3.2 Interferences coextracted from the samples will vary considerably from source to source, depending on the diversity of the industrial complex or municipality being sampled. While general cleanup procedures are provided as part of this method, unique samples may require additional cleanup approaches to achieve the detection limits listed in Table 1.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified^{2,4} for the information of the analyst.

5. APPARATUS AND EQUIPMENT

- 5.1 Sample containers: Narrow-mouth glass bottles, 1-L or 1-quart volume, equipped with polytetrafluoroethylene (PTFE)-lined screw-caps. Wide-mouth glass bottles, 1-quart volume, equipped with PTFE-lined screw-caps may also be used. Prior to use, wash bottles and cap liners with detergent and rinse with tap and distilled water. Allow the bottles and cap liners to air dry, then muffle at 400°C for 1 hour. After cooling, rinse the cap liners with hexane, seal the bottles, and store in a dust-free environment.
 - 5.1.1 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Kuderna-danish (K-D) glassware.

- 5.2.1** Synder column: Three-ball macro (Kontes K-503000-0121 or equivalent) and two-ball micro (Kontes K-569001-0219 or equivalent).
- 5.2.2** Concentrator tube: 10-mL, graduated (Kontes K-570050-1025 or equivalent) with ground-glass stopper.
- 5.2.3** Evaporative flask: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.3 Gas chromatography system.

- 5.3.1** The gas chromatograph must be equipped with a glass-lined injection port compatible with the detector to be used. A data system is recommended for measuring peak areas.

- 5.3.1.1** Column 1: 180 cm long by 2 mm ID, glass, packed with 3% OV-225 on Supelcoport (100/120 mesh) or equivalent.

- 5.3.1.2** Column 2: 120 cm long by 2 mm ID, Pyrex^R glass, packed with 1.5% OV-17/1.95 % QF-1 on Gas Chrom Q, 80/100 mesh or equivalent.

- 5.3.1.3** Column 1 was used to develop the accuracy and precision statements in Section 12. Guidelines for the use of alternative column packings are provided in Section 10.3.1.

- 5.3.1.4** Detector: nitrogen/phosphorus. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 12. Guidelines for the use of alternative detectors are provided in Section 10.3.1.

- 5.4** Chromatographic column: 300 mm long by 10 mm ID Chromaflex, equipped with coarse-fitted bottom plate and PTFE stopcock. (Kontes K-420540-0213 or equivalent).

- 5.5** Drying column: Approximately 400 mm long by 20 mm ID borosilicate glass, equipped with coarse-fitted bottom plate.

- 5.6** Miscellaneous.

- 5.6.1** Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.

- 5.6.2** Separatory funnel: 2-L, equipped with PTFE stopcock.

- 5.6.3** Water bath: Heated with concentric ring cover, capable of temperature control ($\pm 2^{\circ}\text{C}$). The bath should be used in a hood.

- 5.6.4** Standard solution storage containers: 15-mL bottles with PTFE-lined screw-caps.

- 5.6.5** Boiling chips: Approximately 10/40 mesh. Heat to 400°C for 30 minutes or perform a Soxhlet extraction with methylene chloride.

6. REAGENTS AND CONSUMABLE MATERIALS**6.1 Reagents.**

- 6.1.1** Acetone, hexane, and methylene chloride: Demonstrated to be free of analytes.

- 6.1.2** Silica gel: Woelm 70-230 mesh. Activate approximately 100 g of silica gel at 200°C for 6 hours in a tared 500-mL Erlenmeyer flask with ground-glass stopper. Allow to

cool to room temperature, reweigh, and determine the weight of activated silica gel. Deactivate by adding 3% by weight of distilled water. Restopper the flask, and shake on a wrist-action shaker for at least 1 hour. Allow to equilibrate for 3 or more hours at room temperature.

- 6.1.3 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.1.4 Sodium hydroxide (NaOH) solution (10N): Dissolve 40 g NaOH in reagent water and dilute to 100 mL.
- 6.1.5 Sodium sulfate: Granular, anhydrous. Condition by heating at 400°C for 4 hours in a shallow tray.
- 6.1.6 Sulfuric acid (H₂SO₄) solution (1+1): Add measured volume of concentrated H₂SO₄ to equal volume of reagent water.
- 6.2 Standard stock solutions (1.00 µg/µL): These solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedures.
 - 6.2.1 Prepare standard stock solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in hexane and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the standard stock.
 - 6.2.2 Store standard stock solutions at 4°C in 15-mL bottles equipped with PTFE-lined screw-caps. Standard stock solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
 - 6.2.3 Standard stock solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

- 7.1 Collect all samples in duplicate. Grab samples must be collected in glass containers. Conventional sampling practices⁵ should be followed, except that the bottle must not be prewashed with sample before collection.
- 7.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 7.3 Chemical preservatives should not be used in the field unless more than 24 hours will elapse before delivery to the laboratory. If the samples will not be extracted within 48 hours of collection, the sample should be adjusted to a pH range of 6.0 to 8.0 with sodium hydroxide or sulfuric acid.
- 7.4 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.⁶

8. CALIBRATION AND STANDARDIZATION

8.1 Calibration.

- 8.1.1** A set of at least three calibration solutions containing the method analytes is needed. One calibration solution should contain each analyte at a concentration approaching but greater than the estimated detection limit (Table 1) for that compound; the other two solutions should contain analytes at concentrations that bracket the range expected in samples. For example, if the detection limit for a particular analyte is 0.2 µg/L, and a sample expected to contain approximately 5 µg/L is analyzed, solutions of standards should be prepared at concentrations of 0.3 µg/L, 5 µg/L, and 10 µg/L for the particular analyte.
- 8.1.2** To prepare a calibration solution, add an appropriate volume of a standard stock solution to a volumetric flask and dilute to volume with hexane.
- 8.1.3** Starting with the standard of lowest concentration, analyze each calibration standard according to Section 10.3.2 and tabulate peak height or area responses versus the mass of analyte injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to concentration (calibration factor) is a constant over the working range (< 10% relative standard deviation), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.
- 8.1.4** The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any analyte varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a new calibration curve.

9. QUALITY CONTROL

- 9.1** Monitoring for interferences: Analyze a laboratory reagent blank each time a set of samples is extracted. A laboratory reagent blank is a 1-L aliquot of reagent water. If the reagent blank contains a reportable level of any analyte, immediately check the entire analytical system to locate and correct for possible interferences and repeat the test.
- 9.2** Assessing accuracy.
- 9.2.1** After every 10 samples, and preferably in the middle of each day, analyze a laboratory control standard. Calibration standards may not be used for accuracy assessments and the laboratory control standard may not be used for calibration of the analytical system.
- 9.2.1.1** Laboratory control standard concentrate: From stock standards prepared as described in Section 6.2, prepare a laboratory control standard concentrate that contains each analyte of interest at a concentration of 2 µg/ml in acetone or other suitable solvent.⁷
- 9.2.1.2** Laboratory control standard: Using a pipette, add 1.00 mL of the laboratory control standard concentrate to a 1-L aliquot of reagent water.

- 9.2.1.3** Analyze the laboratory control standard as described in Section 10. For each analyte in the laboratory control standard, calculate the percent recovery (P_i) with the equation:

Equation 1

$$P_i = \frac{100S_i}{T_i}$$

where

S_i = The analytical results from the laboratory control standard, in $\mu\text{g/L}$

T_i = The known concentration of the spike, in $\mu\text{g/L}$

- 9.2.2** At least annually, the laboratory should participate in formal performance evaluation studies, where solutions of unknown concentrations are analyzed and the performance of all participants is compared.

9.3 Assessing precision.

- 9.3.1** Precision assessments for this method are based upon the analysis of field duplicates (Section 7.1). Analyze both sample bottles for at least 10% of all samples. To the extent practical, the samples for duplication should contain reportable levels of most of the analytes.

- 9.3.2** For each analyte in each duplicate pair, calculate the relative range⁷ (RR_i) with the equation:

Equation 2

$$RR_i = \frac{100R_i}{X_i}$$

where

R_i = The absolute difference between the duplicate measurements X_1 and X_2 , in $\mu\text{g/L}$

X_i = The average concentration found $\left[\frac{X_1 + X_2}{2} \right]$, in $\mu\text{g/L}$

- 9.3.3** Individual relative range measurements are pooled to determine average relative range or to develop an expression of relative range as a function of concentration.

10. PROCEDURE

10.1 Sample extraction.

- 10.1.1** Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel. Check the pH of the sample with wide-range pH paper and adjust to within the range of 5 to 9 with sodium hydroxide or sulfuric acid.

- 10.1.2** Add 60 mL of 15% methylene chloride/hexane to the sample bottle and shake for 30 seconds to rinse the walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release vapor pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends on the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation. Collect the extract in a 250-mL Erlenmeyer flask.
- 10.1.3** Add an additional 60-mL volume of 15% methylene chloride/hexane to the sample bottle and complete the extraction procedure a second time, combining the extracts in the Erlenmeyer flask.
- 10.1.4** Perform a third extraction in the same manner. Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect it in a 500-mL K-D flask equipped with a 10-mL concentrator tube. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.
- 10.1.5** Add one or two clean boiling chips to the flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (80 to 85°C) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed in steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane. A 5-mL syringe is recommended for this operation. If the extract requires cleanup, proceed to Section 10.2 (cleanup and separation). If cleanup has been performed or if the extract does not require cleanup, proceed with Section 10.1.6.
- 10.1.6** Add a clean boiling chip to the concentrator tube. Attach a two-ball micro-Snyder column. Prewet the micro-Snyder column by adding about 0.5 mL of hexane to the top. Place this micro K-D apparatus on a steaming-water bath (80 to 85°C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation, the balls will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a small volume of hexane. Adjust the final volume to 1.0 mL or to a volume suitable for cleanup or gas chromatography, and stopper the concentrator tube; store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than 2 days, they should be transferred to PTFE-sealed screw-cap bottles. Proceed with gas chromatographic analysis.

10.1.7 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1,000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

10.2 Cleanup and separation.

10.2.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various clean waters and municipal effluents. The silica gel procedure allows for a select fractionation of the compounds and will eliminate non-polar materials. The single-operator precision and accuracy data in Table 2 were gathered using the recommended cleanup procedures. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than that recorded in Table 2.

10.2.2 Prepare silica gel columns using a glass column 200 mm/long by 10 mm ID. Rinse column with hexane. Add approximately 50 mL of hexane to the empty column. Add 3.5 grams of 3% deactivated silica gel. Pack by rotating slowly to release air bubbles. Top with 1.5 cm of Na_2SO_4 . Drain hexane to the top of Na_2SO_4 layer.

10.2.3 Just prior to exposure of the sodium sulfate layer to the air, transfer the sample extract onto the column using an additional 2 mL of hexane to complete the transfer.

10.2.4 Just prior to exposure of the sodium sulfate layer to the air, add 30 mL of 6% methylene chloride/hexane and continue the elution of the column, collecting the eluate in a 500-mL K-D flask equipped with a 10-mL concentration tube. Elution of the column should be at a rate of about 2 mL per minute. Add 50 mL of hexane to the flask and concentrate the collected fraction by the standard technique prescribed in Sections 10.1.5 and 10.1.6.

10.2.5 Continue the elution of the column according to the scheme outlined in Table 3. The elution of the compounds may vary with different sample matrices.

10.2.6 Analyze the fractions by gas chromatography.

10.3 Gas chromatographic analysis.

10.3.1 Recommended columns and detector for the gas chromatography system are described in Section 5.3.1. Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and detection limits that can be achieved by this method. Examples of the separations achieved are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if data quality comparable to Table 2 are achieved. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and data quality comparable to Table 2 are achieved.

10.3.2 Inject 2 to 5 μL of the sample extract using the solvent-flush technique.⁴ Record the volume injected to the nearest 0.05 μL , the total extract volume, and the resulting peak size in area or peak height units.

10.3.3 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of the

day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

10.3.4 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

10.3.5 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

11. CALCULATIONS

11.1 Determine the concentration (C) of individual compounds in the sample in micrograms per liter with the equation:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_e)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_e = Volume of water extracted, in mL

11.2 Report the results for the unknown samples in microgram per Liter. Round off the results to the nearest 0.1 $\mu\text{g/L}$ or two significant figures.

12. METHOD PERFORMANCE

12.1 Estimated detection limits (EDL) and associated chromatographic conditions are listed in Table 1.⁹ The detection limits were calculated from the minimum detectable response of the N/P detector equal to 5 times the GC background noise, assuming a 1.0-mL final extract volume of a 1-L sample and a GC injection of 5 μL .

12.2 Single laboratory accuracy and precision studies were conducted by ESE,⁶ using spiked relevant industrial wastewater samples. The results of these studies are presented in Table 2.

13. GC/MS CONFIRMATION

13.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compounds of interest. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak, but not to exceed 7 scans per peak utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all-glass or glass-lined materials is recommended. A computer system should be interfaced to the mass

spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.

- 13.2** Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved. The calculation of tailing factors is illustrated in Method 625.¹⁰
- 13.3** At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all DFTPP performance criteria are achieved.¹¹
- 13.4** To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
 - 13.4.1** The molecular ion and other ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to $\pm 10\%$. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20 to 40%.
 - 13.4.2** The retention-time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.
 - 13.4.3** Compounds that have similar mass spectra can be explicitly identified by GC/MS only on the basis of retention-time data.
- 13.5** Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.
- 13.6** Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternative packed or capillary GC columns or additional cleanup.

References

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Table 1. Gas Chromatography of Organophosphorus Pesticides

<i>Parameter</i>	<i>Retention Time (minutes)</i>		<i>Detection Limit (µg/L)</i>
	<i>Column 1</i>	<i>Column 2</i>	
Terbufos	1.41	1.9	.004
Dioxathion	2.3	2.3	.01
Ethion	8.3	6.4	0.1
EPN	13.3	8.3	0.2

Column 1: 180 cm long by 2 mm ID, glass, packed with 3% OV-225 on 100/120 Supelcoport; nitrogen carrier gas at a flow rate of 50 mL/min. Column temperature is 200°C for 2 minutes, then programmed at 5°/min to 240°C and held for 5 minutes.

Column 2: 120 cm long by 2 mm ID, Pyrex[®] glass, packed with 1.5% OC-17/1.95% QF-1 on 80/100 mesh Gas Chrom Q or equivalent; nitrogen carrier gas at a flow rate of 30 mL/min. Column temperature is 180°C for 2 minutes, then programmed at 8°/min to 250°C and held for 4 minutes.

Table 2. Single-Laboratory Accuracy and Precision

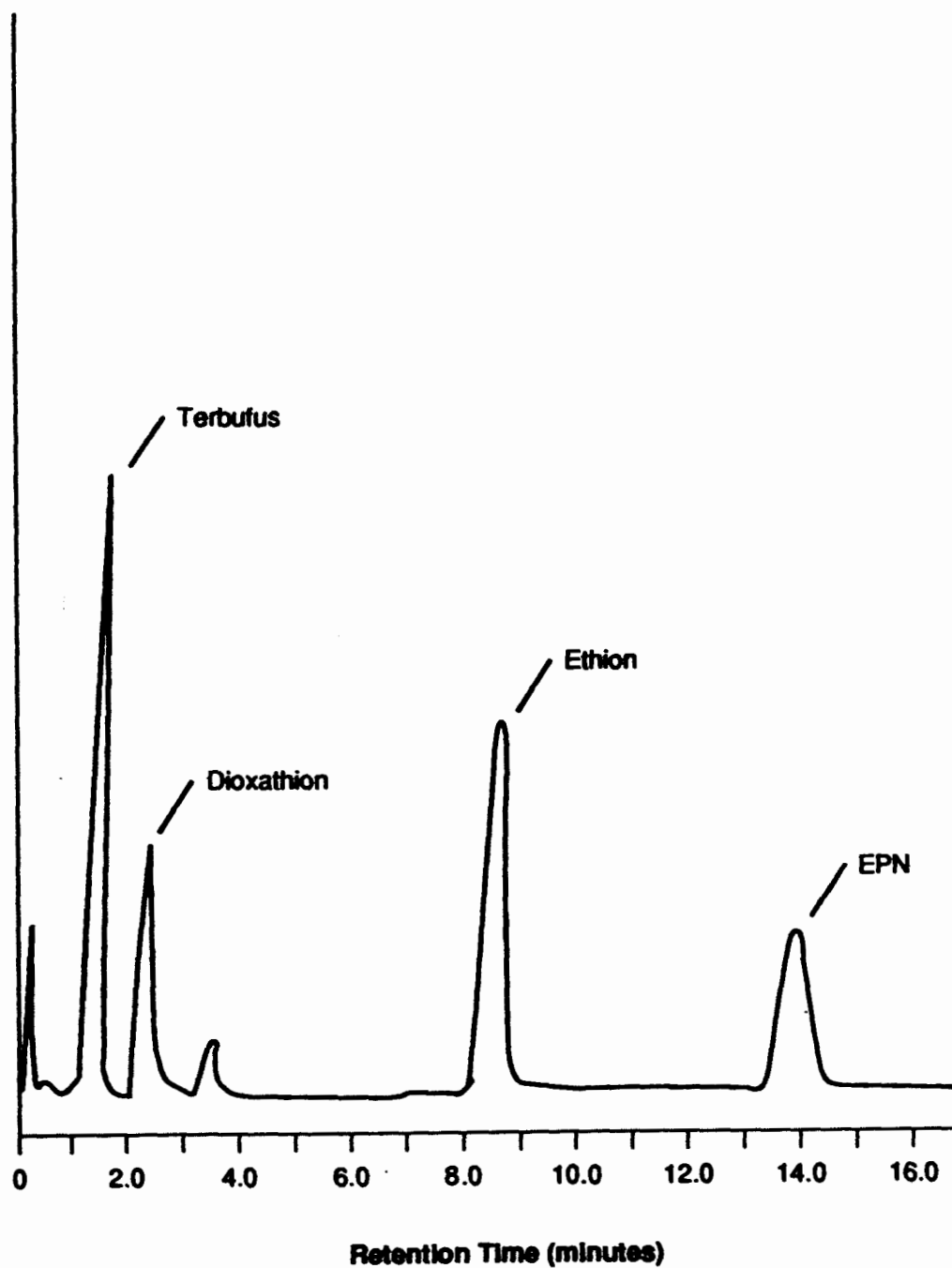
<i>Parameter</i>	<i>Matrix Type*</i>	<i>Spike Range (µg/L)</i>	<i>Number of Replicates</i>	<i>Average Percent Recovery</i>	<i>Standard Deviation (%)</i>
Dioxathion	1	1,978.0	7	94.3	19.9
	1	19.8	7	99.0	27.5
EPN	1	1,293.0	7	96.1	6.1
Ethion	1	1,788.0	7	89.2	4.5
Terbufos	1	15.1	7	101.0	12.4
	1	1,508.0	7	95.0	3.4

*1 = Combined industrial wastewaters

Table 3. Silica Gel Cleanup of Organophosphorus Pesticides

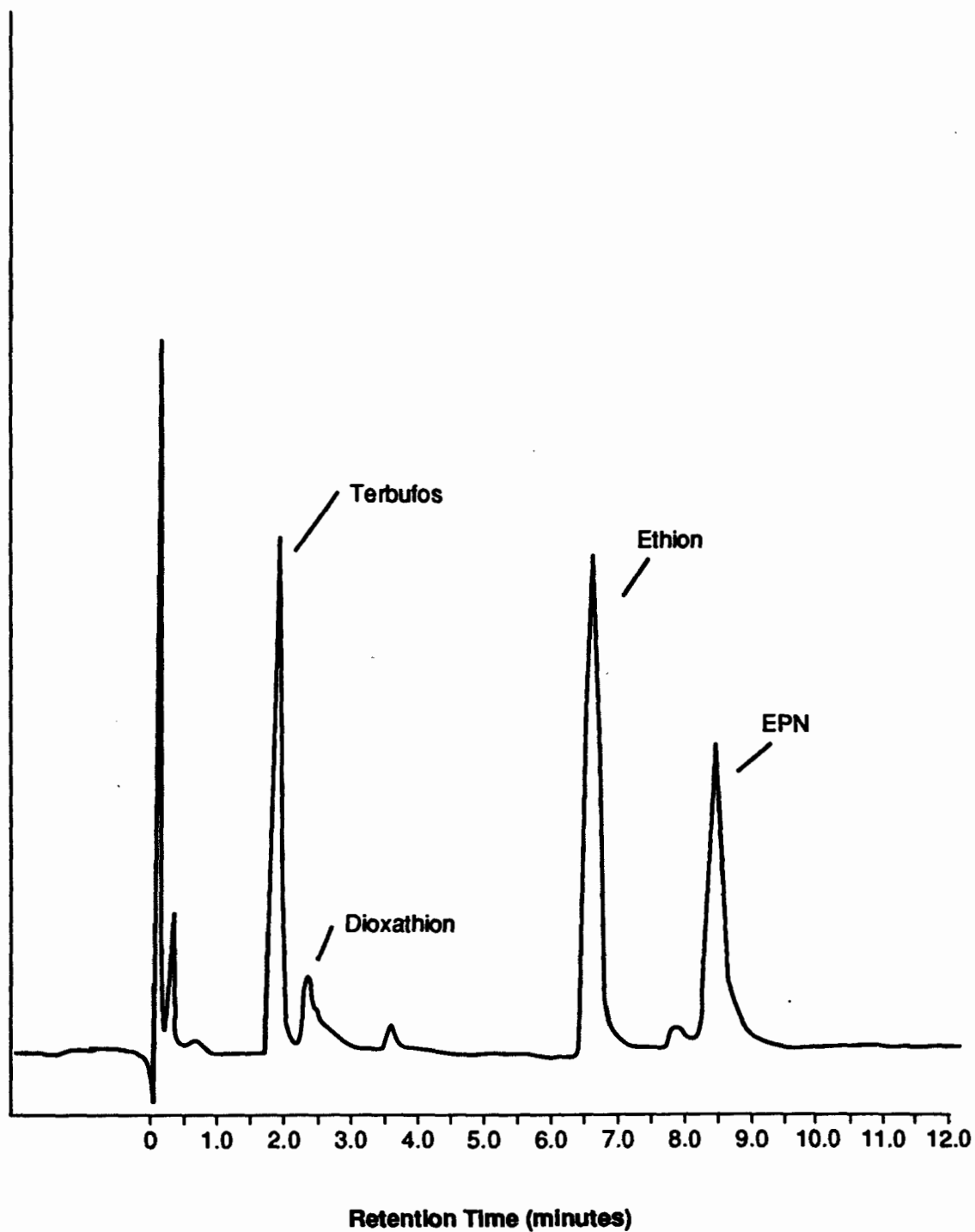
<i>Silica Gel Fraction*</i>	<i>Percent Recoveries</i>			
	<i>Terbufos</i>	<i>Dioxathion</i>	<i>Ethion</i>	<i>EPN</i>
1	0	0	0.8	0
2	0	0	1.9	0
3	93.0	35.1	94.9	46.4
4	0	52.7	3.0	56.0
Total Percent Recoveries	93.0	87.8	101	102

- * Fraction 1 : 30 mL 6% MeCl₂ in hexane
 Fraction 2 : 30 mL 15% MeCl₂ in hexane
 Fraction 3 : 30 mL 50% MeCl₂ in hexane
 Fraction 4 : 30 mL 100% MeCl₂



AS2-002-37

Figure 1. Gas Chromatogram of Organophosphorous Pesticides (Column 1).



A52-002-38

Figure 2. Gas Chromatogram of Organophosphorous Pesticides (Column 2).

Method 615
The Determination of
Chlorinated Herbicides in
Municipal and Industrial
Wastewater

Method 615

The Determination of Chlorinated Herbicides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of certain chlorinated herbicides. The following parent acids can be determined by this method:

<i>Parameter</i>	<i>STORET No.</i>	<i>CAS No.</i>
2,4-D	39736	94-75-7
Dalapon	-	75-99-0
2,4-DB	-	94-82-6
Dicamba	-	1918-00-9
Dichlorprop	-	120-36-5
Dinoseb	-	88-85-7
MCPA	-	94-74-6
MCPP	-	7085-19-0
2,4,5-T	39740	93-76-5
2,4,5-TP	39760	93-72-1

- 1.2 This method is also applicable to the determination of salts and esters of these compounds. These include, but are not limited to: the isobutyl and isooctyl esters of 2,4-D; the isobutyl and isooctyl esters of 2,4-DB; the isooctyl ester of MCPA; and the isooctyl ester of 2,4,5-TP. The actual form of each acid is not distinguished by this method. Results are calculated and reported for each listed parameter as total free acid.
- 1.3 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.4 The method detection limit (MDL, defined in Section 15) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for alternative gas chromatographic columns that can be used to confirm measurements made with the primary column. Section 15 provides gas

chromatograph/ mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. SUMMARY OF METHOD

- 2.1** A measured volume of sample, approximately 1 L, is acidified. The acid herbicides and their esters and salts are extracted with ethyl ether using a separatory funnel. The derivatives are hydrolyzed with potassium hydroxide and extraneous organic material is removed by a solvent wash. After acidification, the acids are extracted and converted to their methyl esters using diazomethane as the derivatizing agent. Excess reagent is removed, and the esters are determined by electron capture (EC) gas chromatography.¹

3. INTERFERENCES

- 3.1** Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.
- 3.1.1** Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with dilute acid, tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.
- 3.1.2** The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.2** The acid forms of the herbicides are strong organic acids, which react readily with alkaline substances and can be lost during analysis. Glassware and glass wool must be acid-rinsed with (1+9) hydrochloric acid and the sodium sulfate must be acidified with sulfuric acid prior to use to avoid this possibility.
- 3.3** Organic acids and phenols, especially chlorinated compounds, cause the most direct interference with the determination. Alkaline hydrolysis and subsequent extraction of the basic solution remove many chlorinated hydrocarbons and phthalate esters that might otherwise interfere with the electron capture analysis.
- 3.4** Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

- 4.1** The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified ³⁻⁵ for the information of the analyst.
- 4.2** Diazomethane is a toxic carcinogen and can explode under certain conditions. The following precautions must be followed:
- 4.2.1** Use only a well-ventilated hood; do not breath vapors.
 - 4.2.2** Use a safety screen.
 - 4.2.3** Use mechanical pipetting aides.
 - 4.2.4** Do not heat above 90°C: EXPLOSION may result.
 - 4.2.5** Avoid grinding surfaces, and avoid the use of ground-glass joints, sleeve bearings, and glass stirrers: EXPLOSION may result.
 - 4.2.6** Do not store near alkali metals: EXPLOSION may result.
 - 4.2.7** Solutions of diazomethane decompose rapidly in the presence of solid materials such as copper powder, calcium chloride, and boiling chips.

5. APPARATUS AND MATERIALS

- 5.1** Sampling equipment, for discrete or composite sampling.
- 5.1.1** Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.
 - 5.1.2** Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.
- 5.2** Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)
- 5.2.1** Separatory funnels: 60-mL and 2000-mL, with TFE-fluorocarbon stopcocks, ground-glass or TFE stoppers.
 - 5.2.2** Concentrator tube, Kuderna-Danish: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground-glass stopper is used to prevent evaporation of extracts.

- 5.2.3 Evaporative flask, Kuderna-Danish: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.
- 5.2.4 Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).
- 5.2.5 Snyder column, Kuderna-Danish: Two-ball micro (Kontes K-569001-0219 or equivalent).
- 5.2.6 Erlenmeyer flask: Pyrex, 250-mL with 24/40 ground-glass joint.
- 5.2.7 Vials: Amber glass, 10- to 15-mL capacity with TFE-fluorocarbon-lined screw-cap.
- 5.3 Boiling chips: Approximately 10/40 mesh. Heat at 400°C for 30 minutes or perform a Soxhlet extraction with methylene chloride.
- 5.4 Water bath: Heated, with concentric ring cover, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.
- 5.5 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6 Diazomethane generator: assemble from two test tubes 150mm long by 20mm ID, two Neoprene rubber stoppers, and a source of nitrogen. The generator assembly is shown in Figure 1.
- 5.7 Glass wool: Acid-washed (Supelco 2-0383 or equivalent).
- 5.8 Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.
 - 5.8.1 Column 1: 180 cm long by 4 mm ID glass, packed with 1.5% SP-2250/1.95% SP-2401 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 16. Alternative columns may be used in accordance with the provisions described in Section 13.1.
 - 5.8.2 Column 2: 180 cm long by 4 mm ID glass, packed with 5% OV-210 on Gas Chrom Q (100/120 mesh) or equivalent.
 - 5.8.3 Column 3: 180 cm long by 2 mm ID glass, packed with 0.1% SP-1000 on Carbowax C (80/100 mesh) or equivalent.
 - 5.8.4 Detector: Electron capture. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 13.1.

6. REAGENTS

- 6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2 Acetone, hexane, methanol: Pesticide-quality or equivalent.
- 6.3 Ethyl ether: Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Cat. No. P1126-8, and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.

- 6.4** Sodium sulfate: ACS, granular, acidified, anhydrous. Condition heating in a shallow tray at 400°C for a minimum of 4 hours to remove phthalates and other interfering organic substances. Alternatively, heat 16 hours at 450 to 500°C in a shallow tray or perform a Soxhlet extraction with methylene chloride for 48 hours. Acidify by slurring 100 g sodium sulfate with enough ethyl ether to just cover the solid. Add 0.1 mL concentrated sulfuric acid and mix thoroughly. Remove the ether under vacuum. Mix 1 g of the resulting solid with 5 mL of reagent water and measure the pH of the mixture. It must be below pH 4. Store at 130°C.
- 6.5** Hydrochloric acid (1+9): Add one volume of concentrated acid (ACS) to 9 volumes reagent water.
- 6.6** Potassium hydroxide solution: 37% aqueous solution (W:V). Dissolve 37 g ACS-grade potassium hydroxide pellets in reagent water and dilute to 100 mL.
- 6.7** Sulfuric acid solution (1+1): Slowly add 50 mL H₂SO₄ (sp. gr. 1.84) to 50 mL of reagent water.
- 6.8** Sulfuric acid solution (1+3): Slowly add 25 mL H₂SO₄ (sp. gr. 1.84) to 75 mL of reagent water. Maintain at 4°C.
- 6.9** Carbitol: Diethylene glycol monoethyl ether, ACS. Available from Aldrich Chemical Co.
- 6.10** Diazald: N-methyl-N-nitroso-p-toluenesulfonamide, ACS. Available from Aldrich Chemical Co.
- 6.11** Silicic acid: Chromatographic grade, nominal 100 mesh. Store at 130°C.
- 6.12** Stock standard solutions (1.00 µg/µL): Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.
- 6.12.1** Prepare stock standard solutions by accurately weighing about 0.0100 g of pure acids. Dissolve the material in pesticide-quality ethyl ether and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 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.
- 6.12.2** Transfer the stock standard solutions into PTFE-sealed screw-cap vials. 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 calibration standards from them.
- 6.12.3** Stock standard solutions must be replaced after 1 week, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1** Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system must be calibrated using the external standard technique.
- 7.2** External standard calibration procedure:
- 7.2.1** For each parameter of interest, prepare working standards of the free acids at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a 10-mL volumetric flask containing 1.0 mL methanol and diluting to volume with ethyl ether. One of the external standards should be representative of a

concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Prepare calibration standards by esterification of 1.00-mL volumes of the working standards as described in Section 11. Using injections of 2 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass of free acid represented by the injection. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the preparation of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. QUALITY CONTROL

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

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

8.2.1 Select a representative spike concentration for each compound (acid or ester) to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone, 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to

determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 2, determine the recovery and single operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁶ that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁶

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 14.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.

8.6 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 monitor the precision of the sampling technique. 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 perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

- 9.1** Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2** The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3** All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

10. SAMPLE EXTRACTION

- 10.1** Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel. Check the pH with wide-range pH paper and adjust to pH less than 2 with sulfuric acid (1+1).
- 10.2** Add 150 mL ethyl ether to the sample bottle, cap the bottle, and shake 30 seconds to rinse the walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical means. Drain the aqueous phase into a 1000-mL Erlenmeyer flask and collect the extract in a 250-mL ground-glass Erlenmeyer flask containing 2 mL of 37% potassium hydroxide solution. Approximately 80 mL of the ethyl ether will remain dissolved in the aqueous phase.
- 10.3** Add a 50-mL volume of ethyl ether to the sample bottle and repeat the extraction a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.
- 10.4** Add 15 mL reagent water and one or two clean boiling chips to the 250-mL flask and attach a three-ball Snyder column. Prewet the Snyder column by adding 1 mL ethyl ether to the top. Place the apparatus on a hot water bath (60 to 65°C), such that the bottom of the flask is bathed in the water vapor. Although the ethyl ether will evaporate in about 15 minutes, continue heating for a total of 60 minutes, beginning from the time the flask is placed on the water bath. Remove the apparatus and let stand at room temperature for at least 10 minutes.
- 10.5** Transfer the solution to a 60-mL separatory funnel using 5 to 10 mL of reagent water. Wash the basic solution twice by shaking for 1 minute with 20-mL portions of ethyl ether. Discard the organic phase. The free acids remain in the aqueous phase.
- 10.6** Acidify the contents of the separatory funnel to pH 2 by adding 2 mL of cold (4°C) sulfuric acid (1+3). Test with pH indicator paper. Add 20 mL ethyl ether and shake vigorously for 2 minutes. Drain the aqueous layer into the 250-mL Erlenmeyer, then pour the organic layer into a 125-mL Erlenmeyer flask containing about 0.5 g of acidified anhydrous sodium sulfate. Repeat

the extraction twice more with 10-mL aliquots of ethyl ether, combining all solvent in the 125-mL flask. Allow the extract to remain in contact with the sodium sulfate for approximately 2 hours.

- 10.7 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.8 Pour the combined extract through a funnel plugged with acid-washed glass wool, and collect the extract in the K-D in concentrator. Use a glass rod to crush any caked sodium sulfate during the transfer. Rinse the Erlenmeyer flask and column with 20 to 30 mL of ethyl ether to complete the quantitative transfer.
- 10.9 Add one to two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL ethyl ether to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.10 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of ethyl ether. A 5-mL syringe is recommended for this operation. Add a fresh boiling chip. Attach a micro-Snyder column to the concentrator tube and prewet the column by adding about 0.5 mL of ethyl ether to the top. Place the micro K-D apparatus on the water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete concentration in 5 to 10 minutes. When the apparent volume of liquid reaches 0.5 mL, remove the micro K-D from the bath and allow it to drain and cool. Remove the micro Snyder column and add 0.1 mL of methanol. Rinse the walls of the concentrator tube while adjusting the volume to 1.0 mL with ethyl ether.

11. *ESTERIFICATION OF ACIDS*

- 11.1 Assemble the diazomethane generator (See Figure 1) in a hood using two test tubes 150 mm long by 20 mm ID. Use neoprene rubber stoppers with holes drilled in them to accommodate glass delivery tubes. The exit tube must be drawn to a point to bubble diazomethane through the sample extract.
- 11.2 Add 5 mL of ethyl ether to the first test tube. Add 1 mL of ethyl ether, 1 mL of carbitol, 1.5 mL of 37% aqueous KOH, and 0.1 to 0.2 g Diazald to the second test tube. Immediately place the exit tube into the concentrator tube containing the sample extract. Apply nitrogen flow (10 mL/min) to bubble diazomethane through the extract for 10 minutes or until the yellow color of diazomethane persists.
- 11.3 Remove the concentrator tube and seal it with a neoprene or PTFE stopper. Store at room temperature in a hood for 20 minutes.
- 11.4 Destroy any unreacted diazomethane by adding 0.1 to 0.2 g silicic acid to the concentrator tube. Allow to stand until the evolution of nitrogen gas has stopped. Adjust the sample volume to 10.0 mL with hexane. Stopper the concentrator tube and store refrigerated if further processing

will not be performed immediately. It is recommended that the methylated extracts be analyzed immediately to minimize any transesterification and other potential reactions that may occur. Analyze by gas chromatography.

- 11.5 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

12. CLEANUP AND SEPARATION

- 12.1 No cleanup procedures were required to analyze the wastewaters described in Section 16. If particular circumstances demand the use of a cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

13. GAS CHROMATOGRAPHY

- 13.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Examples of the separations achieved for the methyl esters are shown in Figures 2 to 3. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.
- 13.2 Calibrate the system daily as described in Section 7.
- 13.3 Inject 1 to 5 μL of the sample extract using the solvent-flush technique.⁸ Record the volume injected to the nearest 0.05 μL , and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.
- 13.4 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 13.5 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 13.6 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

14. CALCULATIONS

- 14.1 Determine the concentration of individual compounds in the sample. Calculate the amount of free acid injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 1

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_i)(V_s)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_s = Volume of water extracted, in mL

- 14.2** Report results in micrograms per liter as acid equivalent without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 14.3** For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

15. GC/MS CONFIRMATION

- 15.1** It is recommended that GC/MS techniques be judiciously employed to support qualitative compound identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the methyl ester of the acid herbicide. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 seconds per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.
- 15.2** Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.⁹
- 15.3** At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.¹⁰
- 15.4** To confirm an identification of a compound, the background-corrected mass spectrum of the methyl ester must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
- 15.4.1** All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to $\pm 10\%$. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20% to 40%.

- 15.4.2** The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.
- 15.4.3** Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.
- 15.5** Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.
- 15.6** Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup.

16. METHOD PERFORMANCE

- 16.1** 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.¹¹ The MDL concentrations listed in Table 1 were obtained from reagent water with an electron capture detector.¹
- 16.2** In a single laboratory (West Coast Technical Services, Inc.), using reagent water and effluents from publicly owned treatment works (POTW), the average recoveries presented in Table 2 were obtained.¹ The standard deviations of the percent recoveries of these measurements are also included in Table 2.

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Table 1. Chromatographic Conditions and Method Detection Limits

<i>Parameter (as methyl ester)</i>	<i>Retention Time</i>			<i>Method Detection Limit µg/L</i>
	<i>Column 1</i>	<i>Column 2</i>	<i>Column 3</i>	
Dicamba	1.2	1.0	—	0.27
2,4-D	2.0	1.6	—	1.20
2,4,5-TP	2.7	2.0	—	0.17
2,4,5-T	3.4	2.4	—	0.20
2,4-DB	4.1	—	—	0.91
Dalapon	—	—	5.0	5.80
MCPP	3.4	—	—	192.00
MCPA	4.1	—	—	249.00
Dichlorprop	4.8	—	—	0.65
Dinoseb	11.2	—	—	0.07

Column 1 conditions: Supelcoport (100/120 mesh) coated with 1.5% SP-2250/ 1.95% SP-2401 packed in a 1.8 m long by 4 mm ID glass column with 95% argon/5% methane carrier gas at a flow rate of 70 mL/min. Column temperature: isothermal at 185°C, except for MCPP, MCPA, dichlorprop and dinoseb, where the column temperature was held at 140°C for 6 minutes and then programmed to 200°C at 10°/min. An electron capture detector was used to measure MDL.

Column 2 conditions: Gas Chrom Q (100/120 mesh) coated with 5% OV-210 packed in a 1.8 m long by 4 mm ID glass column with 95% argon/5% methane carrier gas at a flow rate of 70 mL/min. Column temperature: isothermal at 185°C.

Column 3 conditions: Carbopak C (80/100 mesh) coated with 0.1% SP-1000 packed in a 1.8 m long by 2 mm ID glass column with nitrogen carrier gas at a flow rate of 25 mL/min. Column temperature: programmed at injection from 100°C to 150°C at 10°/min.

Table 2. Single-Operator Accuracy and Precision *

<i>Parameter</i>	<i>Sample Type</i>	<i>Spike ($\mu\text{g/L}$)</i>	<i>Mean Recovery (%)</i>	<i>Standard Deviation (%)</i>
2,4-D	DW	10.9	75	4
	MW	10.1	77	4
	MW	200.0	65	5
Dalapon	DW	23.4	66	8
	MW	23.4	96	13
	MW	468.0	81	9
2,4-DB	DW	10.3	93	3
	MW	10.4	93	3
	MW	208.0	77	6
Dicamba	DW	1.2	79	7
	MW	1.1	86	9
	MW	22.2	82	6
Dichlorprop	DW	10.7	97	2
	MW	10.7	72	3
	MW	213.0	100	2
Dinoseb	MW	0.5	86	4
	MW	102.0	81	3
MCPA	DW	2020.0	98	4
	MW	2020.0	73	3
	MW	21400.0	97	2
MCPP	DW	2080.0	94	4
	MW	2100.0	97	3
	MW	20440.0	95	2
2,4,5-T	DW	1.1	85	6
	MW	1.3	83	4
	MW	25.5	78	5
2,4,5-TP	DW	1.0	88	5
	MW	1.3	88	4
	MW	25.0	72	5

*All results based upon seven replicate analyses.

DW = Reagent water

MW = Municipal water

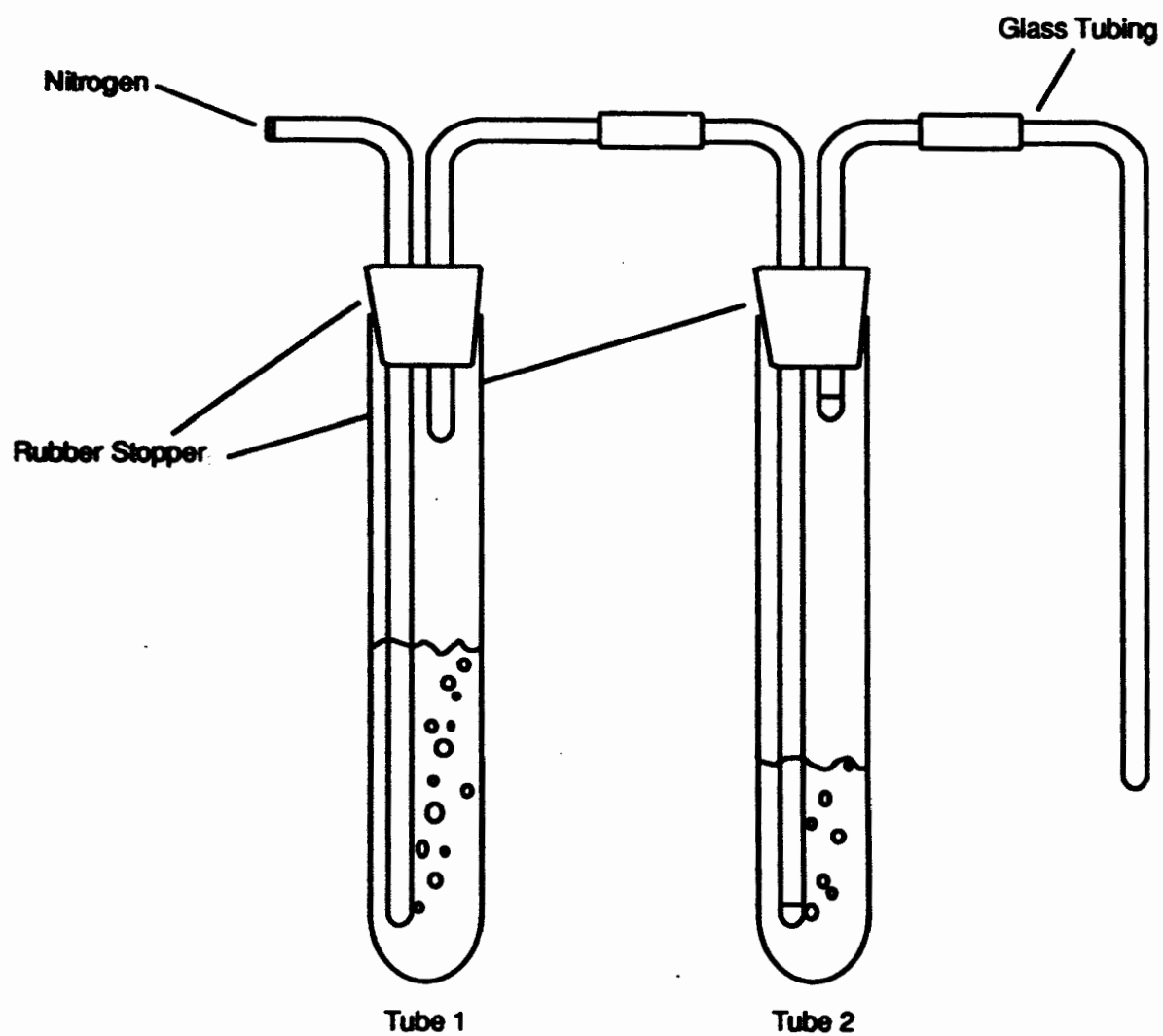
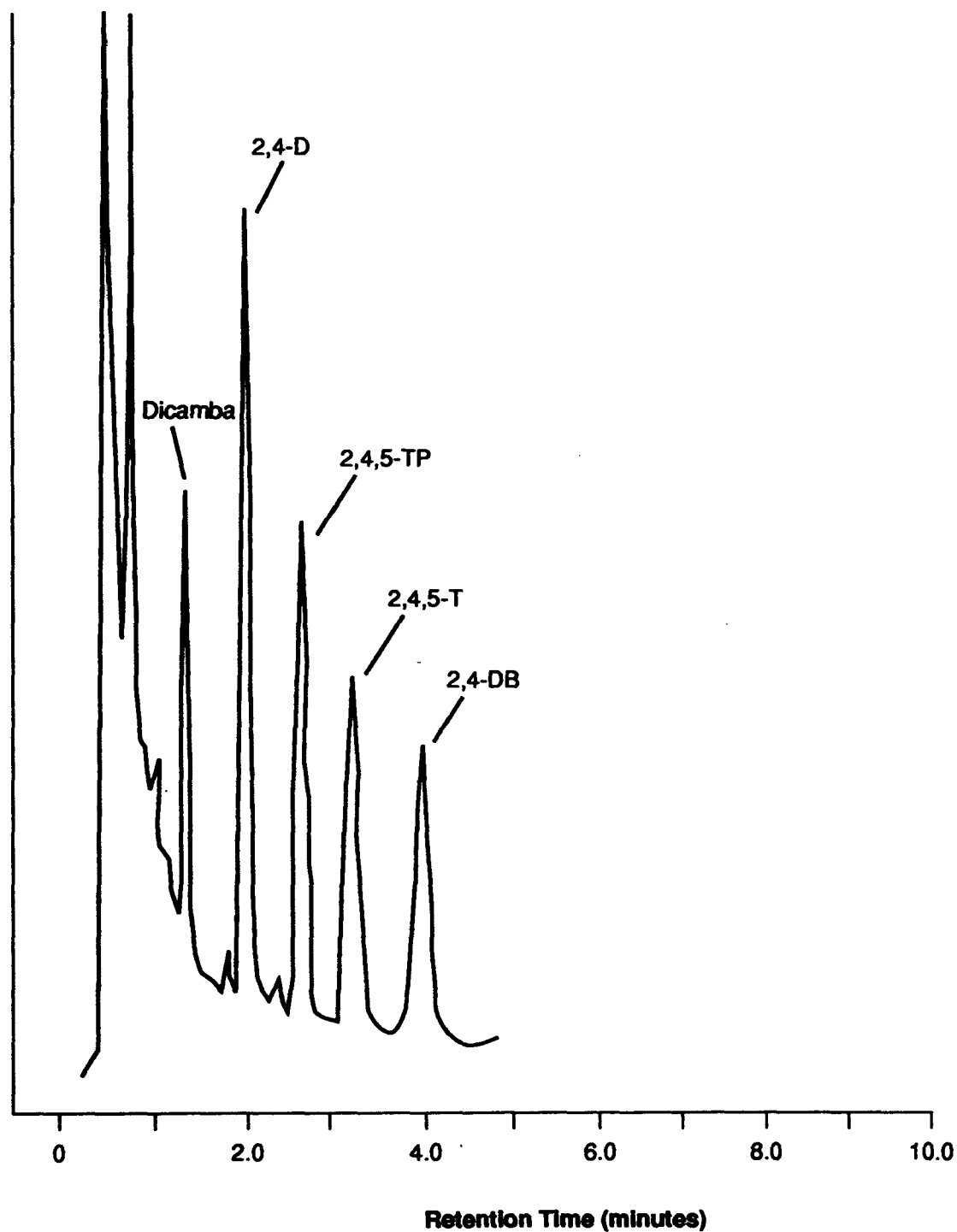


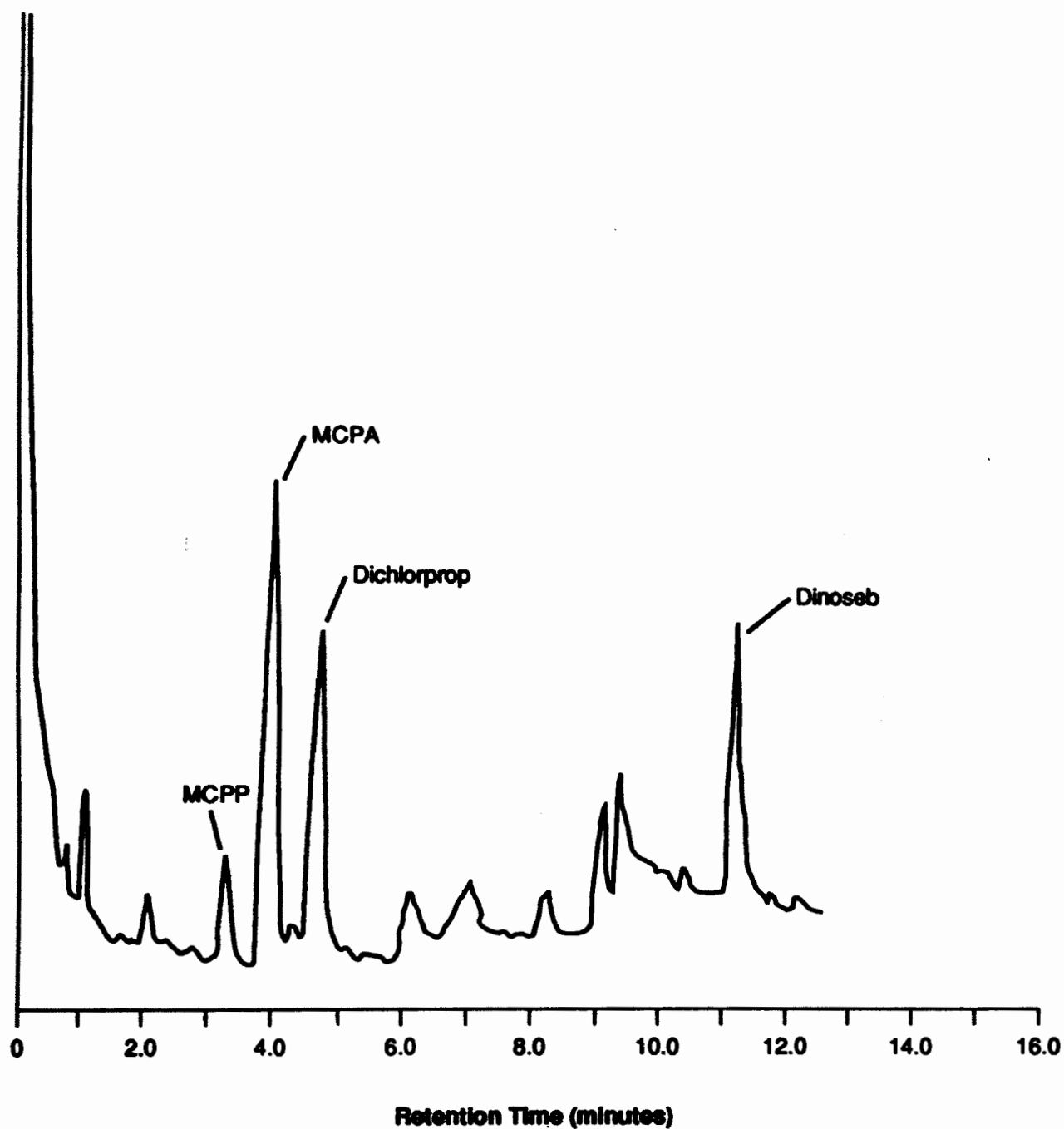
Figure 1. Diazomethane Generator.

AS2-002-17



A52-002-16

Figure 2. Gas Chromatogram of Methyl Esters of Chlorinated Herbicides on Column 1. For Conditions, See Table 1.



AS2-002-15

Figure 3. Gas Chromatogram of Methyl Esters of Chlorinated Herbicides on Column 1. For Conditions, See Table 1.

Method 616
***The Determination of Certain
Carbon-, Hydrogen-, and
Oxygen-Containing Pesticides in
Municipal and Industrial
Wastewater***

Method 616

The Determination of Certain Carbon-, Hydrogen-, and Oxygen-Containing Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of certain carbon-, hydrogen-, and oxygen-containing pesticides. The following parameters can be determined by this method:

<i>Parameter</i>	<i>CAS No.</i>
Cycloprate	54460-46-7
Kinoprene	42588-37-4
Methoprene	40596-69-8
Resmethrin	10453-86-8

- 1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3 The method detection limit (MDL, defined in Section 15) for each parameter is listed in Table 2. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4 The sample extraction and concentration steps in this method are essentially the same as certain other 600-series methods. Thus, a single sample may be extracted to measure the compounds included in the scope of the methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures.
- 1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. SUMMARY OF METHOD

- 2.1** A measured volume of sample, approximately 1 L, is extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated to 1.0 mL. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by flame ionization detector/gas chromatography (GC/FID).¹
- 2.2** This method provides a silica gel column cleanup procedure to aid in the elimination of interferences which may be encountered.

3. INTERFERENCES

- 3.1** Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.
- 3.1.1** Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 4 hours. Do not heat volumetric ware. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.
- 3.1.2** The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.2** Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 2.

4. SAFETY

- 4.1** The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified³⁻⁵ for the information of the analyst.

5. APPARATUS AND MATERIALS

- 5.1 Sampling equipment, for discrete or composite sampling.
- 5.1.1 Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with PTFE. Foil may be substituted for PTFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.
- 5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.
- 5.2 Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)
- 5.2.1 Separatory funnel: 2000-mL, with PTFE stopcock.
- 5.2.2 Drying column: Chromatographic column 400 mm long by 10 mm ID with coarse frit.
- 5.2.3 Chromatographic column: 400 mm long by 19 mm ID with 250-mL reservoir at the top and PTFE stopcock (Kontes K-420290 or equivalent).
- 5.2.4 Concentrator tube, Kuderna-Danish: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. A ground-glass stopper is used to prevent evaporation of extracts.
- 5.2.5 Evaporative flask, Kuderna-Danish: 250 mL (Kontes K-570001-0250 or equivalent). Attach to concentrator or tube with springs.
- 5.2.6 Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).
- 5.2.7 Snyder column, Kuderna-Danish: Two-ball micro (Kontes K-569001-0219 or equivalent).
- 5.2.8 Vials: Amber glass, 10- to 15-mL capacity with PTFE-lined screw-cap.
- 5.2.9 Graduated cylinder: 1000-mL.
- 5.2.10 Erlenmeyer flask: 250-mL.
- 5.3 Boiling chips: Approximately 10/40 mesh carborundum. Heat at 400°C for 4 hours or perform a Soxhlet extraction with methylene chloride.
- 5.4 Water bath: Heated, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.
- 5.5 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6 Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

- 5.6.1 Column 1: 180 cm long by 2 mm ID glass, packed with 3% SP2250 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 12.1.
- 5.6.2 Column 2: 180 cm long by 2 mm ID glass, packed with 10% OV-210 on Supelcoport (100/120 mesh) or equivalent.
- 5.6.3 Detector: Flame ionization detector (FID). This detector has proven effective in the analysis of wastewaters for the compounds listed in the scope and was used to develop the method performance statements in Section 15.

6. REAGENTS

- 6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2 Methylene chloride, methanol, petroleum ether, ethyl ether, methyl t-butyl ether, distilled-in-glass quality or equivalent. Ethyl ether must be free of peroxides as indicated by EK Quant Test Strips (available from Scientific Products Co., Catalog No. P1126-8 and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips.
- 6.3 Sodium sulfate: ACS, granular, anhydrous; heated in a muffle furnace at 400°C overnight.
- 6.4 Silica gel: Davison Grade 923 (100/120 mesh). Purchase activated. To prepare for use, place in a wide-mouth jar and heat overnight at 120 to 130°C. Seal tightly with PTFE or aluminum-foil-lined screw-cap and cool to room temperature.
- 6.5 Sodium phosphate: Monobasic, monohydrate.
- 6.6 Sodium phosphate: Dibasic.
- 6.7 Stock standard solutions (1.00 µg/µL): Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.
 - 6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in distilled-in-glass quality methyl t-butyl ether and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 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.
 - 6.7.2 Transfer the stock standard solutions into PTFE-sealed screw-cap bottles. Store at 4°C and protect from light. Frequently check standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
 - 6.7.3 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 2. The gas chromatographic system can be calibrated using the external standard technique (Section 7.2)

or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure.

7.2.1 For each compound of interest, prepare calibration standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with methyl t-butyl ether. One of the external standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrations or should define the working range of the detector.

7.2.2 Using injection of 1 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each compound at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than 10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each compound 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 methyl t-butyl ether. One of the standards should be at a concentration near, but above, the method detection limit. The other concentrations expected in the sample concentrates or should define the working range of the detector.

7.3.2 Using injections of 1 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

7.3.3 If the RF value over the working range is constant, less than 10% relative standard deviation, 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 or response ratios, A_s/A_{is} against RF. The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than 10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. QUALITY CONTROL

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

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

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methyl t-butyl ether, 1000 times more concentrated than the selected concentrations.

- 8.2.2** Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
- 8.2.3** Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.
- 8.2.4** Using the appropriate data from Table 3, determine the recovery and single operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.
- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
- 8.3.1** Calculate upper and lower control limits for method performance as follows:
- Upper Control Limit (UCL): $R + 3s$
Lower Control Limit (LCL): $R - 3s$
- where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁶ that are useful in observing trends in performance.
- 8.3.2** The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.
- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.
- 8.5** Before processing any samples, the analyst should demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

- 8.6** 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 monitor the precision of the sampling technique. 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 perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. SAMPLES COLLECTION, PRESERVATION, AND HANDLING

- 9.1** Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2** The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3** Adjust the pH of the sample to 6.8 by addition of 2 g each of monobasic and dibasic sodium phosphate per liter of sample.

10. SAMPLE EXTRACTION

- 10.1** Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel. Add 2 g each of monobasic sodium phosphate and dibasic sodium phosphate to the sample to adjust the pH to 6.8.
- 10.2** Add 60 mL of methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.
- 10.3** Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.
- 10.4** Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 250-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.5** Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with 30 to 40 mL of methylene chloride.

- 10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 3 to 4 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.7 Remove the macro-Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. Adjust the sample extract volume to 5 mL with methylene chloride.
- 10.8 Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract is to be stored longer than 2 days, transfer the extract to a screw-capped vial with a PTFE-lined cap. If the sample extract requires no further cleanup, proceed to Section 10.9. If the sample requires cleanup, proceed to Section 11.
- 10.9 Add one or two boiling chips and attach a two-ball micro-Snyder column to the concentrator tube. Prewet the micro-Snyder column with methylene chloride and concentrate the solvent extract to 1 mL as before.
- 10.10 Add 20 mL of methyl t-butyl ether to the concentrator tube and reconcentrate the solvent extract as before. When an apparent volume of 0.5 mL is reached, or the solution stops boiling, remove the K-D apparatus and allow it to drain and cool for 10 minutes.
- 10.11 Remove the micro-Snyder column and adjust the volume of the extract to 1.0 mL with methyl t-butyl ether. Transfer the extract to an appropriate container for subsequent GC analysis.
- 10.12 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.
- 11.2 The following silica gel column cleanup procedure has been demonstrated to be applicable to the four C, H, and O pesticides listed in Table 1.
 - 11.2.1 Deactivate silica gel by mixing 100 mL of acetone, 1.2 mL of distilled water, and 20 g of silica gel thoroughly for 30 minutes in a 250-mL beaker. Transfer the slurry to a chromatographic column (silica gel is retained with a plug of glass wool). Allow the solvent to elute from the column until the silica gel is almost exposed to the air. Wash the column sequentially with 10 mL of acetone, two 10-mL portions of methylene chloride, and three 10-mL portions of petroleum ether. Use a column flow rate of 2 to

2.5 mL/min throughout the wash and elution profiles. Add an additional 50 mL of petroleum ether to the head of the column.

11.2.2 Quantitatively add the methylene chloride extract from Section 10.8 to the head of the column. Allow the solvent to elute from the column until the silica gel is almost exposed to the air. Elute the column with 25 mL of petroleum ether. Discard this fraction.

11.2.3 Elute the column with 50 mL of 6% ethyl ether in petroleum ether (Fraction 1) and collect eluate in a K-D apparatus. Repeat process with 50 mL of 15% ethyl ether in petroleum ether (Fraction 2), add 100 mL of 50% ethyl ether in petroleum ether (Fraction 3). Collect each fraction in a separate K-D apparatus. The elution patterns for the C, H, and O pesticides are shown in Table 1. Concentrate each fraction to 1 mL as described in Sections 10.9, 10.10, and 10.11. Proceed with gas chromatographic analysis.

11.2.4 The above-mentioned fractions can be combined before concentration at the discretion of the analyst.

12. GAS CHROMATOGRAPHY

12.1 Table 2 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Examples of the separations achieved by Columns 1 and 2 are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the gas chromatographic system daily as described in Section 7.

12.3 If the internal standard approach is being used, the analyst must not add the internal standard to the sample extracts until immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 1 to 5 μL of the sample extract using the solvent flush technique. Record the volume injected to the nearest 0.05 μL , and the resulting peak sizes in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.

12.5 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_i)(V_s)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_s = Volume of water extracted, in mL

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where

A_s = Response for parameter to be measured

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_o = Volume of water extracted, in L

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

14. GC/MS CONFIRMATION

14.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 seconds per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all glass or glass-lined

materials is recommended. When using a fused silica capillary column, the column outlet should be threaded through the interface to within a few millimeters of the entrance to the source ionization chamber. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.

- 14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.¹⁰
- 14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all DFTPP performance criteria are achieved.⁹
- 14.4 To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
 - 14.4.1 The molecular ion and all other ions that are present above 10% relative abundance in the mass be present in the mass spectrum of the sample with agreement to $\pm 10\%$. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20 to 40%.
 - 14.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.
 - 14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.
- 14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.
- 14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. METHOD PERFORMANCE

- 15.1 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.¹¹ The MDL concentrations listed in Table 2.¹ Similar results were obtained using reagent water were achieved using representative wastewaters.
- 15.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range 10 x MDL to 1000 x MDL.
- 15.3 In a single laboratory, Battelle Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 3 were obtained after silica gel cleanup. Seven replicates of each of two different wastewaters were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 3.¹

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Table 1. Elution Characteristics of the C, H, And O Compounds from 6% Deactivated Silica Gel

<i>Parameter</i>	<i>Recovery in Specified Fraction (a)</i>			
	<i>F1</i>	<i>F2</i>	<i>F3</i>	<i>Total</i>
Cycloprate	97	ND(a)	ND	97
Kinoprene	100	ND	ND	100
Methoprene	ND	101	<1	101
Resmethrin	65	27	ND	92

(a) Elution solvents are 50 mL each of the following:

F1 = 6% ethyl ether in petroleum ether

F2 = 15% ethyl ether in petroleum ether

F3 = 50% ethyl ether

(b) ND = Not detected

Table 2. Chromatographic Conditions and Method Detection Limits

<i>Parameter</i>	<i>Retention Time (Min.)</i>		<i>MDL</i>
	<i>Column 1</i>	<i>Column 2</i>	<i>(µg/L)</i>
Cycloprate	3.6	3.9	21
Kinoprene	4.4	5.5	18
Methoprene	5.5	6.5	22
Resmethrin	8.4	8.9	36

Column 1 conditions: Supelcoport (100/120 mesh) coated with 3% SP-2250 packed in a 1.8 m long by 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. Column temperature is programmed from 180°C to 240°C at 8°C/min, injector temperature is 280°C and detector is 300°C. A flame ionization detector is used.

Column 2 conditions: Supelcoport (100/120 mesh) coated with 10% OV-210 packed in a 1.8 m long by 2 mm ID glass columns with helium carrier gas at a flow rate of 30 mL/min. Column temperature is programmed from 180°C to 240°C at 4°C/min, injector temperature is 280°C and detector is 300°C. A flame ionization detector is used.

Table 3. Single-Laboratory Accuracy and Precision(a)

<i>Parameter</i>	<i>Sample Type(b)</i>	<i>Background $\mu\text{g/L(c)}$</i>	<i>Spike $\mu\text{g/L}$</i>	<i>Mean Recovery %</i>	<i>Standard Deviation %</i>	<i>Number of Replicates</i>
Cycloprate	1	ND	100	84	14	7
	1	ND	1000	94	4	7
Kinoprene	1	ND	100	89	6	7
	1	ND	1000	92	6	7
Methoprene	1	ND	100	93	13	7
	1	ND	1000	90	4	7
Resmethrin	1	ND	100	86	8	7
	1	ND	1000	91	3	7

(a) Column 1 conditions were used.

(b) 1 = Columbus POTW secondary effluent

(c) ND = Not detected

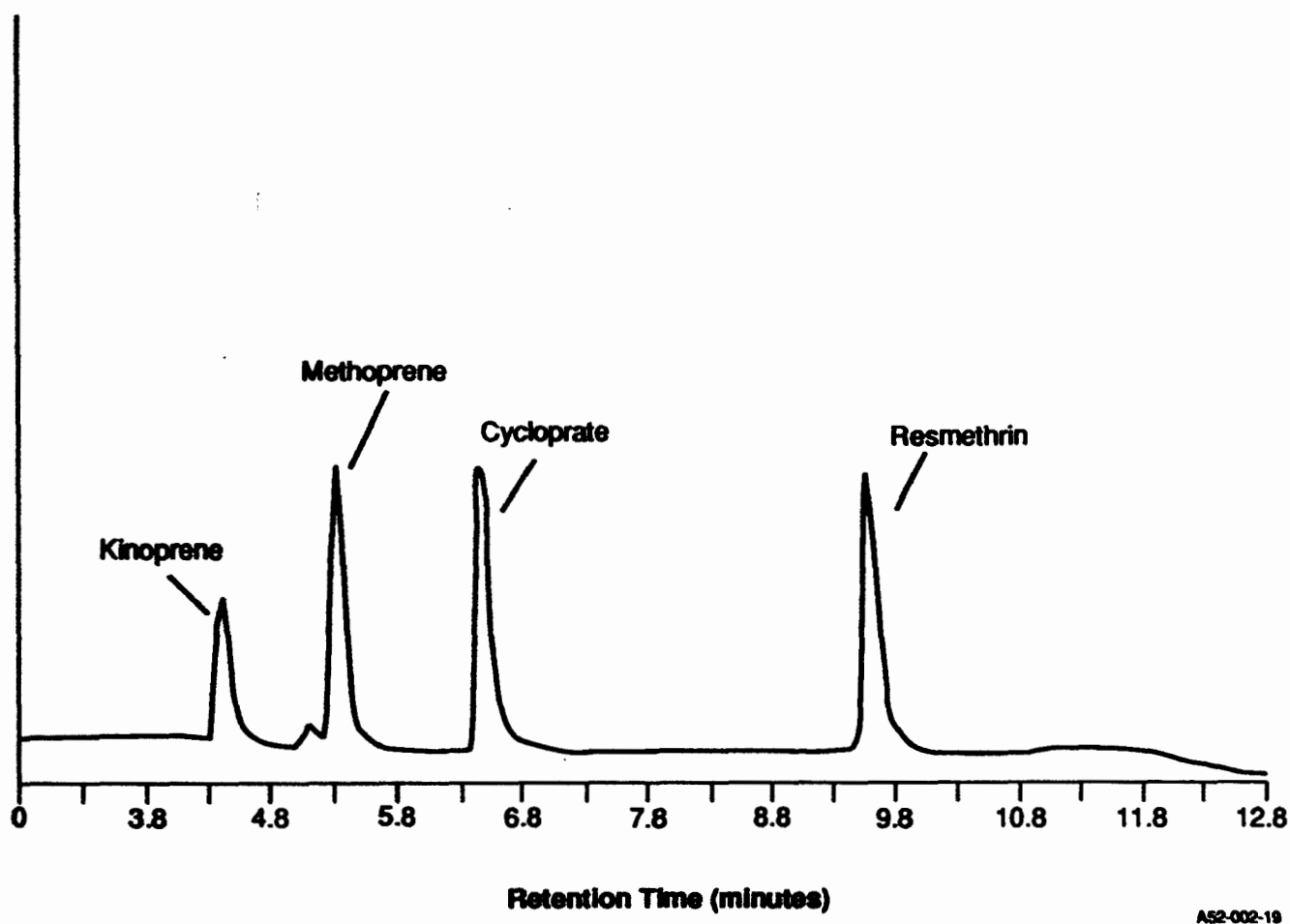
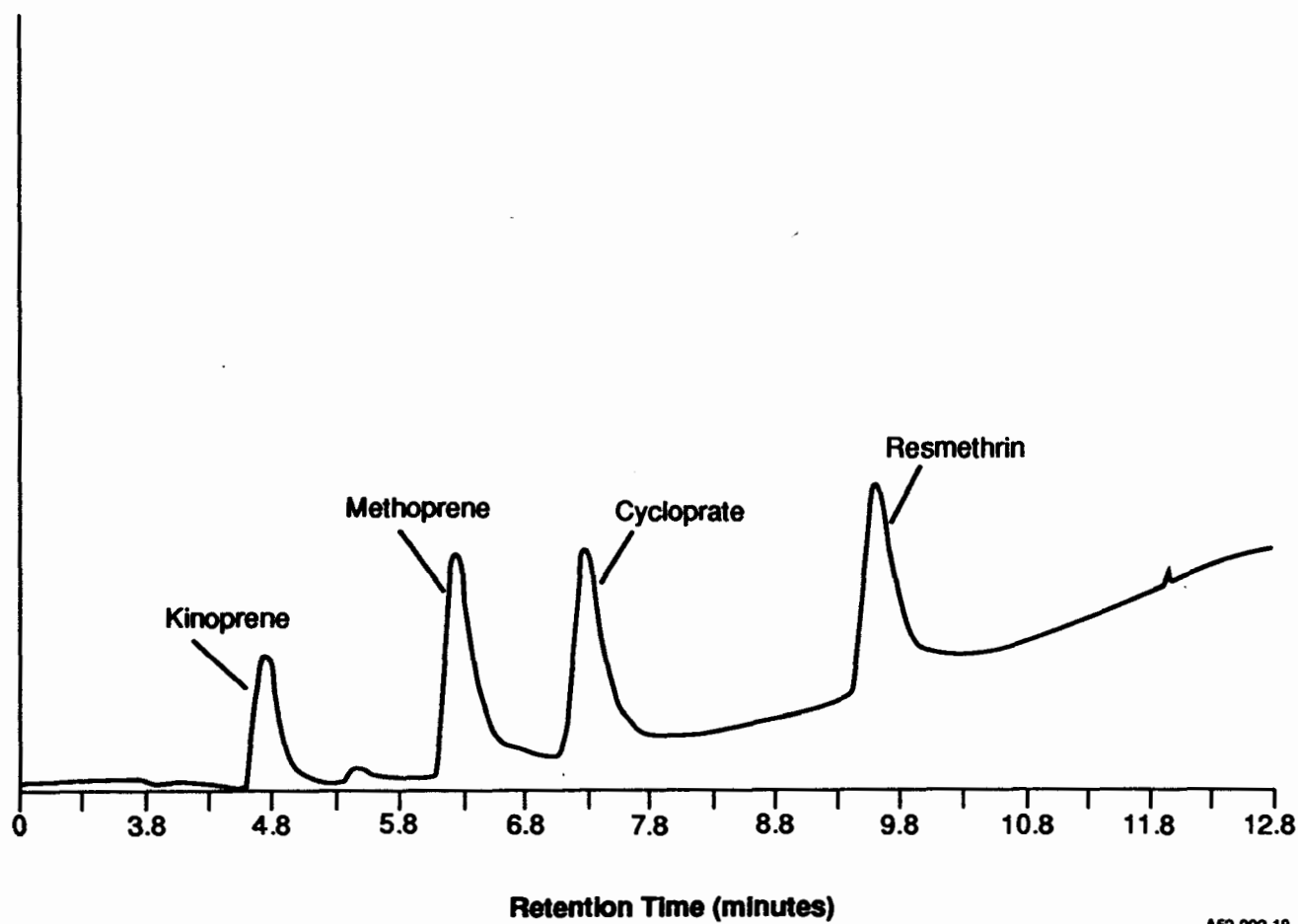


Figure 1. GC-FID Chromatogram of 200 ng Each of C, H, and O Compounds (Column 1).



A52-002-18

Figure 2. GC-FID Chromatogram of 200 ng Each of C, H, and O Compounds (Column 2).

Method 617
The Determination of
Organohalide Pesticides and
PCBs in Municipal and Industrial
Wastewater

Method 617

The Determination of Organohalide Pesticides and PCBS in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1** This method covers the determination of certain organohalide pesticides and PCBs. The following parameters can be determined by this method:

<i>Parameter</i>	<i>Storet No.</i>	<i>CAS No.</i>
Aldrin	39330	309-00-2
α -BHC	39337	319-84-6
β -BHC	39338	319-85-7
δ -BHC	39259	319-86-8
γ -BHC	39340	58-89-9
Captan	39640	133-06-2
Carbophenothion	--	786-19-6
Chlordane	39350	5103-74-2
4,4'-DDD	39310	72-54-8
4,4'-DDE	39320	72-55-9
4,4'-DDT	39300	50-29-3
Dichloran	--	99-30-9
Dicofol	39780	115-32-2
Dieldrin	39380	60-57-1
Endosulfan I	34356	959-98-8
Endosulfan II	34361	33213-65-9
Endosulfansulfate	34351	1031-07-8
Endrin	39390	72-20-8
Endrinaldehyde	34366	7421-93-4
Heptachlor	39410	76-44-8
Heptachlor epoxide	39420	1024-57-3
Isodrin	39430	465-73-6

<i>Parameter</i>	<i>Storet No.</i>	<i>CAS No.</i>
Methoxychlor	39480	72-43-5
Mirex	39755	2385-85-5
PCNB	39029	82-68-8
Perthane	39034	72-56-0
Strobane	—	8001-50-1
Toxaphene	39400	8001-35-2
Trifluralin	39030	1582-09-8
PCB-1016	34671	12674-11-2
PCB-1221	39488	11104-28-2
PCB-1232	39492	11141-16-5
PCB-1242	39496	53469-21-9
PCB-1248	39500	12672-29-6
PCB-1254	39504	11097-69-1
PCB-1260	39508	11096-82-5

- 1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3 The method detection limit (MDL, defined in Section 15) for many of the parameters are listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4 The sample extraction and concentration steps in this method are essentially the same as in Method 614. Thus, a single sample may be extracted to measure the parameters included in the scope of both of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures. Under gas chromatography, the analyst is allowed the latitude to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters (see Section 12).
- 1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique.

This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. SUMMARY OF METHOD

- 2.1 A measured volume of sample, approximately 1 L, is extracted with 15% methylene chloride in hexane using a separatory funnel. The extract is dried and concentrated to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by electron capture gas chromatography.
- 2.2 Method 617 represents an editorial revision of two previously promulgated U.S. EPA methods for pesticides and for PCBs.¹ While complete method validation data is not presented herein, the method has been in widespread use since its promulgation, and represents the state of the art for the analysis of such materials.
- 2.3 This method provides selected cleanup procedures to aid in the elimination of interferences which may be encountered.

3. INTERFERENCES

- 3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.
 - 3.1.1 Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.
 - 3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.2 Interferences by phthalate esters can pose a major problem in pesticide analysis when the EC detector is used. These compounds generally appear in the chromatogram as large late-eluting peaks, especially in the 15 and 50% fractions from the Florisil column cleanup. Common flexible plastics contain varying amounts of phthalates. These phthalates are easily extracted or leached from such materials during laboratory operations. Cross-contamination of clean glassware occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Interferences from phthalates can be minimized by avoiding the use of plastics in the laboratory. Exhaustive cleanup of reagents and glassware may be required to eliminate

background phthalate contamination.^{3,4} The interferences from phthalate esters can be avoided by using a microcoulometric or electrolytic conductivity detector.

- 3.3** Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

- 4.1** The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified⁵⁻⁷ for the information of the analyst.
- 4.2** The following parameters covered by this method have been tentatively classified as known or suspected human or mammalian carcinogens: aldrin, benzene hexachlorides, chlordane, heptachlor, PCNB, PCBs, and toxaphene. Primary standards of these toxic materials should be prepared in a hood.

5. APPARATUS AND MATERIALS

- 5.1** Sampling equipment, for discrete or composite sampling.
- 5.1.1** Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.
- 5.1.2** Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.
- 5.2** Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)
- 5.2.1** Separatory funnel: 125-mL, 1000-mL, and 2000-mL, with TFE-fluorocarbon stopcock, ground-glass or TFE stopper.
- 5.2.2** Drying column: Chromatographic column 400 mm long by 19mm ID with coarse-fritted disc.

- 5.2.3 Chromatographic column: 400 mm long by 19 mm ID with coarse-fritted disc at bottom and TFE-fluorocarbon stopcock (Kontes K-420540-0224 or equivalent).
- 5.2.4 Concentrator tube, Kuderna-Danish: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground-glass stopper is used to prevent evaporation of extracts.
- 5.2.5 Evaporative flask, Kuderna-Danish: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.
- 5.2.6 Snyder column, Kuderna-Danish: three-ball macro (Kontes K-503000-0121 or equivalent).
- 5.2.7 Vials: Amber glass, 10- to 15-mL capacity with TFE-fluorocarbon-lined screw-cap.
- 5.3 Boiling chips: Approximately 10/40 mesh. Heat at 400°C for 30 minutes or perform a Soxhlet extraction with methylene chloride.
- 5.4 Water bath: Heated, with concentric ring cover, capable of temperature control ($\pm 2^{\circ}\text{C}$). The bath should be used in a hood.
- 5.5 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6 Shaker: Laboratory, reciprocal action.
- 5.7 Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.
 - 5.6.1 Column 1: 180 cm long by 4 mm ID glass, packed with 1.5% SP-2250/1.95% SP-2401 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 12.1.
 - 5.6.2 Column 2: 180 cm long by 4 mm ID glass, packed with 3% OV-1 on Supelcoport (100/120 mesh) or equivalent.
 - 5.6.3 Detector: Electron capture. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. REAGENTS

- 6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2 Acetone, hexane, isooctane, methylene chloride: Pesticide-quality or equivalent.
- 6.3 Ethyl ether: Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Cat. No. P1126-8, and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.
- 6.4 Acetonitrile, hexane-saturated: Mix pesticide-quality acetonitrile with an excess of hexane until equilibrium is established.

- 6.5 Sodium sulfate: ACS, granular, anhydrous. Heat in a shallow tray at 400°C for a minimum of 4 hours to remove phthalates and other interfering organic substances. Alternatively, heat 16 hours at 450 to 500°C in a shallow tray or Soxhlet extract with methylene chloride for 48 hours.
- 6.6 Sodium chloride solution, saturated: Prepare saturated solution of NaCl in reagent water and extract with hexane to remove impurities.
- 6.7 Sodium hydroxide solution (10N): Dissolve 40 g ACS grade NaOH in reagent water and dilute to 100 mL.
- 6.8 Sulfuric acid solution (1 + 1): Slowly add 50 mL H₂SO₄ (sp. gr. 1.84) to 50 mL of reagent water.
- 6.9 Mercury: Triple-distill.
- 6.10 Florisil: PR grade (60/100 mesh). Purchase activated at 675°C and store in dark in glass container with ground-glass stopper or foil-lined screw-cap. Before use, activate each batch at least 16 hours at 130°C in a foil-covered glass container.
- 6.11 Stock standard solutions (1.00 µg/µL): Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.
 - 6.11.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in pesticide-quality isooctane and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially-prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.
 - 6.11.2 Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw-cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
 - 6.11.3 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2 External standard calibration procedure.
 - 7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with isooctane. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

- 7.2.2** Using injections of 1 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.
- 7.2.3** The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.
- 7.3** Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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.
- 7.3.1** Prepare calibration standards at a minimum of three concentration levels for each parameter 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 isooctane. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.
- 7.3.2** Using injections of 1 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} against RF.

- 7.3.3** The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.
- 7.4** The cleanup procedure in Section 11 utilizes Florisil chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil which is used, the use of the lauric acid value is suggested. This procedure⁸ determines the adsorption from hexane solution of lauric acid, in milligrams, per gram of Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.
- 7.5** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.
- 7.6** The multippeak materials included in this method present a special calibration problem. Recommended procedures for calibration, separation and measurement of PCBs is discussed in detail in the previous edition of this method.¹ Illustrated methods for the calibration and measurement of chlordane and strobane/toxaphene are available elsewhere.⁹

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.
- 8.1.1** Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2** In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
- 8.1.3** The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2** To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
- 8.2.1** Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone, 1000 times more concentrated than the selected concentrations.
- 8.2.2** Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level

for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Table 2 provides single-operator recovery and precision for many of the organohalide pesticides. Similar results should be expected from reagent water for all parameters listed in this method. Compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts¹⁰ that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.¹⁰

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.

8.6 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 monitor the precision of the sampling technique. 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 perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

- 9.1** Grab samples must be collected in glass containers. Conventional sampling practices¹¹ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2** The samples must be iced or refrigerated at 4°C from the time of collection until extraction. Chemical preservatives should not be used in the field unless more than 24 hours will elapse before delivery to the laboratory. If the samples will not be extracted within 48 hours of collection, the sample should be adjusted to a pH range of 6.0 to 8.0 with sodium hydroxide or sulfuric acid. Record the volume of acid or base used.
- 9.3** All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

10. SAMPLE EXTRACTION

- 10.1** Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel.
- 10.2** Add 60 mL 15% methylene chloride in hexane (V:V) to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Drain the aqueous phase into a 1000-mL Erlenmeyer flask and collect the extract in a 250-mL Erlenmeyer flask. Return the aqueous phase to the separatory funnel.
- 10.3** Add a second 60-mL volume of 15% methylene chloride in hexane to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the 250-mL Erlenmeyer flask. Perform a third extraction in the same manner.
- 10.4** Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.5** Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of hexane to complete the quantitative transfer.
- 10.6** Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 80 to 85°C, so that the concentrator tube is partially immersed in

the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

- 10.7** Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to PTFE-sealed screw-cap bottles. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis. If the sample requires cleanup, proceed to Section 11.
- 10.8** Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1** Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various industrial and municipal effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.
- 11.2** Acetonitrile partition: The following acetonitrile partitioning procedure may be used to isolate fats and oils from the sample extracts. This procedure is applicable to all of the parameters in this method except mirex.
- 11.2.1** Quantitatively transfer the previously concentrated extract to a 125-mL separatory funnel with enough hexane to bring the final volume to 15 mL. Extract the sample four times by shaking vigorously for 1 minute with 30-mL portions of hexane-saturated acetonitrile.
- 11.2.2** Combine and transfer the acetonitrile phases to a 1-L separatory funnel and add 650 mL of reagent water and 40 mL of saturated sodium chloride solution. Mix thoroughly for 30 to 45 seconds. Extract with two 100-mL portions of hexane by vigorously shaking for 15 seconds.
- 11.2.3** Combine the hexane extracts in a 1-L separatory funnel and wash with two 100-mL portions of reagent water. Discard the water layer and pour the hexane layer through a drying column containing 7 to 10 cm of anhydrous sodium sulfate into a 500-mL K-D flask equipped with a 10-mL concentrator tube. Rinse the separatory funnel and column with three 10-mL portions of hexane.
- 11.2.4** Concentrate the extracts to 6 to 10 mL in the K-D as directed in Section 10.6. Adjust the extract volume to 10 mL with hexane.
- 11.2.5** Analyze by gas chromatography unless a need for further cleanup is indicated.

- 11.3 Florisil column cleanup:** The following Florisil column cleanup procedure has been demonstrated to be applicable to most of the organochlorine pesticides and PCBs listed in Table 3. It should also be applicable to the cleanup of extracts for PCNB, strobane, and trifluralin.
- 11.3.1** Add a weight of Florisil (nominally 20 g), predetermined by calibration (Section 7.4 and 7.5), to a chromatographic column. Settle the Florisil by tapping the column. Add anhydrous sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatography column. Discard the eluate.
- 11.3.2** Adjust the sample extract volume to 10 mL with hexane and transfer it from the K-D concentrator tube to the Florisil column. Rinse the tube twice with 1 to 2 mL hexane, adding each rinse to the column.
- 11.3.3** Place a 500-mL K-D flask and clean concentrator tube under the chromatography column. Drain the column into the flask until the sodium sulfate layer is nearly exposed. Elute the column with 200 mL of 6% ethyl ether in hexane (V/V) (Fraction 1) using a drip rate of about 5 mL/min. Remove the K-D flask and set aside for later concentration. Elute the column again, using 200 mL of 15% ethyl ether in hexane (V/V) (Fraction 2), into a second K-D flask. Perform a third elution using 200 mL of 50% ethyl ether in hexane (V/V) (Fraction 3) into a separate K-D flask. The elution patterns for the pesticides and PCBs are shown in Table 3.
- 11.3.4** Concentrate the eluates by standard K-D techniques (Section 10.6), using the water bath at about 85°C. Adjust final volume to 10 mL with hexane. Analyze by gas chromatography.
- 11.4 Removal of sulfur:** Elemental sulfur will elute in Fraction 1 of the Florisil cleanup procedure. If a large amount of sulfur is present in the extract, it may elute in all fractions. If so, each fraction must be further treated to remove the sulfur. This procedure cannot be used with heptachlor, endosulfans, or endrin aldehyde.
- 11.4.1** Pipette 1.00 mL of the concentrated extract into a clean concentrator tube or a vial with a TFE-fluorocarbon seal. Add 1 to 3 drops of mercury and seal.
- 11.4.2** Agitate the contents of the vial for 15 to 30 seconds.
- 11.4.3** Place the vial in an upright position on a reciprocal laboratory shaker and shake for up to 2 hours.
- 11.4.4** If the mercury appears shiny after this treatment, analyze the extract by gas chromatography. If the mercury is black, decant the extract into a clean vial and repeat the cleanup with fresh mercury.

12. GAS CHROMATOGRAPHY

- 12.1** Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the

relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

- 12.2 Calibrate the system daily as described in Section 7.
- 12.3 If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.
- 12.4 Inject 1 to 5 μL of the sample extract using the solvent-flush technique.¹² Record the volume injected to the nearest 0.05 μL , and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used. Multipeak materials present a special analytical problem beyond the scope of this discussion. Illustrated procedures for calibration and measurement are available for PCBs¹ and pesticides.⁹
- 12.5 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

- 13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_s)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_s = Volume of water extracted, in mL

- 13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_e)}$$

where:

A_s = Response for parameter to be measured.

A_{is} = Response for the internal standard.

I_s = Amount of internal standard added to each extract, in μg .

V_e = Volume of water extracted, in liters.

- 13.2** Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 13.3** For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. GC/MS CONFIRMATION

- 14.1** It is recommended that GC/MS techniques be judiciously employed to support qualitative compound identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least five scans per peak but not to exceed seven per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.
- 14.2** Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.¹³
- 14.3** At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.¹⁴
- 14.4** To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
- 14.4.1** All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to $\pm 10\%$. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20% to 40%.

14.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.

14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.

14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.

14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. METHOD PERFORMANCE

15.1 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.¹⁵ The MDL concentrations listed in Table 1 were obtained using reagent water.¹⁶

15.2 In a single laboratory, Susquehanna University, using spiked tap water samples, the average recoveries presented in Table 2 were obtained. The standard deviation of the percent recovery is also included in Table 2.¹⁶

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Table 1. Gas Chromatography of Pesticides and PCBs

<i>Parameter</i>	<i>Retention Time (min)</i>		<i>Method</i>
	<i>Column 1</i>	<i>Column 2</i>	<i>Detection Limit</i> ($\mu\text{g/L}$)
Aldrin	2.40	4.10	0.009
α -BHC	1.35	1.82	0.004
β -BHC	1.90	1.97	ND
δ -BHC	2.15	2.20	ND
γ -BHC	1.70	2.13	0.002
Captan	6.22	5.00	ND
Carbophenothion	10.9	10.90	ND
4,4'-DDD	7.83	9.08	0.012
4,4'-DDE	5.13	7.15	0.004
4,4'-DDT	9.40	11.75	0.032
Dichloran	1.85	2.01	ND
Dicofol	2.86	4.59	ND
Dieldrin	5.45	7.23	0.011
Endosulfan I	4.50	6.20	0.11
Endosulfan II	8.00	8.28	0.17
Endosulfan sulfate	14.22	10.70	ND
Endrin	6.55	8.19	ND
Endrin aldehyde	11.82	9.30	ND
Heptachlor	2.00	3.35	0.004
Heptachlor epoxide	3.50	5.00	0.003
Isodrin	3.00	4.83	ND
Methoxychlor	18.20	26.60	0.176
Mirex	14.60	15.50	0.015
PCNB	1.63	2.01	0.002
Trifluralin	0.94	1.35	0.013

*For multippeak materials, see Figures 2 to 10 for chromatographic conditions and retention patterns.

ND = Not Determined

Column 1 conditions: Supelcoport (100/120 mesh) coated with 1.5% SP-2250/1.95% SP-2401 in a 1.8 m long by 4 mm ID glass column with 95% argon/5% methane carrier gas at a flow rate of 60 mL/min. Column temperature: isothermal at 200°C. An electron capture detector was used with this column to determine the MDL.

Column 2 conditions: Supelcoport (100/120 mesh) coated with 3% OV-1 packed in a 1.8 m long by 4 mm ID glass column with 95% argon/5% methane carrier gas at a flow rate of 60 mL/min. Column temperature: isothermal at 200°C.

Table 2. Single-Operator Accuracy and Precision for Tap Water

<i>Parameter</i>	<i>Average Percent Recovery</i>	<i>Standard Deviation (%)</i>	<i>Spike Range (µg/L)</i>	<i>Number of Analyses</i>
Aldrin	78.1	5.4	0.03-3.0	21
δ-BHC	95.3	8.9	0.01-1.0	21
γ-BHC	95.1	7.2	0.01-1.0	21
4,4'-DDD	94.4	5.0	0.08-8.0	21
4,4'-DDE	89.8	3.7	0.05-5.0	21
4,4'-DDT	91.0	4.5	0.2-20	21
Dieldrin	98.2	4.9	0.06-6.0	21
Endosulfan I	101.0	7.6	0.05-5.0	21
Endosulfan II	92.9	4.8	0.09-9.0	21
Heptachlor	84.4	6.4	0.02-2.0	21
Heptachlor epoxide	93.7	3.9	0.03-3.0	21
Methoxychlor	96.6	6.7	0.6-60	21
Mirex	89.1	4.8	0.2-20	21
PCNB	82.6	6.2	0.01-1.0	21
Trifluralin	94.3	10.5	0.03-3.0	21

Table 3. Distribution and Recovery of Chlorinated Pesticides and PCBs Using Florisil Column Chromatography

<i>Parameter</i>	<i>Percent Recovery by Fraction</i>		
	<i>No. 1</i>	<i>No. 2</i>	<i>No. 3</i>
Aldrin	100		
α -BHC	100		
β -BHC	97		
δ -BHC	98		
γ -BHC	100		
Captan	+	+	
Carbofenthion	100		
Chlordane	100		
4,4'-DDD	99		
4,4'-DDE	98		
4,4'-DDT	100		
Dicofol	+	+	
Dieldrin	0	100	
Endosulfan II	37	64	91
Endosulfan II	0	7	106
Endosulfan sulfate	0	0	
Endrin	4	96	
Endrin aldehyde	0	68	26
Heptachlor	100		
Heptachlor epoxide	100		
Isodrin	100		
Methoxychlor	100		
Mirex	100		
Perthane	100		
Toxaphene	96		
PCB-1016	97		
PCB-1221	97		
PCB-1232	95		
PCB-1242	97		
PCB-1248	103		
PCB-1254	90		
PCB-1260	95		

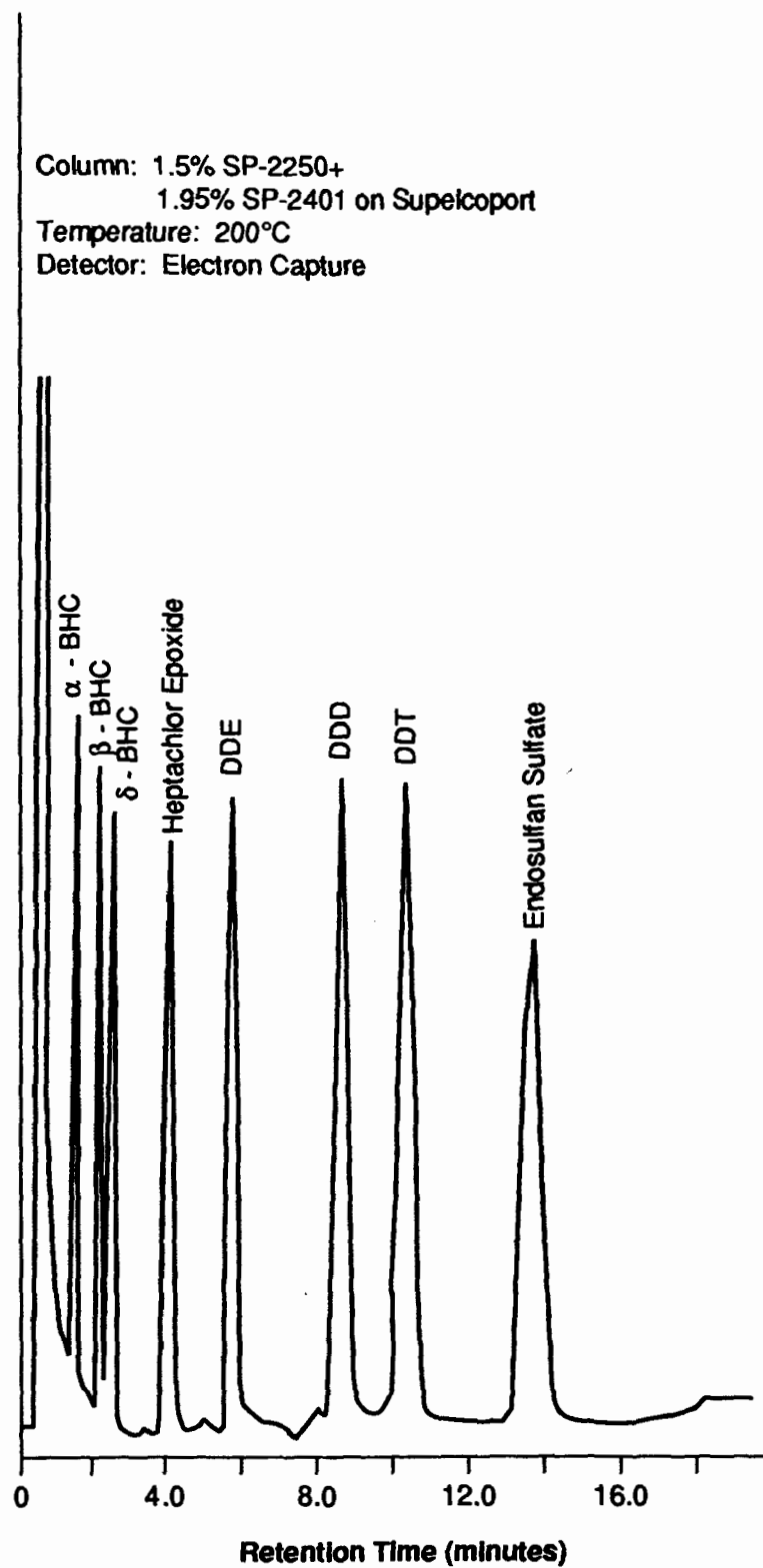
+Compound occurs in both 6% and 15% fractions.

Florisil eluate composition by fraction:

Fraction 1 - 200 mL of 65 ethyl ether in hexane

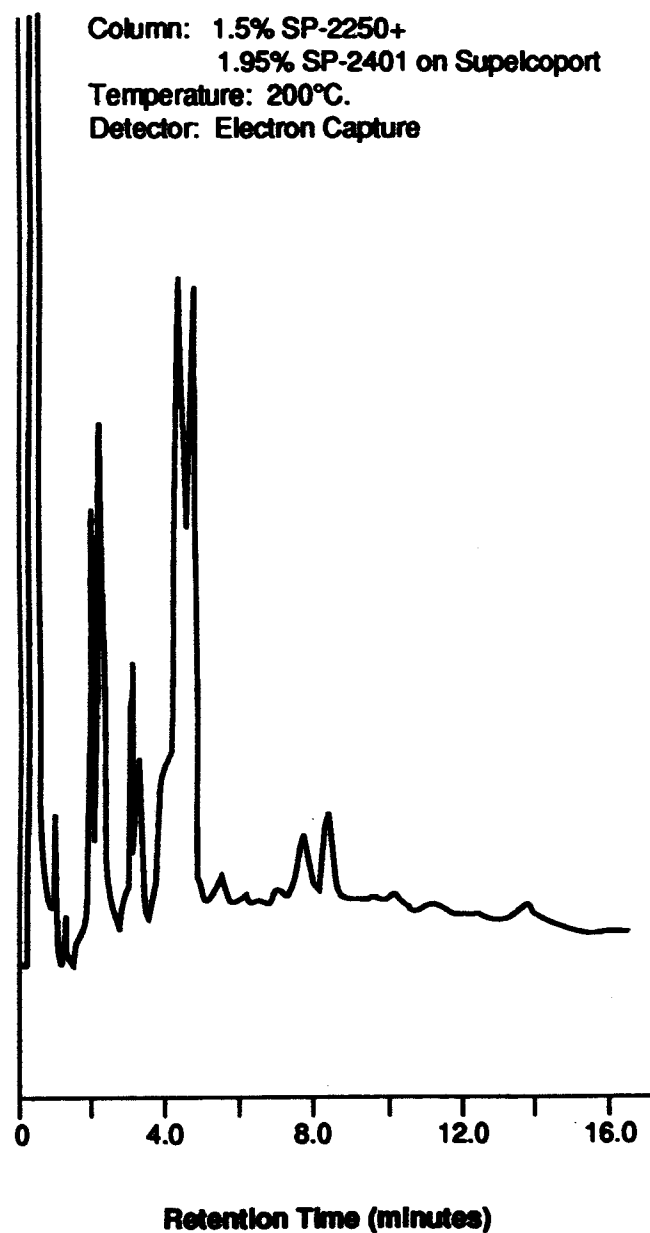
Fraction 2 - 200 mL of 15% ethyl ether in hexane

Fraction 3 - 200 mL of 50% ethyl ether in hexane



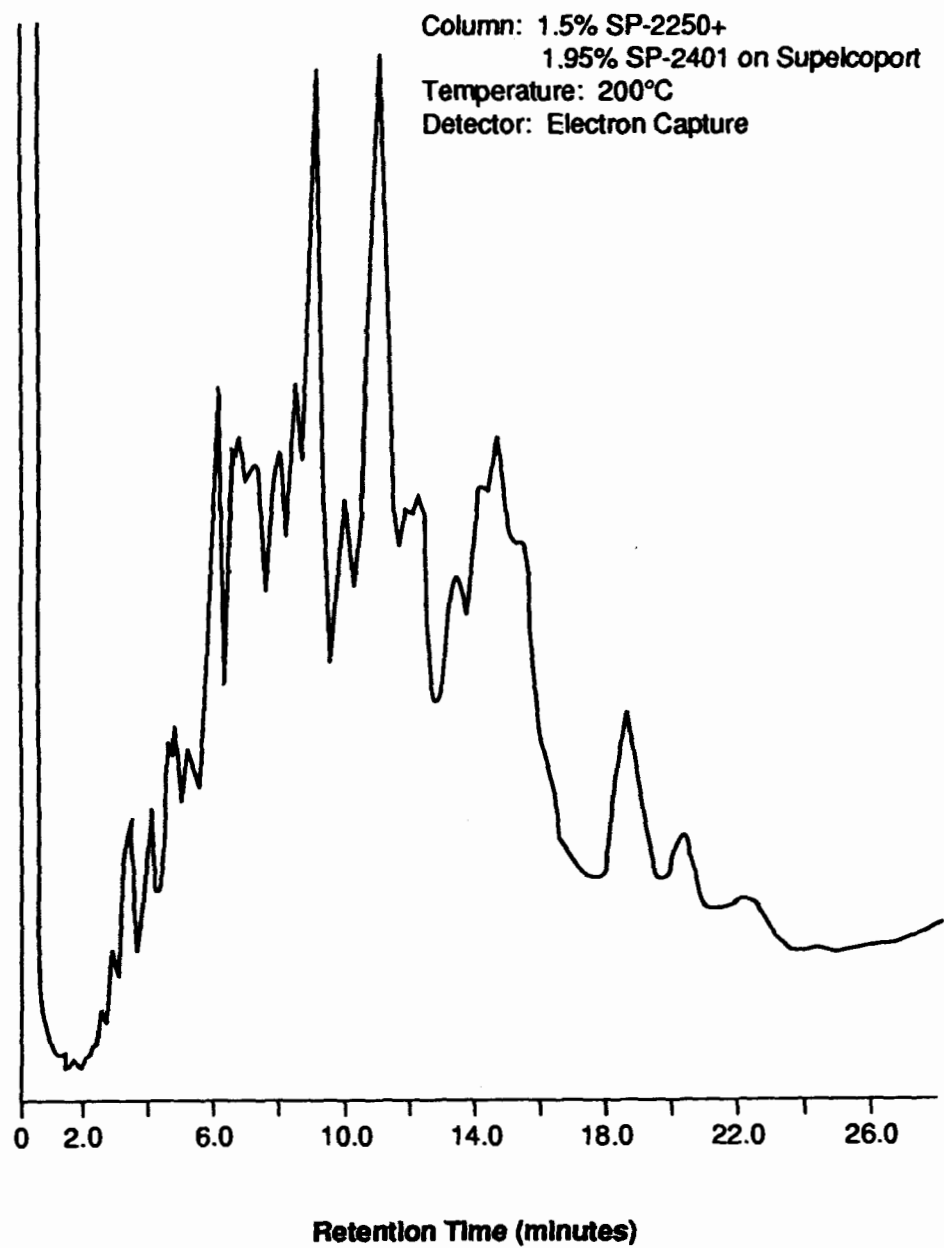
AS2-002-39

Figure 1. Gas Chromatogram of Pesticides.



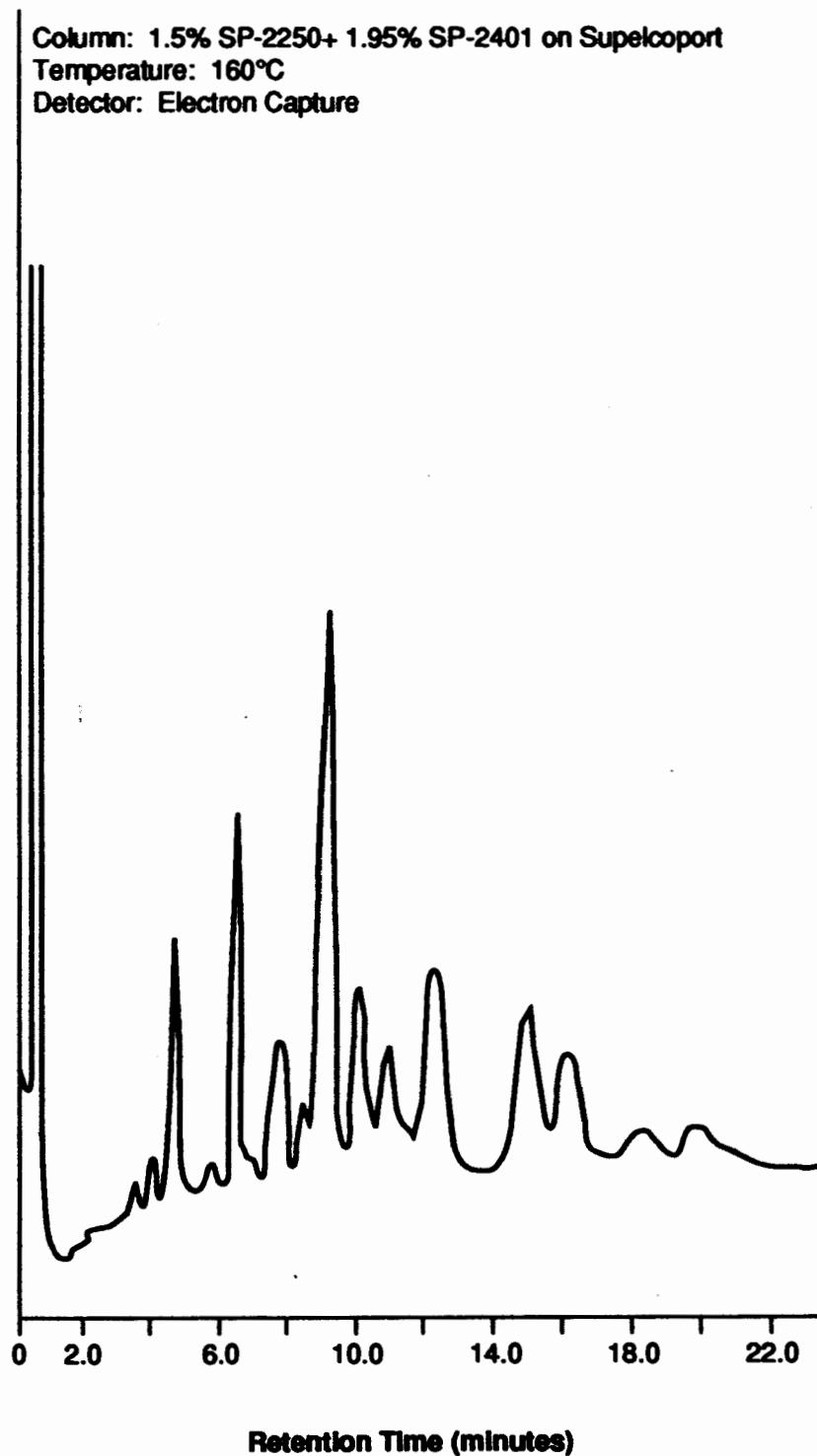
A52-002-40

Figure 2. Gas Chromatogram of Chlordane



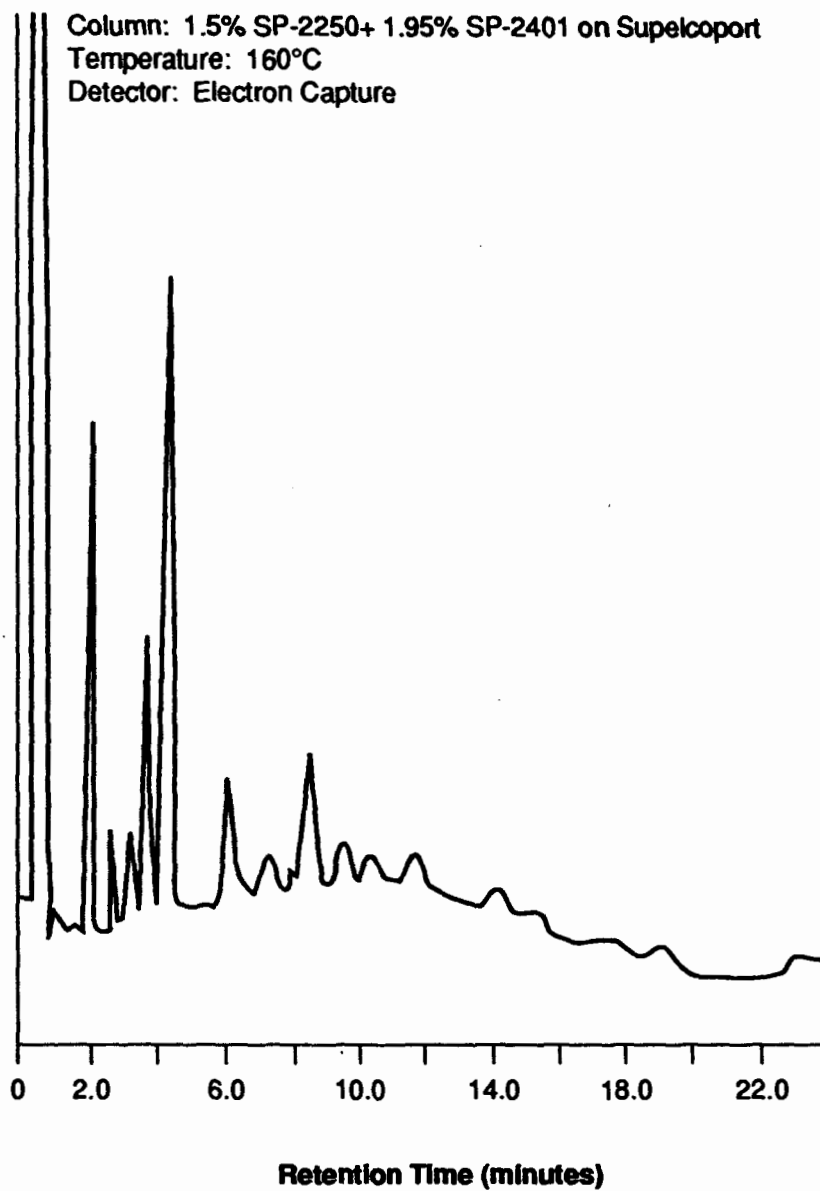
A52-002-41

Figure 3. Gas Chromatogram of Toxaphene.



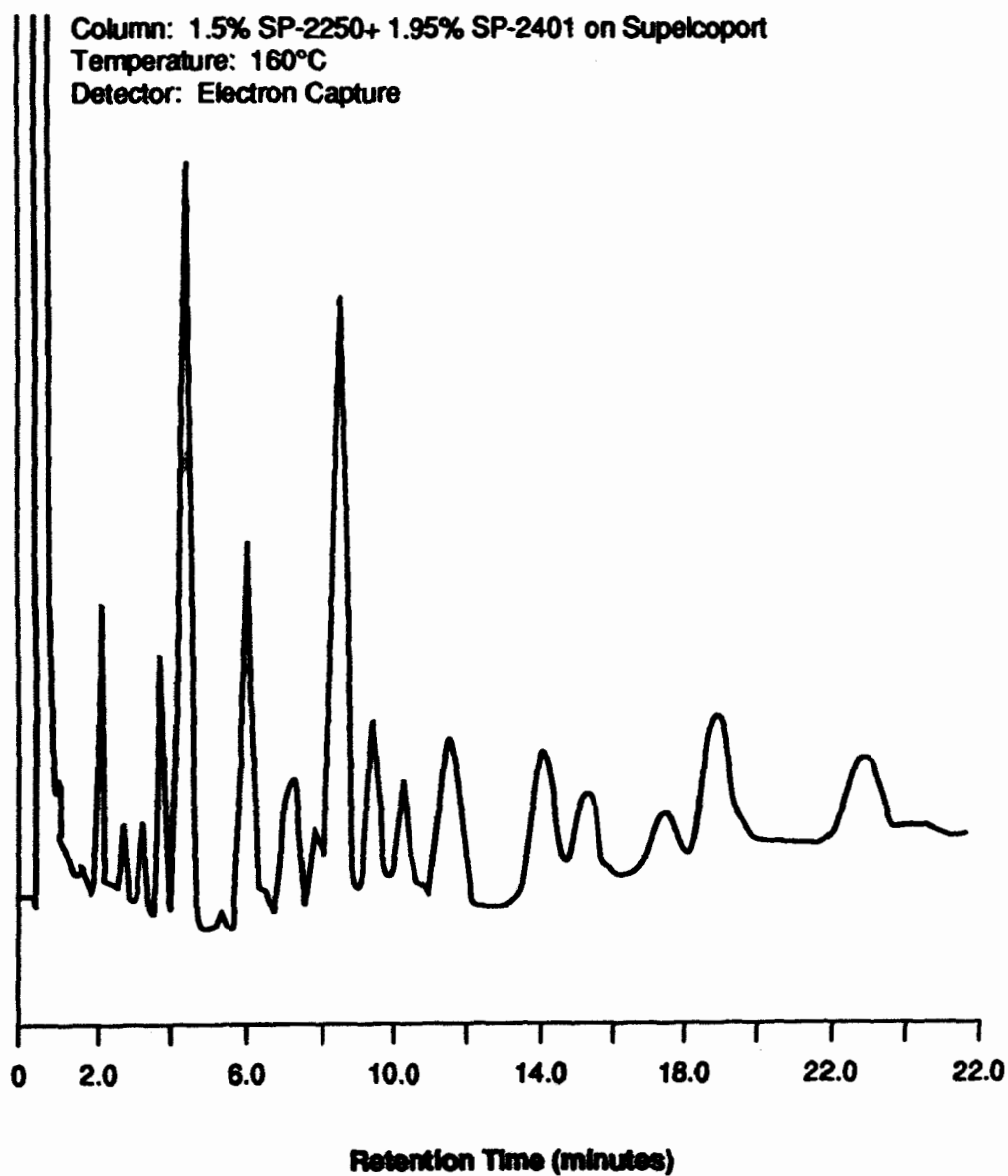
AS2-002-42

Figure 4. Gas Chromatogram of PCB-1016.



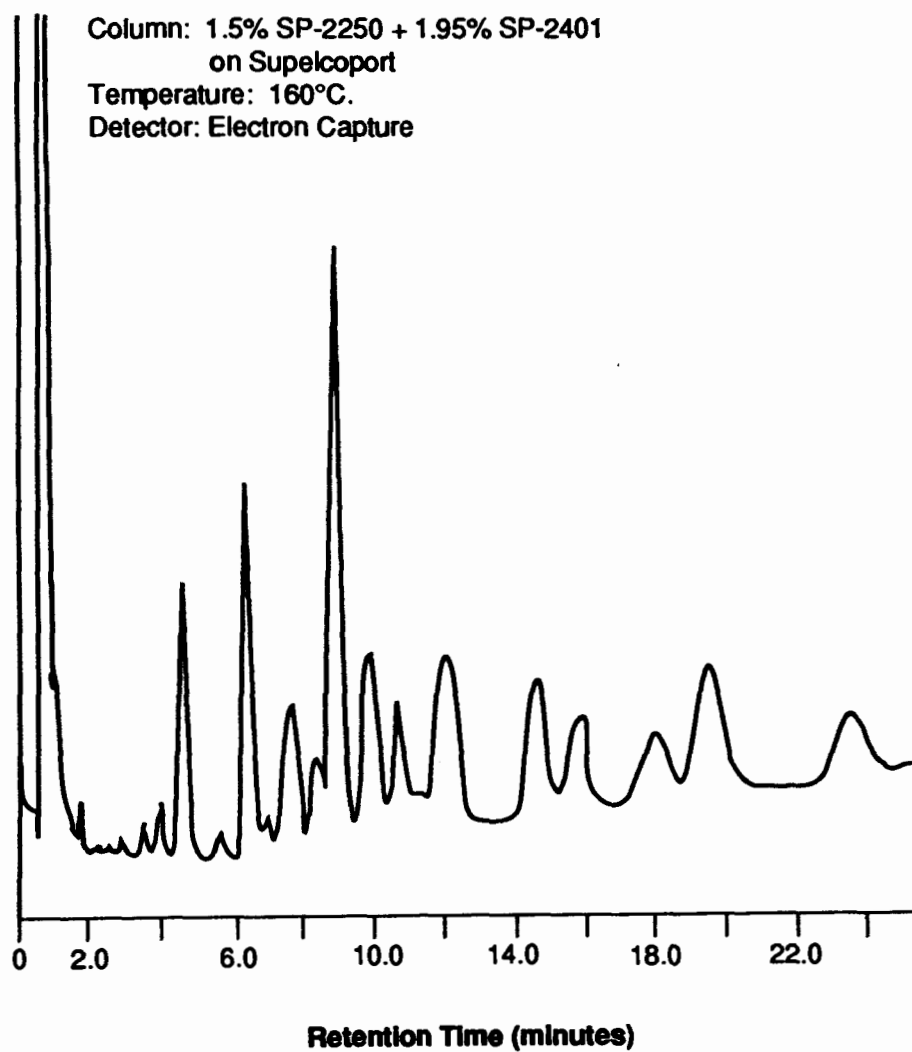
AS2-002-43

Figure 5. Gas Chromatogram of PCB-1221.



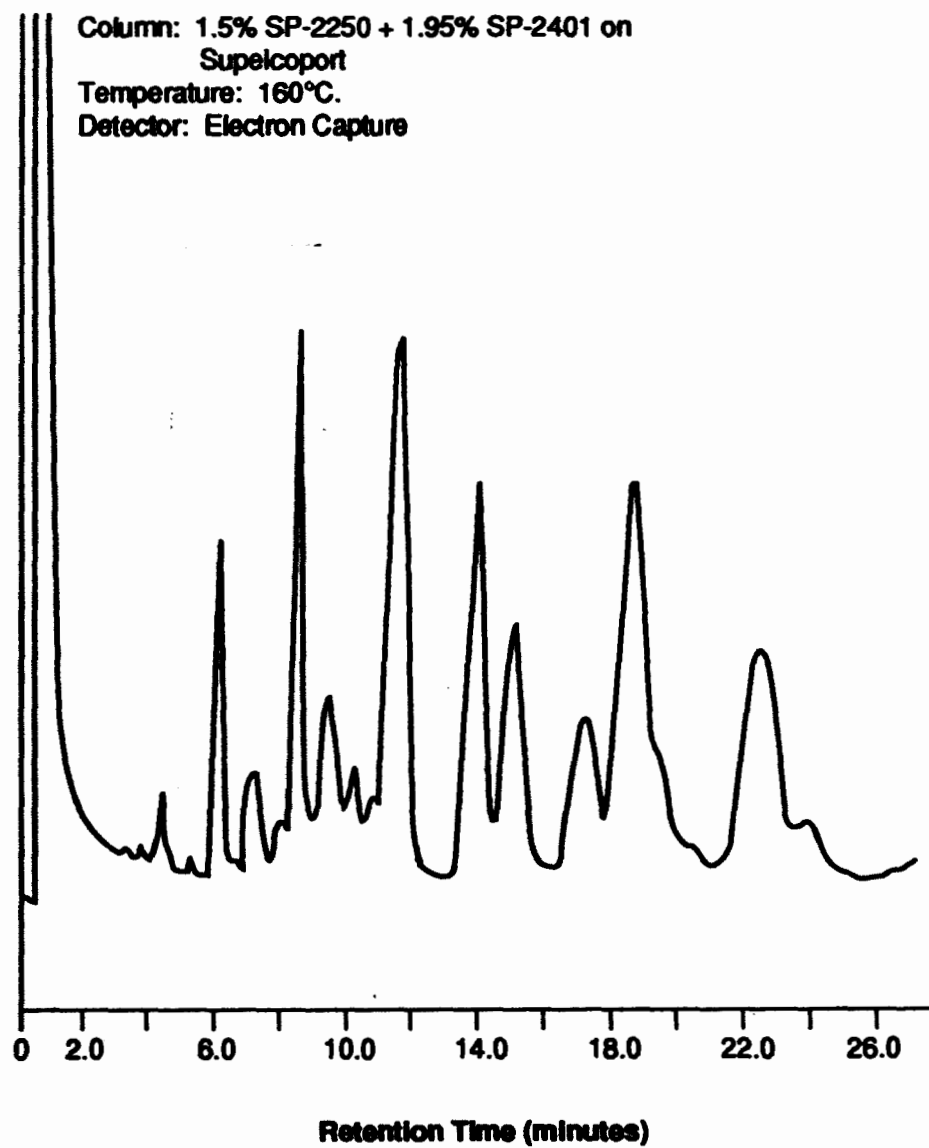
A52-002-44

Figure 6. Gas Chromatogram of PCB-1232.



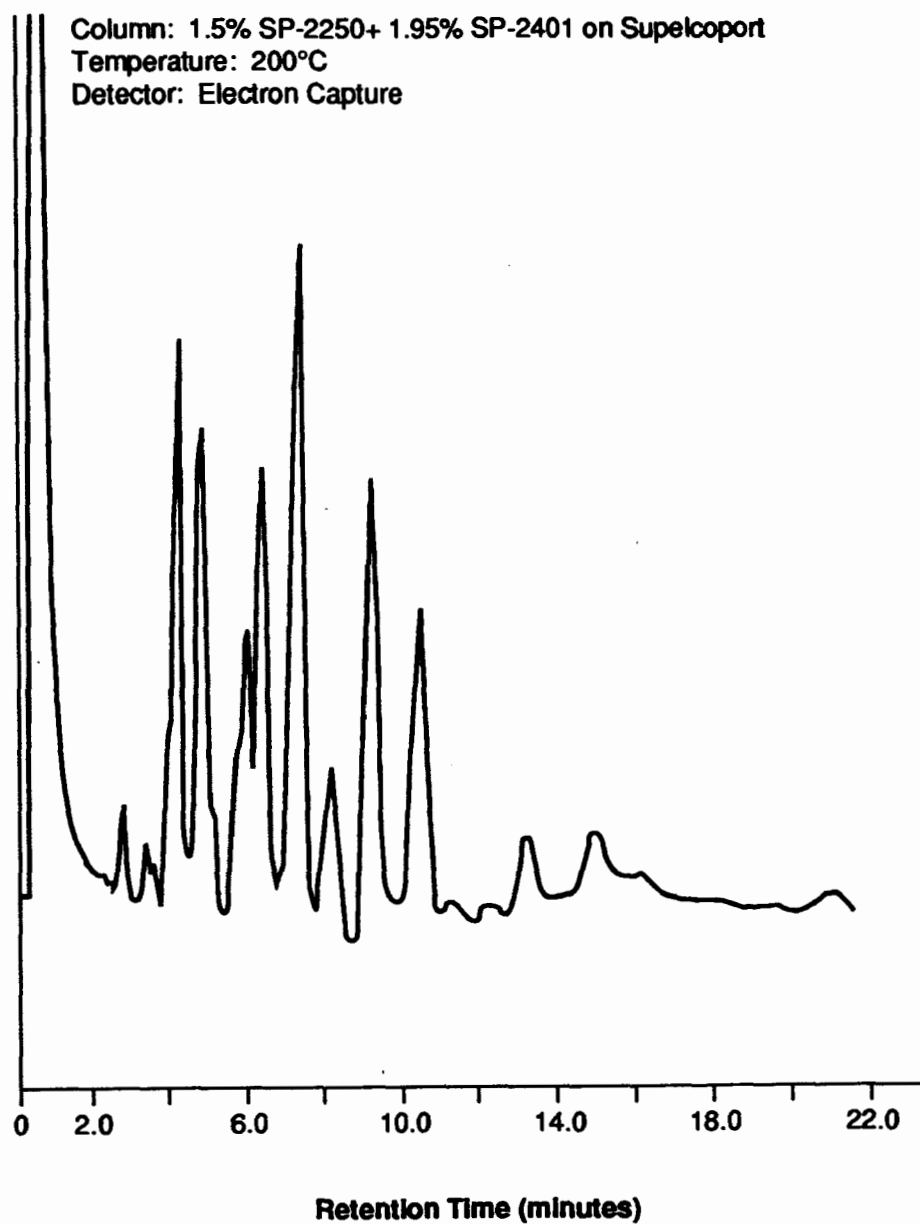
A52-002-45

Figure 7. Gas Chromatogram of PCB-1242



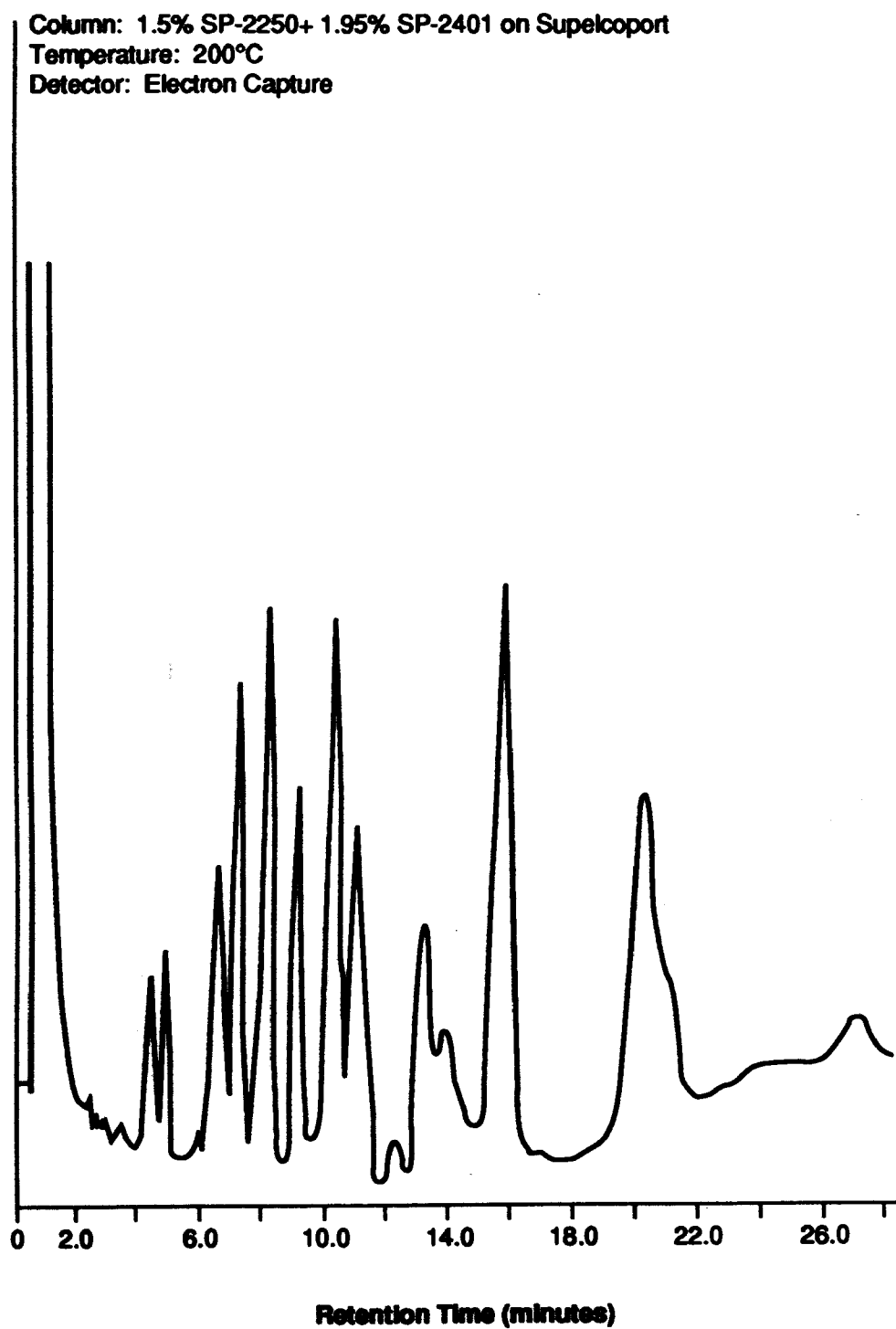
A62-002-46

Figure 8. Gas Chromatogram of PCB-1248



A52-002-47

Figure 9. Gas Chromatogram of PCB-1254.



A82-002-48

Figure 10. Gas Chromatogram of PCB-1260.

Method 618
***The Determination of Volatile
Pesticides in Municipal and
Industrial Wastewater***

Method 618

The Determination of Volatile Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of certain volatile pesticides. The following parameters can be determined by this method:

<i>Parameter</i>	<i>CAS No.</i>
Chloropicrin	76-06-2
Ethylene dibromide	106-93-4

- 1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges.
- 1.3 The method detection limit (MDL, defined in Section 15) for each compound is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.5 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. SUMMARY OF METHOD

- 2.1 A measured volume of sample, 20 mL, is extracted with cyclohexane. The cyclohexane extract is analyzed with no additional treatment. Gas chromatographic conditions are described which permit the separation of the compounds in the extract and their measurement by an electron capture detector.

3. INTERFERENCES

- 3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in gaschromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.¹ Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Thorough rinsing with acetone may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. Some samples may require a cleanup approach to achieve the MDL listed in Table 1.

4. SAFETY

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. Chloropicrin produces severe sensory irritation in upper respiratory passages. It has strong lacrimatory properties and produces increased sensitivity after frequent exposures. Taken orally, chloropicrin causes severe nausea, vomiting, colic, and diarrhea. Chloropicrin is a potent skin irritant. Ethylene dibromide liquid on the skin causes blisters if evaporation is delayed. Inhalation of ethylene dibromide causes delayed pulmonary lesions. Prolonged exposure may also result in liver and kidney injury. Exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified^{2,4} for the information of the analyst.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment, for discrete sampling.

5.1.1 Vial: 25-mL capacity or larger, equipped with a screw-cap with hole in center (Pierce No. 13075 or equivalent). Detergent wash, rinse with tap and distilled water, and dry at 105°C before use.

5.1.2 Septum: PTFE-faced silicone (Pierce No. 12722 or equivalent). Detergent wash, rinse with tap and distilled water, and dry at 105°C before use.

5.2 Glassware (all specifications are suggested).

5.2.1 Centrifuge tube: 40-mL, with screw-cap lined with PTFE.

5.2.2 Pipette: 4-mL graduated.

5.2.3 Graduated cylinder: 25-mL.

5.2.4 Volumetric flask: 10-mL, ground-glass stoppered.

- 5.3** Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.4** Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.
- 5.4.1** Column 1: 180 cm long by 2 mm ID glass, packed with 1% SP-1000 on Carbopak B (60/80 mesh) or equivalent. This column was used to develop the method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 11.1.
- 5.4.2** Column 2: 180 cm long by 2 mm ID glass, packed with 30% OV-17 on Gas Chrom Q (100/120 mesh) or equivalent.
- 5.4.3** Detector: electron capture. This detector has proven effective in the analysis of wastewaters for the compounds listed in the scope and was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. REAGENTS

- 6.1** Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each compound of interest.
- 6.2** Cyclohexane: Pesticide-quality or equivalent. Because of the frequent occurrence of contaminants in solvents, interfering with electron capture several lots of solvent, or a different solvent, e.g., hexane, heptane, or isooctane, may have to be analyzed to find a suitable extraction solvent.
- 6.3** Sodium hydroxide: 6N in distilled water.
- 6.4** Sulfuric acid: 6N in distilled water.
- 6.5** Stock standard solutions (20 mg/ml): Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions. Prepare stock solutions in cyclohexane using assayed liquids.
- 6.5.1** Place about 9.5 mL of pesticide-quality cyclohexane in a 10-mL volumetric flask. Allow the flask to stand, unstoppered, for about 5 minutes or until all cyclohexane-wetted surfaces have dried. Weigh the flask to the nearest 0.1 mg. Using a 250- μ L syringe, immediately add 121 μ L of chloropicrin ($d_4^{20} = 1.66$) and/or 92 μ L of ethylene dibromide ($d_4^{20} = 2.18$). The liquid must fall directly into the cyclohexane without contacting the neck of the flask. Reweigh, dilute to volume, stopper, and mix by inverting the flask several times. Calculate the concentration in milligrams per milliliter (mg/mL) from the net gain in weight. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 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.
- 6.5.2** Transfer the stock standard solutions into PTFE-sealed screw-cap bottles. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

- 6.5.3** Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1** Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2** External standard calibration procedure.
- 7.2.1** For each compound of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with cyclohexane. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
- 7.2.2** Using injections of 1 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each compound at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.
- 7.2.3** The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.
- 7.3** Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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 specified; however, bromoform has been shown to be satisfactory in some cases.
- 7.3.1** Prepare calibration standards at a minimum of three concentration levels for each compound 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 cyclohexane. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
- 7.3.2** Using injections of 1 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, 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} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. QUALITY CONTROL

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

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

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol such that

a 4- μ L aliquot of the check sample concentrate in 20 mL of water gives the selected concentration.

8.2.2 Using a 10- μ L syringe, add 4 μ L of the check sample concentrate to each of a minimum of four 20-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 2, determine the recovery and single-operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and compound being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁵ that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁵

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a 20-mL aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

- 8.6 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 monitor the precision of the sampling technique. 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 perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

- 9.1 Grab samples must be collected in glass containers having a total volume of at least 25 mL. Fill the sample bottle just to overflowing in such a manner that no air bubbles pass through the sample as the bottle is being filled. Seal the bottle so that no air bubbles are entrapped in it. Store the sample in an inverted position and maintain the hermetic seal on the sample bottle until the time of analysis.
- 9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

10. SAMPLE EXTRACTION

- 10.1 Measure 20 mL of sample by pouring the sample into a 40-mL centrifuge tube equipped with a PTFE-lined screw-cap to a predetermined 20-mL mark. Adjust pH of sample to 6 to 8 by addition of 6N sodium hydroxide or 6N sulfuric acid. Measure 4.0 mL of extraction solvent with a 4-mL graduated pipette and add to the centrifuge tube.
- 10.2 Shake the tube vigorously for 1 minute. Allow the layers to separate for at least 10 minutes. Centrifuge, if necessary, to facilitate phase separation.
- 10.3 Withdraw an aliquot of the solvent layer and proceed with gas chromatographic analysis.

11. CLEANUP AND SEPARATION

- 11.1 Cleanup procedures are not generally necessary. If particular circumstances demand the use of a cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.

12. GAS CHROMATOGRAPHY

- 12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Examples of the separations achieved by Columns 1 and 2 are shown in Figures 1 and 2, respectively. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.
- 12.2 Calibrate the system daily as described in Section 7.
- 12.3 If the internal standard approach is being used, the analyst must not add the internal standard to sample extracts until immediately before injection into the instrument. Mix thoroughly.

- 12.4** Inject 1 to 5 μL of the sample extract using the solvent-flush technique.⁶ Record the volume injected to the nearest 0.05 μL , and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.
- 12.5** The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6** If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7** If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

- 13.1** Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_e)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_e = Volume of water extracted, in mL

13.1.2 If the internal standard calibration procedure is used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_i)}{(A_e)(\text{RF})(V_e)}$$

where

A_s = Response for parameter to be measured

A_e = Response for the internal standard

I_i = Amount of internal standard added to each extract, in μg

V_e = Volume of water extracted, in L

- 13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

14. GC/MS CONFIRMATION

- 14.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 seconds per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.
- 14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.⁹
- 14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.⁷
- 14.4 To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
- 14.4.1 All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to $\pm 10\%$. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20 to 40%.
- 14.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.
- 14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.
- 14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.
- 14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. METHOD PERFORMANCE

- 15.1** 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.¹⁰ The MDL concentrations listed in Table 1 were obtained using reagent water.⁸ Similar results were achieved using representative wastewaters.
- 15.2** This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from $10 \times \text{MDL}$ to $1000 \times \text{MDL}$.
- 15.3** In a single laboratory, Battelle Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 2 were obtained. Seven replicates each of two different wastewaters were spiked and analyzed. The relative standard deviations of the percent recovery of these measurements are also included in Table 2.

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6. Burke, J.A., "Gas Chromatography for Pesticide Residue Analysis; Some Practical Aspects," *Journal of the Association of Official Analytical Chemists*, 48 1037 (1965).
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8. "Development of Methods for Pesticides in Wastewaters," Report from Battelle's Columbus Laboratories for EPA Contract 68-03-2956 (in preparation).
9. McNair, H.M., and Bonelli, E.J., *Basic Chromatography*, Consolidated Printing, Berkeley, California, 52 (1969).
10. Glaser, J.A. et al., "Trace Analysis for Wastewaters," *Environmental Science and Technology*, 15, 1426 (1981).

Table 1. Chromatographic Conditions and Estimated Method Detection Limits

<i>Parameter</i>	<i>Retention Time (Minutes)</i>		<i>Method Detection Limits (μ/L)</i>
	<i>Column 1</i>	<i>Column 2</i>	
Chloropicrin	5.60	2.03	0.8
Ethylene Dibromide	9.90	3.15	0.2

Column 1 Conditions: Carbopak B (60/80 mesh) coated with 1% SP-1000 packed in a 1.8 m long by 2 mm ID glass column with nitrogen carrier gas at a flow rate of 30 mL/minutes. Column temperature, isothermal at 135°C. An electron capture detector was used with this column to determine the MDL.

Column 2 Conditions: Gas Chrom Q (100/120 mesh) coated with 30% OV-17 packed in a 1.8 m long by 2 mm ID glass column with helium carrier gas at a flow rate of 25 mL/minutes. Column temperature, isothermal at 95°C.

Table 2. Single-Laboratory Accuracy and Precision (a)

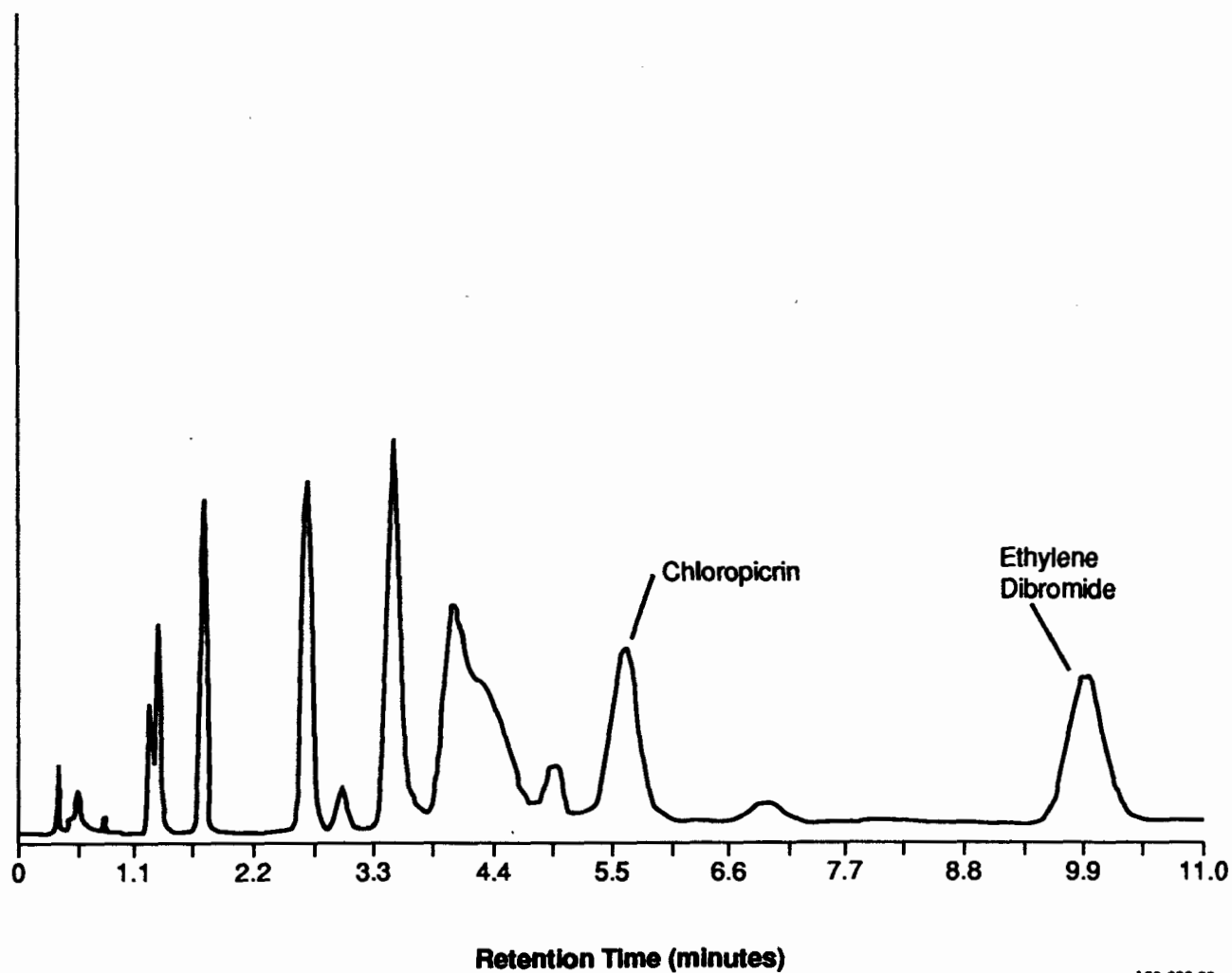
<i>Parameter</i>	<i>Sample Type(b)</i>	<i>Background μg/L(c)</i>	<i>Spike Level μg/L</i>	<i>Mean Recovery (%)</i>	<i>Standard Deviation (%)</i>	<i>No. of Replicates</i>
Chloropicrin	1	ND	5	98	12	7
	2	ND	50	98	3.3	7
Ethylene Dibromide	1	ND	5	69	6.9	7
	2	ND	50	108	4.8	7

(a) Column 1 conditions were used.

(b) 1 = Low background relevant industrial effluent

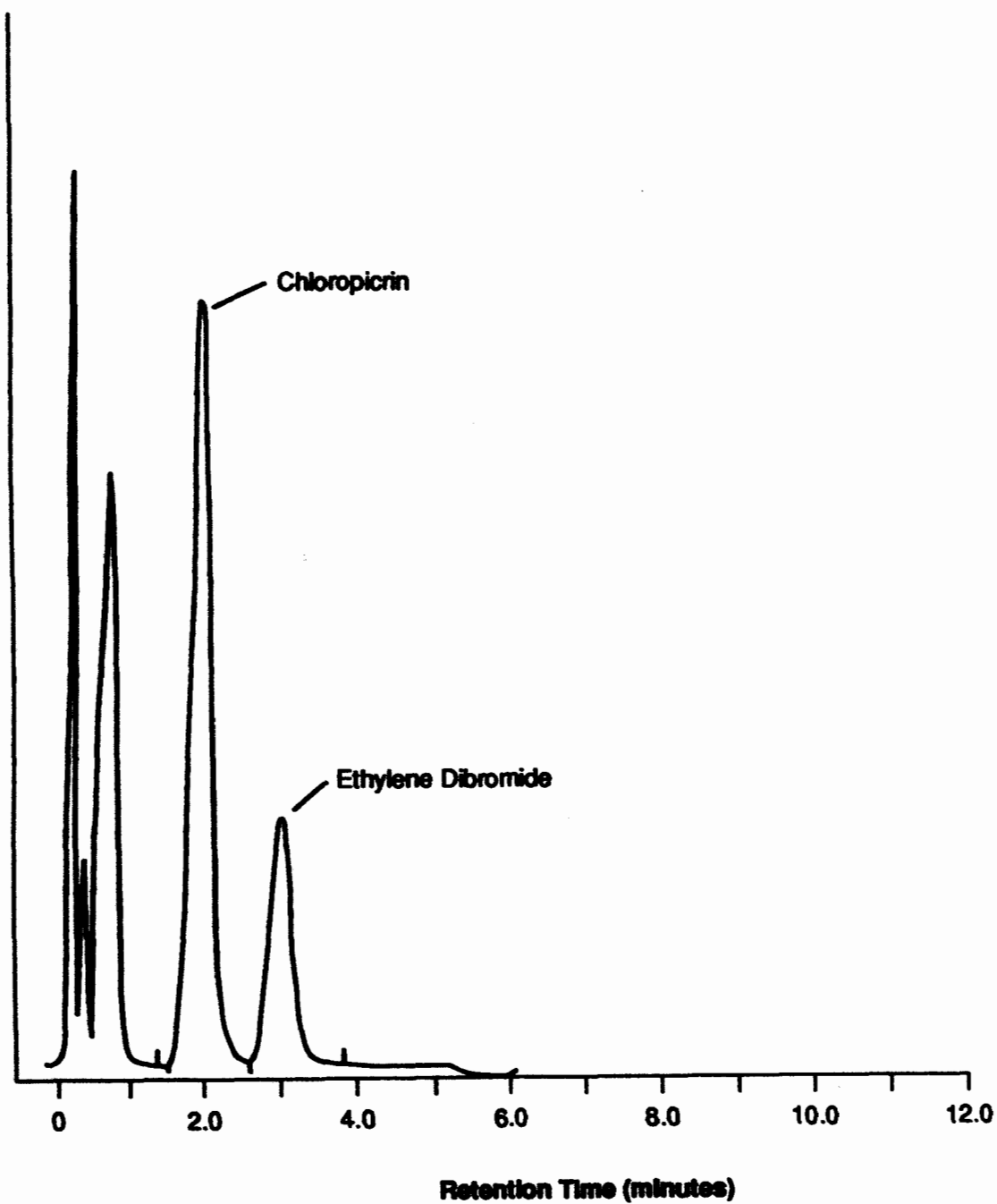
2 = High background relevant industrial effluent

(c) ND = Not detected



A52-002-20

Figure 1. GC-ECD Chromatogram of 200 ng Chloropicrin and Ethylene Dibromide in Cyclohexane (Column 1).



A62-002-21

Figure 2. GC-ECD Chromatogram of 400 ng Chloropicrin and Ethylene Dibromide in Cyclohexane (Column 2).

Method 619
The Determination of
Triazine Pesticides in
Municipal and Industrial
Wastewater

Method 619

The Determination of Triazine Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of certain triazine pesticides. The following parameters can be determined by this method:

<i>Parameter</i>	<i>STORET No.</i>	<i>CAS No.</i>
Ametryn	—	834-12-8
Atraton	—	1610-17-9
Atrazine	39033	1912-24-9
Prometon	39056	1610-18-0
Prometryn	39057	7287-19-6
Propazine	39024	139-40-2
Secbumeton	—	26259-45-0
Simetryn	39054	1014-70-6
Simazine	39055	122-34-9
Terbuthylazine	—	5915-41-3
Terbutryn	—	86-50-0

- 1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3 The estimated method detection limit (MDL, defined in Section 15) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4 The sample extraction and concentration steps in this method are essentially the same as several others in 600-series methods. Thus, a single sample may be extracted to measure the parameters included in the scope of each of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures. Under gas chromatography, the analyst is allowed the latitude to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters (see Section 12).
- 1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

- 1.6** When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. SUMMARY OF METHOD

- 2.1** A measured volume of sample, approximately 1 L, is extracted with 15% methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to hexane during concentration to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by gas chromatography with a thermionic bead detector in the nitrogen mode.^{1,2}
- 2.2** Method 619 represents an editorial revision of a previously promulgated U.S. EPA method for organophosphorus pesticides.³ While complete method validation data is not presented herein, the method has been in widespread use since its promulgation, and represents the state of the art for the analysis of such materials.
- 2.3** This method provides an optional Florisil column cleanup procedure to aid in the elimination or reduction of interferences which may be encountered.

3. INTERFERENCES

- 3.1** Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.
- 3.1.1** Glassware must be scrupulously cleaned.⁴ Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.
- 3.1.2** The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.2** Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

- 4.1** The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified⁵⁻⁷ for the information of the analyst.

5. APPARATUS AND MATERIALS

- 5.1** Sampling equipment, for discrete or composite sampling.
- 5.1.1** Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.
- 5.1.2** Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.
- 5.2** Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)
- 5.2.1** Separatory funnel: 2000-mL, with TFE-fluorocarbon stopcock, ground-glass or TFE stopper.
- 5.2.2** Drying column: Chromatographic column 400 mm long by 19 mm ID with coarse-fritted disc.
- 5.2.3** Chromatographic column: 400 mm long by 19 mm ID with coarse-fritted disc at bottom and TFE-fluorocarbon stopcock (Kontes K-420540-0224 or equivalent).
- 5.2.4** Concentrator tube, Kuderna-Danish: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground-glass stopper is used to prevent evaporation of extracts.
- 5.2.5** Evaporative flask, Kuderna-Danish: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.
- 5.2.6** Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).
- 5.2.7** Vials: Amber glass, 10- to 15-mL capacity with TFE-fluorocarbon-lined screw-cap.
- 5.3** Boiling chips: Approximately 10/40 mesh. Heat at 400°C for 30 minutes or perform a Soxhlet extraction with methylene chloride.

- 5.4 Water bath: Heated, with concentric ring cover, capable of temperature control ($\pm 2^{\circ}\text{C}$). The bath should be used in a hood.
- 5.5 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6 Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.
 - 5.6.1 Column 1: 180 cm long by 2 mm ID glass, packed with 5% Carbowax 20M-TPA on Supelcoport (80/100 mesh) or equivalent. This column was used to develop the method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 12.1.
 - 5.6.2 Column 2: 180 cm long by 4 mm ID glass, packed with 1.0% Carbowax 20M on Gas Chrom Q (100/120 mesh) or equivalent.
 - 5.6.3 Detector: Thermionic bead in the nitrogen mode. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. REAGENTS

- 6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2 Acetone, hexane, methylene chloride, methanol: Pesticide-quality or equivalent.
- 6.3 Ethyl ether: Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Cat. No. Pl126-8, and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.
- 6.4 Sodium sulfate: ACS, granular, anhydrous. Condition by heating in a shallow tray at 400°C for a minimum of 4 hours to remove phthalates and other interfering organic substances. Alternatively, heat 16 hours at 450 to 500°C in a shallow tray or perform a Soxhlet extraction with methylene chloride for 48 hours.
- 6.5 Florisil: PR grade (60/100 mesh). Purchase activated at 675°C and store in the dark in glass container with ground-glass stopper or foil-lined screw-cap. Before use, activate each batch at least 16 hours at 130°C in a foil-covered glass container.
- 6.6 Stock standard solutions ($1.00\ \mu\text{g}/\mu\text{L}$): Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.
 - 6.6.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in pesticide-quality hexane or other suitable solvent and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

- 6.6.2 Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw-cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
- 6.6.3 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2 External standard calibration procedure:
 - 7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with hexane or other suitable solvent. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
 - 7.2.2 Using injections of 1 to 5 μ L of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.
 - 7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.
- 7.3 Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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.
 - 7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter 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 hexane or other suitable solvent. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of

concentrations expected in the sample concentrates, or should define the working range of the detector.

- 7.3.2** Using injections of 1 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} against RF.

- 7.3.3** The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.
- 7.4** The cleanup procedure in Section 11 utilizes Florisil chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil which is used, the use of the lauric acid value is suggested. This procedure⁸ determines the adsorption from hexane solution of lauric acid, in milligrams, per gram of Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.
- 7.5** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.
- 8.1.1** Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

- 8.1.2** In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
- 8.1.3** The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2** To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
- 8.2.1** Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone, 1000 times more concentrated than the selected concentrations.
- 8.2.2** Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
- 8.2.3** Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.
- 8.2.4** Using the data from Table 2, estimate the recovery and single-operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.
- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
- 8.3.1** Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁹ that are useful in observing trends in performance.

- 8.3.2** The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁹
- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and

analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

- 8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.
- 8.6 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 monitor the precision of the sampling technique. 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 perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

- 9.1 Grab samples must be collected in glass containers. Conventional sampling practices¹⁰ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

10. SAMPLE EXTRACTION

- 10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel.
- 10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.
- 10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

- 10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.
- 10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.7 Increase the temperature of the hot water bath to about 80°C. Momentarily remove the Snyder column, add 50 mL of hexane and a new boiling chip, and reattach the Snyder column. Pour about 1 mL of hexane into the top of the Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 minutes. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.8 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation.

NOTE: Precipitation of triazines in the hexane may occur if the concentration in the original sample exceeded 500 µg/L. If this occurs, redissolve the triazines in methylene chloride and analyze the extract using flame ionization gas chromatography. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to TFE-fluorocarbon-sealed screw-cap vials. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis. If the sample requires cleanup, proceed to Section 11.

- 10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various industrial and municipal effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

- 11.2** The following Florisil column cleanup procedure has been demonstrated to be applicable to the nine triazine pesticides listed in Table 3.
- 11.2.1** Add a weight of Florisil (nominally 20 g) predetermined by calibration (Section 7.4 and 7.5) to a chromatographic column. Settle the Florisil by tapping the column. Add anhydrous sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatography column. Discard the eluate.
- 11.2.2** Adjust the sample extract volume to 10 mL with hexane and transfer it from the K-D concentrator tube to the Florisil column. Rinse the tube twice with 1 to 2 mL hexane, adding each rinse to the column.
- 11.2.3** Drain the column until the sodium sulfate layer is nearly exposed. Elute the column with 200 mL of 6% ethyl ether in hexane (V/V) (Fraction 1) using a drip rate of about 5 mL/min. This fraction may be discarded. Place a 500-mL K-D flask and clean concentrator tube under the chromatography column. Elute the column into the flask, using 200 mL of 15% ethyl ether in hexane (V/V) (Fraction 2). Perform a third elution using 200 mL of 50% ethyl ether in hexane (V/V) (Fraction 3), and a final elution with 200 mL of 100% ethyl ether (Fraction 4), into separate K-D flasks. The elution patterns for nine of the pesticides are shown in Table 3.
- 11.2.4** Concentrate the eluates by standard K-D techniques (Section 10.6), substituting hexane for the glassware rinses and using the water bath at about 85°C. Adjust final volume to 10 mL with hexane. Analyze by gas chromatography.

12. GAS CHROMATOGRAPHY

- 12.1** Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. An example of the separation achieved by Column 1 is shown in Figure 1. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.
- 12.2** Calibrate the system daily as described in Section 7.
- 12.3** If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.
- 12.4** Inject 1 to 5 μL of the sample extract using the solvent-flush technique.¹¹ Record the volume injected to the nearest 0.05 μL , and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.
- 12.5** The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

- 12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

- 13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_i)(V_s)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_s = Volume of water extracted, in mL

- 13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where

A_s = Response for parameter to be measured

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_o = Volume of water extracted, in L

- 13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. GC/MS CONFIRMATION

- 14.1** It is recommended that GC/MS techniques be judiciously employed to support qualitative compound identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 seconds per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.
- 14.2** Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.¹²
- 14.3** At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.¹³
- 14.4** To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
- 14.4.1** All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to $\pm 10\%$. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20% to 40%.
- 14.4.2** The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.
- 14.4.3** Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.
- 14.5** Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.
- 14.6** Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. METHOD PERFORMANCE

- 15.1** 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.¹⁴ The MDL concentrations listed in Table 1 were estimated from the response of the thermionic bead nitrogen detector to each compound. The estimate is based upon the amount of material required to yield

a signal 5 times the GC background noise, assuming a 5- μ L injection from a 10-mL final extract of a 1-L sample.

- 15.2** In a single laboratory (either West Cost Technical Services, Inc., or Midwest Research Institute), using effluents from pesticide manufacturers and publicly owned treatment works (POTW), the average recoveries presented in Table 2 were obtained after Florisil cleanup.^{1,2} The standard deviations of the percent recoveries of these measurements are also included in Table 2.

References

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Table 1. Chromatographic Conditions and Method Detection Limits

<i>Parameter</i>	<i>Retention Time (Min.)</i>		<i>Method Detection Limit (µg/L)</i>
	<i>Column 1</i>	<i>Column 2</i>	
Prometon	6.9	4.9	0.03
Atraton	—	6.3	ND
Propazine	9.2	6.7	0.03
Terbuthylazine	10.2	7.3	0.03
Sebumeton	—	8.3	ND
Atrazine	12.4	9.4	0.05
Prometryn	13.8	10.3	0.06
Terbutryn	15.4	—	0.05
Simazine	16.3	12.7	0.06
Ametryn	17.7	14.0	0.06
Simetryn	23.0	—	0.07

ND = Not determined

Column 1 conditions: Supelcoport (80/100 mesh) coated with 5% Carbowax 20M-TPA packed in a 1.8 m long by 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. Column temperature, isothermal at 200°C. A thermionic bead detector was used with this column to determine the MDL.

Column 2 conditions: Gas Chrom Q (100/120 mesh) coated with 1.0% Carbowax 20 M packed in a 1.8 m long by 4 mm ID glass column with helium carrier gas at 80 mL/min flow rate. Column temperature, isothermal at 155°C.

Table 2. Single-Laboratory Accuracy and Precision

<i>Parameter</i>	<i>Sample Type*</i>	<i>Spike (µg/L)</i>	<i>Number of Replicates</i>	<i>Mean Recovery (%)</i>	<i>Standard Deviation (%)</i>
Ametryn	3	4,000	2	104	—
	3	2,000	2	118	—
Atrazine	3	300	2	108	—
	1	1,000	7	177	15.2
Prometon	1	130	7	67	3.9
	2	260	7	51	3.0
Prometryn	3	2,000	2	76	—
	3	50	2	110	—
Propazine	1	516	7	54	6.5
	3	15		116	—
Simatryn	3	30	2	183	—
	3	15	2	182	—
Simazine	1	115	7	152	24.3
	3	10	2	99	—
Terbuthylazine	3	100	2	114	—
	3	15	2	100	—
Terbutryn	1	968	7	83	10.0
	2	169	7	89	24.0

* Sample Type

1 - Industrial process water

2 - Industrial effluent

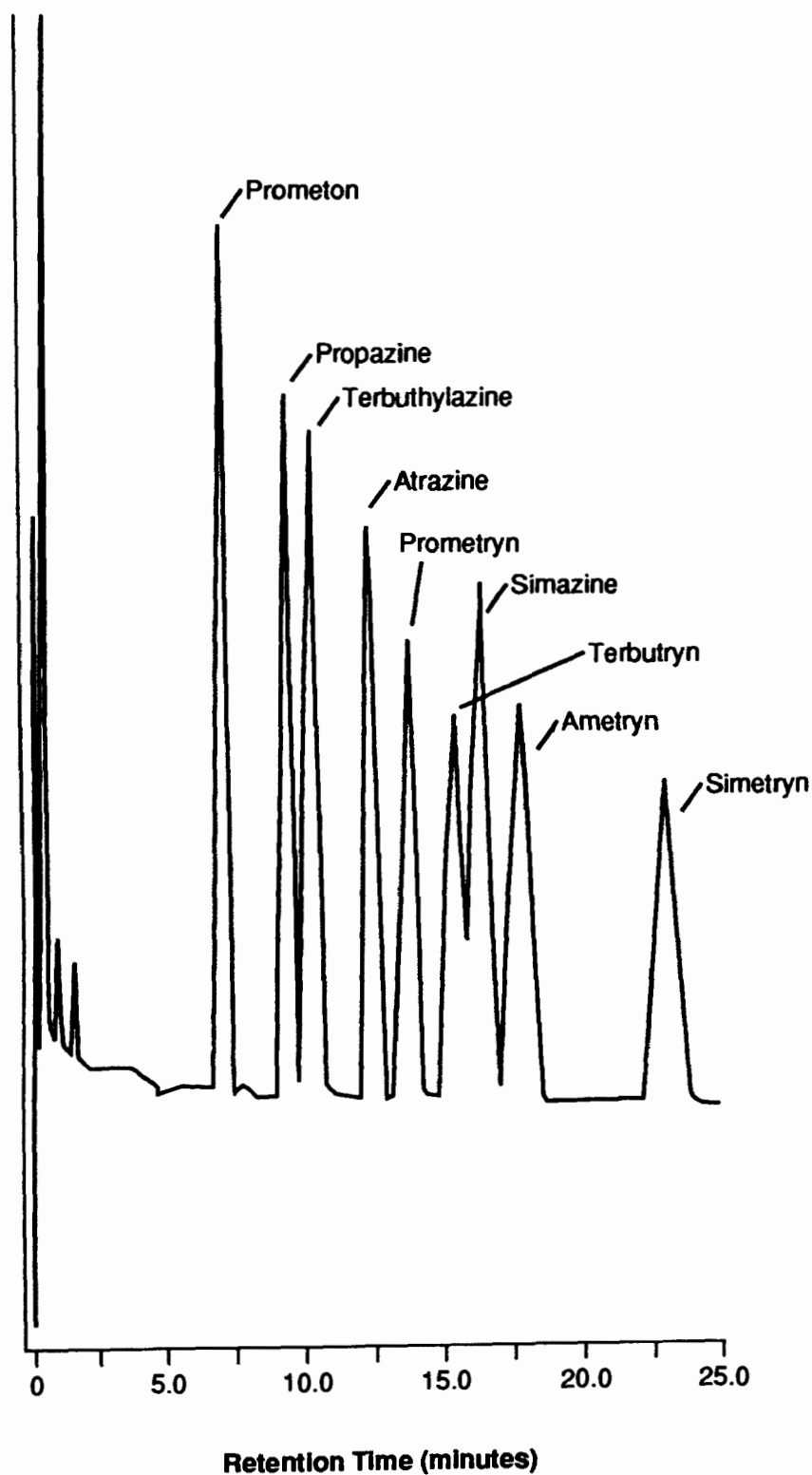
3 - 80% Industrial process water/20% industrial effluent

Table 3. Florisil Fractionation Patterns

<i>Parameter</i>	<i>Percent Recovery by Fraction</i>			
	<i>No. 1</i>	<i>No. 2</i>	<i>No. 3</i>	<i>No. 4</i>
Propazine	0	90	10	
Terbuthylazine	0	30	70	
Atrazine	0	20	80	
Ametryn			100	
Prometryn			100	
Simazine			100	
Atraton			100	
Secbumeton				100
Prometon				100

Florisil eluate composition by fraction

Fraction 1 - 200 mL of 6% ethyl ether in hexane
 Fraction 2 - 200 mL of 15% ethyl ether in hexane
 Fraction 3 - 200 mL of 50% ethyl ether in hexane
 Fraction 4 - 200 mL of ethyl ether



A52-002-49

Figure 1. Gas Chromatogram of Triazine Pesticides on Column 1.
For Conditions, See Table 1.

Method 620
The Determination of
Diphenylamine in Municipal
and Industrial Wastewater

Method 620

The Determination of Diphenylamine in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of diphenylamine, CAS. No. 122-39-4.
- 1.2 This is a gas chromatographic (GC) method applicable to the determination of diphenylamine in municipal and industrial discharges.
- 1.3 The method detection limit (MDL, defined in Section 15) for diphenylamine is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4 The sample extraction and concentration steps in this method are similar to those of other 600-series methods. Thus, a single sample may be extracted to measure the compounds included in the scope of the methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures.
- 1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. SUMMARY OF METHOD

- 2.1 A measured volume of sample, approximately 1 L, is extracted with methylene chloride using a continuous extractor. The methylene chloride extract is dried and concentrated to 5.0 mL. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by alkali flame detector (AFD) gas chromatography.¹
- 2.2 This method provides an optional silica gel column cleanup procedure to aid in the elimination of interferences which may be encountered.

3. INTERFERENCES

- 3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from

interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified for the information of the analyst.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with PTFE/PIFE. Aluminum foil may be substituted for PTFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

- 5.2** Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)
- 5.2.1** Continuous extractor: 2000-mL, available from Paxton Woods Glass Shop, Cincinnati, Ohio, or equivalent.
 - 5.2.2** Drying column: Chromatographic column 400 mm long by 10 mm ID.
 - 5.2.3** Chromatographic column: 400 mm long by 19 mm ID with 250-mL reservoir at the top and PTFE stopcock (Kontes K-420290 or equivalent).
 - 5.2.4** Concentrator tube, Kuderna-Danish: 25-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. A ground-glass stopper is used to prevent evaporation of extracts.
 - 5.2.5** Evaporative flask, Kuderna-Danish: 500-mL (Kontes K570001-0500 or equivalent). Attach to concentrator tube with springs.
 - 5.2.6** Snyder column, Kuderna-Danish: Three-ball macro (Kontes K503000-0121 or equivalent).
 - 5.2.7** Snyder column, Kuderna-Danish: Two-ball micro (Kontes K569001-0219 or equivalent).
 - 5.2.8** Vials: Amber glass, 10- to 15-mL capacity with PTFE-lined screw-cap.
 - 5.2.9** Volumetric flask: 5-mL with glass stopper.
- 5.3** Boiling chips: Approximately 10/40 mesh carborundum. Heat to 400°C for 4 hours or extract in a Soxhlet extractor with methylene chloride.
- 5.4** Water bath: Heated, capable of temperature control $\pm 2^{\circ}\text{C}$. The bath should be used in a hood.
- 5.5** Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6** Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.
- 5.6.1** Column 1: 180 cm long by 2 mm ID glass, packed with 3% SP2250 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 15. Guidelines for the use of alternative columns are provided in Section 12.1.
 - 5.6.2** Column 2: 180 cm long by 2 mm ID glass, packed with 3% SP 1000 on Supelcoport (100/120 mesh) or equivalent.
 - 5.6.3** Detector: Alkali-flame detector (AFD), sometimes referred to as a nitrogen-phosphorous detector (NPD) or a thermionic-specific detector (TSD). This detector has proven effective in the analysis of wastewaters for the compounds listed in the scope and was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. REAGENTS

- 6.1** Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2** Methylene chloride, acetone, methanol, petroleum ether, ethyl ether, toluene (distilled-in-glass quality or equivalent). Ethyl ether must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Catalog No. P1126-8, and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips.
- 6.3** Sodium sulfate: ACS, granular, anhydrous; heated in a muffle furnace at 400°C overnight.
- 6.4** Silica gel: Davison Grade 923, 100-200 mesh; activated by heating for 24 hours at 150°C.
- 6.5** 6N sulfuric acid: Slowly add 16.7 mL of concentrated H₂SO₄ (94%) to about 50 mL of reagent water. Dilute to 100 mL with reagent water.
- 6.6** 6N sodium hydroxide: Dissolve 24.0 grams of sodium hydroxide in 100 mL of reagent water.
- 6.7** Stock standard solutions (1.00 µg/µL): Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.
- 6.7.1** Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in distilled-in-glass quality methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 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.
- 6.7.2** Transfer the stock standard solutions into PTFE-sealed screw-cap bottles. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
- 6.7.3** Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1** Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2** External standard calibration procedure.
- 7.2.1** For each compound of interest, prepare calibration standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with toluene. 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.
- 7.2.2** Using injections of 2 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration

curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each compound at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

- 7.2.3** The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.
- 7.3** Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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, although carbazole has been used successfully in some instances.
- 7.3.1** Prepare calibration standards at a minimum of three concentration levels for each parameter 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 toluene. 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.
- 7.3.2** Using injections of 2 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, 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} against RF.

- 7.3.3** The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using

a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

- 7.4** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

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

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol, 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 2, determine the recovery and single-operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.

- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁶ that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation R and s . Alternatively, the analyst must use four wastewater datapoints gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements⁶ should be updated regularly.

- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.
- 8.5** Before processing any samples, the analyst should demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.
- 8.6** 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 monitor the precision of the sampling technique. 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 perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. SAMPLES COLLECTION, PRESERVATION, AND HANDLING

- 9.1** Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2** The samples must be iced or refrigerated at 40°C, from the time of collection until extraction.
- 9.3** Adjust the pH of the sample to 6 to 8 with 6N sodium hydroxide or 6N sulfuric acid immediately after sampling.

10. SAMPLE EXTRACTION

- 10.1 Assemble continuous extraction apparatus by placing five to ten carborundum chips into the 500-mL round-bottom flask and attaching to the extraction flask.
- 10.2 Add 400 mL methylene chloride to the extraction flask. Some methylene chloride should displace into the round-bottom flask.
- 10.3 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into the extraction flask and add sufficient distilled water to fill the extraction flask (2 L total volume aqueous phase).
- 10.4 Check the pH of the sample with wide-range pH paper and adjust to 6 to 8 with 6N sodium hydroxide or 6N sulfuric acid.
- 10.5 Connect the stirring apparatus to the extraction flask without the frit touching the sample. Heat methylene chloride in round-bottom flask to continuous reflux and continue heating for 30 minutes to 1 hour until frit is thoroughly wetted with methylene chloride.
- 10.6 Lower frit until it just touches the sample and start the stirring apparatus rotating.
- 10.7 Continuously extract sample for 18 to 24 hours.
- 10.8 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.9 Pour the extract from the round-bottom flask through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with 30 to 40 mL of methylene chloride.
- 10.10 Add one to two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches approximately 4 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.11 Remove the Snyder column and flask and adjust the volume of the extract to 5.0 mL with methylene chloride. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract is to be stored longer than two days, transfer the extract to a screw-capped vial with a PTFE-lined cap. If the sample extract requires no further cleanup, proceed with solvent exchange to toluene and gas chromatographic analysis as described in Sections 11.5 and 12 respectively. If the sample requires cleanup, proceed to Section 11.
- 10.12 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.
- 11.2 Stir 20 g of silica gel in 100 mL of acetone and 1.2 mL of reagent water for 30 minutes on a stirring plate. Transfer the slurry to a chromatographic column (silica gel may be retained with a plug of glass wool). Wash the column with 20 mL of methylene chloride and then with 30 mL of petroleum ether. Use a column flow rate of 2 to 2.5 mL/min throughout the wash and elution profiles. Add an additional 50 mL of petroleum ether to the head of the column.
- 11.3 Add the extract from Section 10.11 to the head of the column. Allow the solvent to elute from the column until the Florisil is almost exposed to the air. Elute the column with 50 mL of 6% ethyl ether in petroleum ether. Discard this fraction.
- 11.4 Elute the column with 100 mL of 15% ethyl ether in petroleum ether and collect in a K-D apparatus.
- 11.5 Add 2.5 mL of toluene to the fraction. Concentrate the fraction to approximately 4 mL with the water bath at 75 to 80°C as described in Section 10.10. Transfer the sample to a 5-mL volumetric flask and dilute to 5 mL with toluene. Proceed with gas chromatographic analysis.

12. GAS CHROMATOGRAPHY

- 12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. An example of the separations achieved by Column 1 and Column 2 are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.
- 12.2 Calibrate the gas chromatographic system daily as described in Section 7.
- 12.3 If an internal standard approach is being used, the analyst must not add the internal standard to the sample extracts until immediately before injection into the instrument. Mix thoroughly.
- 12.4 Inject 2 to 5 μL of the sample extract using the solvent flush technique.⁸ Record the volume injected to the nearest 0.05 μL and the resulting peak sizes in area or peak height units.
- 12.5 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

- 12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

- 13.1 Determine the concentration of individual compounds in the sample.

- 13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_e)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_e = Volume of water extracted, in mL

- 13.1.2 The internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_e)(\text{RF})(V_e)}$$

where

A_s = Response for parameter to be measured

A_e = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_e = Volume of water extracted, in L

- 13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

14. GC/MS CONFIRMATION

- 14.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative identifications made with this method. The mass spectrometer should be capable of scanning the

mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 seconds per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all glass or glass-lined materials is recommended. When using a fused-silica capillary column, the column outlet should be threaded through the interface to within a few millimeter of the entrance to the source ionization chamber. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.

- 14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved. The calculation of tailing factors is illustrated in Method 625.
- 14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all DFTPP performance criteria are achieved.⁹
- 14.4 To confirm an identification of a compound, the background corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
 - 14.4.1 The molecular ion and all other ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to $\pm 10\%$. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20 to 40%.
 - 14.4.2 The retention time of the compound in the sample must be within 30 seconds of the same compound in the standard solution.
 - 14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.
- 14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.
- 14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. METHOD PERFORMANCE

- 15.1 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 Table 1 were obtained using reagent water.¹ Similar results were achieved using representative wastewaters.
- 15.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from $10 \times \text{MDL}$ to $1000 \times \text{MDL}$.

- 15.3** In a single laboratory, Battelle Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 2 were obtained. Seven replicates of each of two different wastewaters were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 2.¹

References

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9. Eichelberger, J.W., Harris, L. E., and Budde, W.L., "Reference Compound to Calibrate Ion Abundance Measurement in Gas Chromatography - Mass Spectrometry, *Analytical Chemistry*, 47, 995 (1975).

Table 1. Chromatographic Conditions and Method Detection Limits

<i>Parameter</i>	<i>Retention Time (Minutes)</i>		<i>Method Detection Limit (µg/L)</i>
	<i>Column 1</i>	<i>Column 2</i>	
Diphenylamine	18.1	19.3	1.6

Column 1 conditions: Supelcoport (100/120 mesh) coated with 3% SP-2250 packed in a 1.8 m long by 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. Column temperature is held at 80°C for 4 minutes, programmed from 80°C to 300°C at 8°C/min and held at 300°C for 4 minutes.

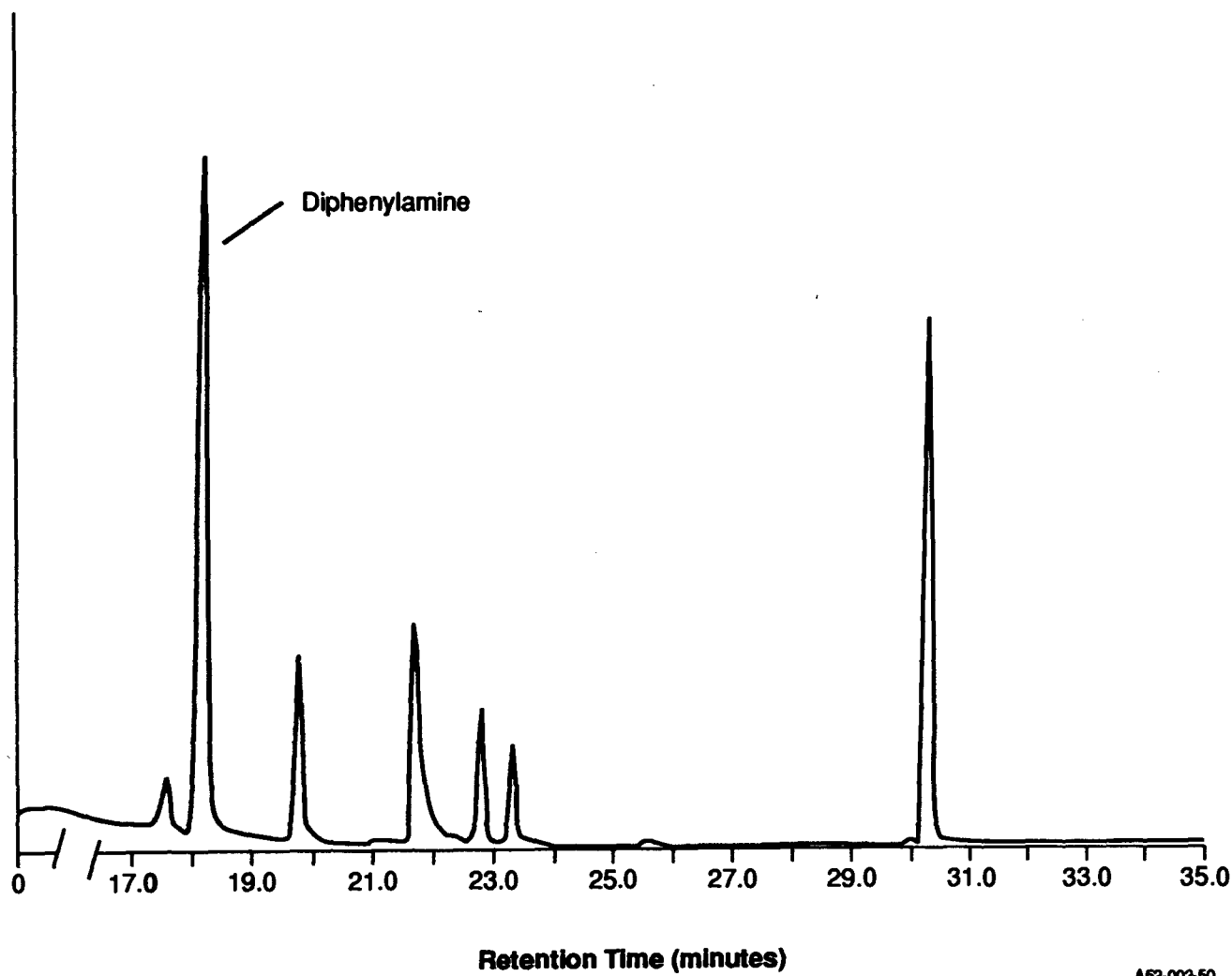
Column 2 conditions: Supelcoport (100/120 mesh) coated with 3% SP-1000 packed in a 1.8 m long by 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. Column temperature is held at 80°C for 4 minutes, programmed from 80°C to 250°C at 8°C/min and held at 250°C for 4 minutes.

Table 2. Single-Laboratory Accuracy and Precision*

<i>Parameter</i>	<i>Average Percent Recovery</i>	<i>Relative Standard Deviation %</i>	<i>Spike Level (µg/L)</i>	<i>Number of Analyses</i>	<i>Matrix Type (b)</i>
Diphenylamine	120	25	5.0	7	1
	89	11	50.0	7	1

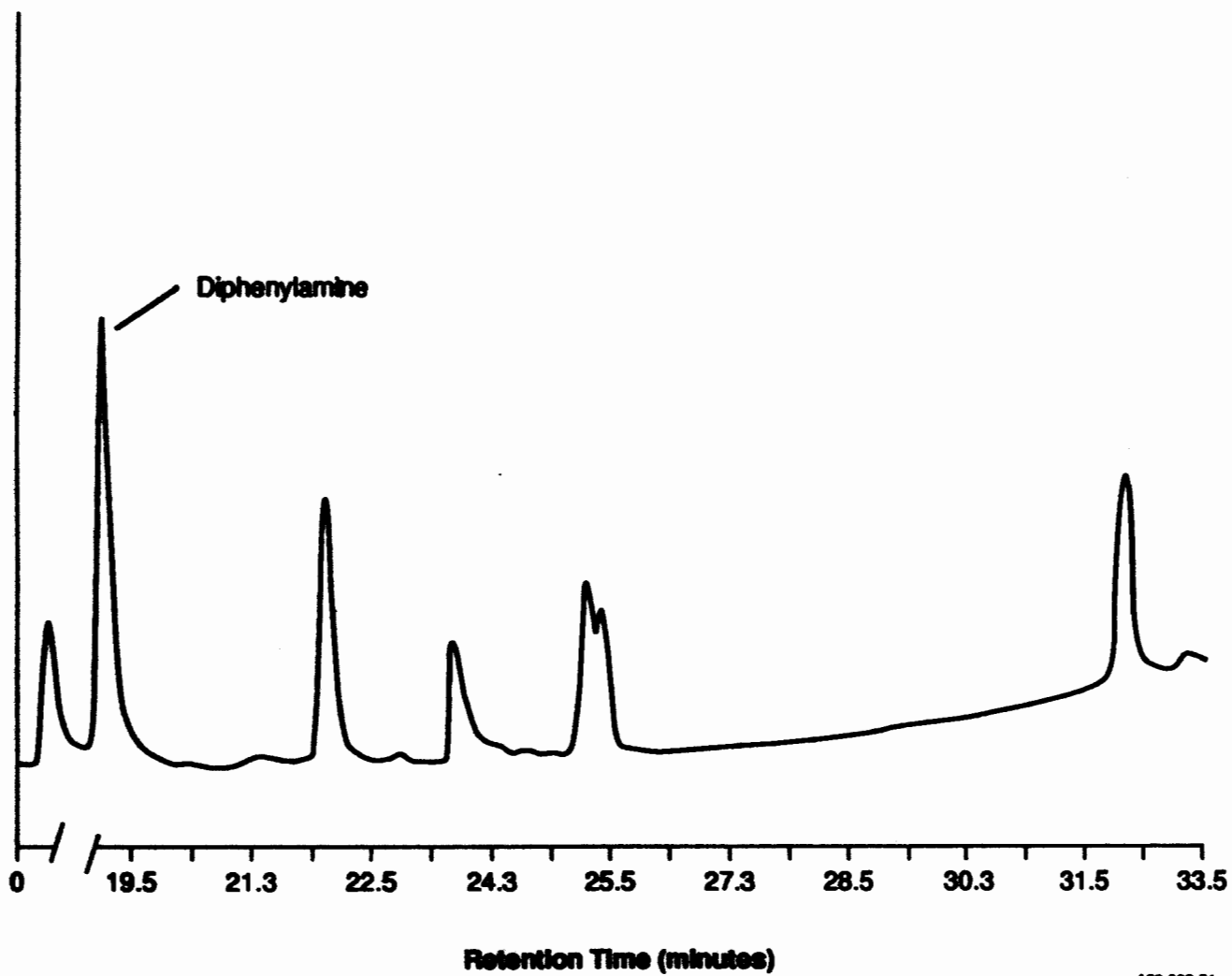
(a) Column 1 conditions were used.

(b) 1 = Columbus secondary POTW effluent.



A52-002-50

Figure 1. GC-AFD Chromatogram of 100 ng of Diphenylamine (Column 1).



A52-002-61

Figure 2. GC-FID Chromatogram of 200 ng of Diphenylamine (Column 2).

Method 622
The Determination of
Organophosphorus
Pesticides in Municipal and
Industrial Wastewater

Method 622

The Determination of Organophosphorus Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of certain organophosphorus pesticides. The following parameters can be determined by this method:

<i>Parameter</i>	<i>STORET No.</i>	<i>CAS No.</i>
Azinphos methyl	39580	86-50-0
Bolstar	—	35400-43-2
Chlorpyrifos	—	2921-88-2
Chlorpyrifos methyl	—	5598-13-0
Coumaphos	81293	56-72-4
Demeton	39560	8065-48-3
Diazinon	39570	333-41-5
Dichlorvos	—	62-73-7
Disulfoton	39010	298-04-4
Ethoprop	—	13194-48-4
Fensulfothion	—	115-90-2
Fenthion	39016	55-38-9
Merphos	39019	150-50-5
Mevinphos	—	7786-34-7
Naled	—	300-76-5
Parathion methyl	39600	298-00-0
Phorate	39023	298-02-2
Ronnel	39357	299-84-3
Stirofos	—	961-11-5
Tokuthion	—	34643-46-4
Trichloronate	—	327-98-0

- 1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternate test procedures under 40 *CFR* 136.4 and 136.5.

- 1.3 The estimated method detection limit (MDL, defined in Section 15) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4 The sample extraction and concentration steps in this method are essentially the same as several others in the 600-series methods. Thus, a single sample may be extracted to measure the parameters included in the scope of each of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures. Under gas chromatography, the analyst is allowed the latitude to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters (see Section 12).
- 1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. SUMMARY OF METHOD

- 2.1 A measured volume of sample, approximately 1 L, is extracted with 15% methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to hexane during concentration to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by gas chromatography with a thermionic bead or flame photometric detector in the phosphorus mode.¹

3. INTERFERENCES

- 3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.
 - 3.1.1 Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.
 - 3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

- 3.2** Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. Unique samples may require special cleanup approaches or selective GC detectors to achieve the MDL listed in Table 1. Use of a flame photometric detector in the phosphorus mode will minimize interferences from materials that do not contain phosphorus. Elemental sulfur, however, may interfere with the determination of certain organophosphorus pesticides by flame photometric gas chromatography. A halogen-specific detector (electrolytic conductivity or microcoulometric) is very selective for the halogen-containing pesticides and has been shown to be effective in the analysis of wastewater for dichlorvos, naled, and stirofos.

4. SAFETY

- 4.1** The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified³⁻⁵ for the information of the analyst.

5. APPARATUS AND MATERIALS

- 5.1** Sampling equipment, for discrete or composite sampling.
- 5.1.1** Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.
- 5.1.2** Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.
- 5.2** Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)
- 5.2.1** Separatory funnel: 2000-mL, with TFE-fluorocarbon stopcock, ground-glass or TFE stopper.
- 5.2.2** Drying column: Chromatographic column 400 mm long by 19 mm ID with coarse-fritted disc.

- 5.2.3 Concentrator tube, Kuderna-Danish: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground-glass stopper is used to prevent evaporation of extracts.
- 5.2.4 Evaporative flask, Kuderna-Danish: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.
- 5.2.5 Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).
- 5.2.6 Vials: Amber glass, 10- to 15-mL capacity with TFE-fluorocarbon-lined screw-cap.
- 5.3 Boiling chips: Approximately 10/40 mesh. Heat at 400°C for 30 minutes or perform soxhlet extraction with methylene chloride.
- 5.4 Water bath: Heated, with concentric ring cover, capable of temperature control ($\pm 2^{\circ}\text{C}$). The bath should be used in a hood.
- 5.5 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6 Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector and strip-chart recorder. A data system is recommended for measuring peak areas.
 - 5.6.1 Columns: These columns were used to develop the method performance statements in Section 15. Alternate columns may be used in accordance with the provisions described in Section 12.1.
 - 5.6.1.1 Column 1: 180 cm long by 2 mm ID glass, packed with 5% SP-2401 on Supelcoport (100/120 mesh) or equivalent.
 - 5.6.1.2 Column 2: 180 cm long by 2 mm ID glass, packed with 3% SP-2401 on Supelcoport (100/120 mesh) or equivalent.
 - 5.6.1.3 Column 3: 50 cm long by 1/8 inch OD PTFE, packed with 15% SE-54 on Gas Chrom Q (80/100 mesh) or equivalent.
 - 5.6.2 Detector: Thermionic bead or flame photometric in the phosphorus mode. These detectors have proven effective in the analysis of wastewaters for the parameters listed in the scope and were used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. REAGENTS

- 6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2 Acetone, hexane, methylene chloride: Pesticide-quality or equivalent.
- 6.3 Sodium sulfate: ACS, granular, anhydrous. Condition by heating in a shallow tray at 400°C for a minimum of 4 hours to remove phthalates and other interfering organic substances. Alternatively, heat 16 hours at 450 to 500°C in a shallow tray or perform a Soxhlet extraction with methylene chloride for 48 hours.
- 6.5 Stock standard solutions (1.00 $\mu\text{g}/\mu\text{L}$): Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.

- 6.5.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in pesticide-quality hexane or other suitable solvent and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially-prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.
- 6.5.2 Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw-cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
- 6.6.3 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2 External standard calibration procedure.
 - 7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with hexane or other suitable solvent. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
 - 7.2.2 Using injections of 1 to 5 μ L of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.
 - 7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.
- 7.3 Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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.

- 7.3.1** Prepare calibration standards at a minimum of three concentration levels for each parameter 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 hexane or other suitable solvent. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.
- 7.3.2** Using injections of 1 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} , against RF.

- 7.3.3** The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.
- 7.4** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.
- 8.1.1** Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

- 8.1.2** In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
- 8.1.3** The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2** To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
- 8.2.1** Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone, 1000 times more concentrated than the selected concentrations.
- 8.2.2** Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
- 8.2.3** Calculate the average % recovery (R), and the standard deviation of the % recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.
- 8.2.4** Using the appropriate data from Table 2, determine the recovery and single-operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.
- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
- 8.3.1** Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁶ that are useful in observing trends in performance.

- 8.3.2** The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁶
- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one

spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

- 8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.
- 8.6 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 monitor the precision of the sampling technique. 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 perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

- 9.1 Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

10. SAMPLE EXTRACTION

- 10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel.
- 10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.
- 10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

- 10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.
- 10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.7 Increase the temperature of the hot water bath to about 80°C. Momentarily remove the Snyder column, add 50 mL of hexane and a new boiling chip, and reattach the Snyder column. Pour about 1 mL of hexane into the top of the Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 minutes. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.8 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to TFE-fluorocarbon-sealed screw-cap vials. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis. If the sample requires cleanup, proceed to Section 11.
- 10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix and were not required for the analysis of the wastewaters reported in Section 15. If particular circumstances demand the use of a cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

12. GAS CHROMATOGRAPHY

- 12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Naled is partially converted to dichlorvos on GC Columns 1 and 2 but not on Column 3.

Therefore, if naled is to be measured in the sample, GC analysis for dichlorvos and naled must be performed using Column 3. Examples of the separations achieved are shown in Figures 1 through 4. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

- 12.2 Calibrate the system daily as described in Section 7.
- 12.3 If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.
- 12.4 Inject 1 to 5 μL of the sample extract using the solvent-flush technique.⁸ Record the volume injected to the nearest 0.05 μL , and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.
- 12.5 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7 If the measurement of the peak response is prevented by the presence of interferences, cleanup is required.

13. CALCULATIONS

- 13.1 Determine the concentration of individual compounds in the sample.

- 13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_s)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_s = Volume of water extracted, in mL

- 13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where

A_s = Response for parameter to be measured

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_o = Volume of water extracted, in L

- 13.2** Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 13.3** For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. GC/MS CONFIRMATION

- 14.1** It is recommended that GC/MS techniques be judiciously employed to support qualitative compound identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 seconds per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.
- 14.2** Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.⁹
- 14.3** At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.¹⁰
- 14.4** To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
- 14.4.1** All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to $\pm 10\%$. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20% to 40%.

- 14.4.2** The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.
- 14.4.3** Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.
- 14.5** Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.
- 14.6** Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. METHOD PERFORMANCE

- 15.1** 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.¹¹ The MDL concentrations listed in Table 1 were estimated from the response of the detector to each compound. The estimate is based upon the amount of material required to yield a signal 5 times the GC background noise, assuming a 5- μ L injection from a 10-mL final extract of a 1-L sample.
- 15.2** In a single laboratory (West Cost Technical Services, Inc.), using effluents from pesticide manufacturers and publicly-owned treatment works (POTW), the average recoveries presented in Table 2 were obtained.¹ The standard deviations of the percent recoveries of these measurements are also included in Table 2.

References

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7. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, Pennsylvania, p. 76, 1980.
8. Burke, J.A., "Gas Chromatography for Pesticide Residue Analysis; Some Practical Aspects," *Journal of the Association of Official Analytical Chemists*, 48, 1037 (1965).
9. McNair, H.M., and Bonelli, E. J., *Basic Chromatography*, Consolidated Printing, Berkeley, California, p. 52, 1969.
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Table 1. Chromatographic Conditions and Estimated Method Detection Limits

<i>Parameter</i>	<i>GC Column</i>	<i>Retention Time (min)</i>	<i>Estimated MDL (µg/L)</i>
Demeton	1a	1.16 2.53	0.25
Phorate	1a	1.43	0.15
Disulfoton	1a	2.10	0.20
Trichloronate	1a	2.94	0.15
Fenthion	1a	3.12	0.10
Tokuthion	1a	3.40	0.5
Bolstar	1a	4.23	0.15
Fensulfothion	1a	6.41	1.5
Azinphos methyl	1a	6.80	1.5
Coumaphos	1a	11.6	1.5
Dichlorvos	1b	0.8	0.1
Mevinphos	1b	2.41	0.3
Stirofos	1b	8.52	5.0
Ethoprop	2	3.02	0.25
Parathion methyl	2	3.37	0.3
Rommel	2	5.57	0.3
Chlorpyrifos methyl	2	5.72	0.3
Chlorpyrifos	2	6.16	0.3
Merphos	2	7.45	0.25
Diazinon	2	7.73	0.6
Dichlorvos	3	1.50	0.1
Naled	3	3.28	0.1
Stirofos	3	5.51	5.0

Column 1a Conditions: Supelcoport (100/120 mesh) coated with 5% SP-2401 packed in a 180 cm long by 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. Column temperature, programmed: Initial 150°C, hold for 1 minute, then program at 25°C/min to 220°C and hold. A flame photometric detector was used with this column to estimate the MDL.

Column 1b Conditions: Same as Column 1a, except nitrogen carrier gas at a flow rate of 30 mL/min. Temperature, programmed: Initial 170°C, hold 2 minutes, then program at 20°C/min to 220°C and hold.

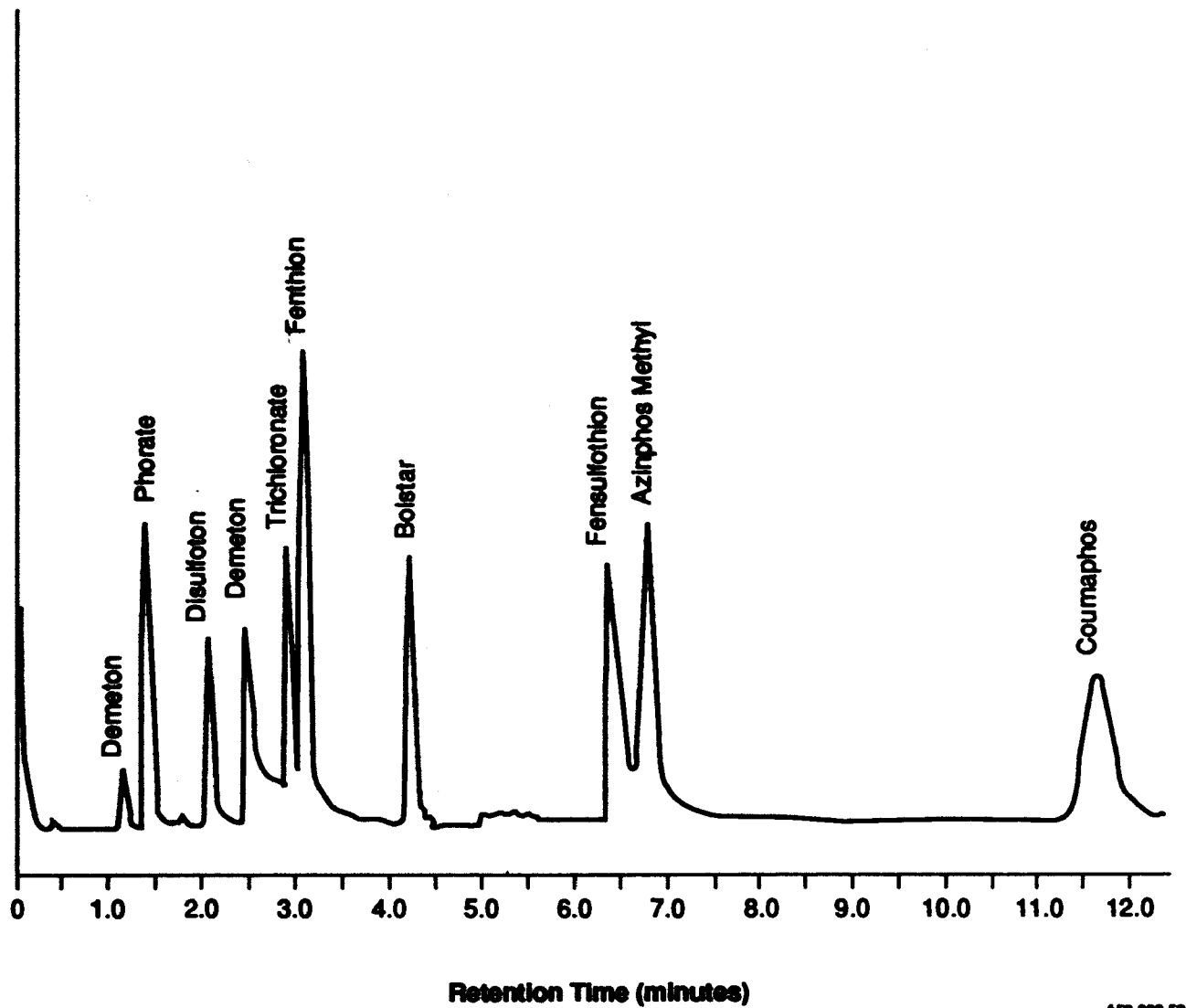
Column 2 Conditions: Supelcoport (100/120 mesh) coated with 3% SP-2401 packed in a 180 cm long by 2 mm ID glass column with helium carrier gas at a flow rate of 25 mL/min. Column temperature, programmed, initial 170°C, hold for 7 minutes, then program at 10°C/min to 250°C and hold. A thermionic bead detector was used with this column to estimate the MDL.

Table 1. Chromatographic Conditions and Estimated Method Detection Limits (cont.)

Column 3 Conditions: Gas Chrom Q (100/120 mesh) coated with 15% SE-54 packed in a 50 cm long by 1/8-inch OD PTFE column with nitrogen carrier gas at a flow rate of 30 mL/min. Temperature, programmed: Initial 100°C, then program immediately at 25°C/min to 200°C and hold. An electrolytic detector in the halogen mode was used with this column to estimate the MDL.

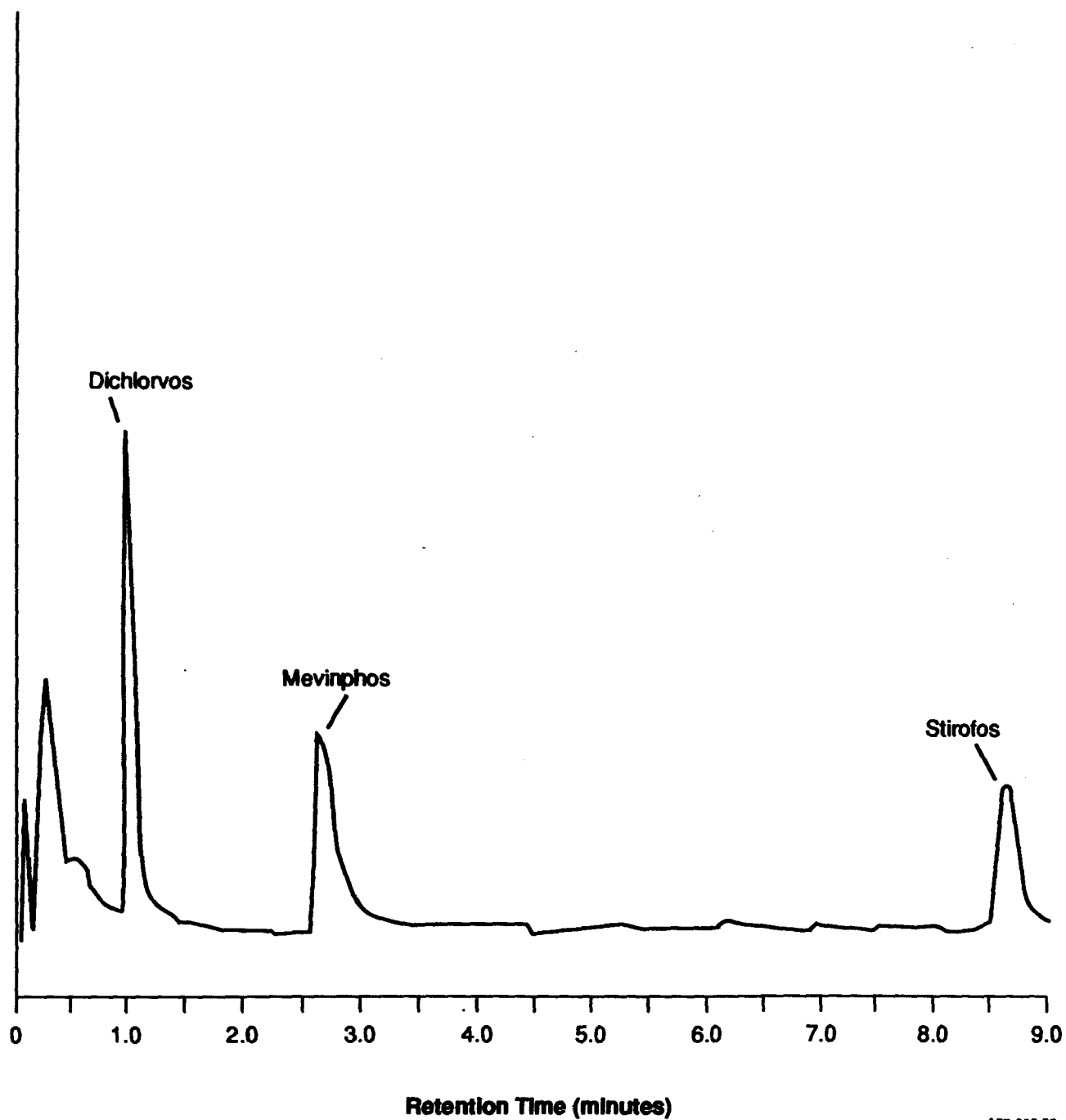
Table 2. Single-Operator Accuracy and Precision

<i>Parameter</i>	<i>Average Percent Recovery</i>	<i>Standard Deviation (%)</i>	<i>Spike Range (µg/L)</i>	<i>Number of Analyses</i>	<i>Types</i>
Azinphos methyl	72.7	18.8	21-250	17	3
Bolstar	64.6	6.3	4.9-46	17	3
Chlorpyrifos	98.3	5.5	1.0-50.5	18	3
Coumaphos	109.0	12.7	25-225	17	3
Demeton	67.4	10.5	11.9-314	17	3
Diazinon	67.0	6.0	5.6	7	1
Dichlorvos	72.1	7.7	15.6-517	16	3
Disulfoton	81.9	9.0	5.2-92	17	3
Ethoprop	100.5	4.1	1.0-51.5	18	3
Fensulfothion	94.1	17.1	23.9-110	17	3
Fenthion	68.7	19.9	5.3-64	17	3
Merphos	120.7	7.9	1.0-50	18	3
Mevinphos	56.5	7.8	15.5-520	16	3
Naled	78.0	8.1	25.8-294	16	3
Parathion methyl	96.0	5.3	0.5-500	21	3
Phorate	62.7	8.9	4.9-47	17	3
Ronnel	99.2	5.6	1.0-50	18	3
Stirofos	66.1	5.9	30.3-505	16	3
Tokuthion	64.6	6.8	5.3-64	17	3
Trichloronate	105.0	18.6	20	3	1



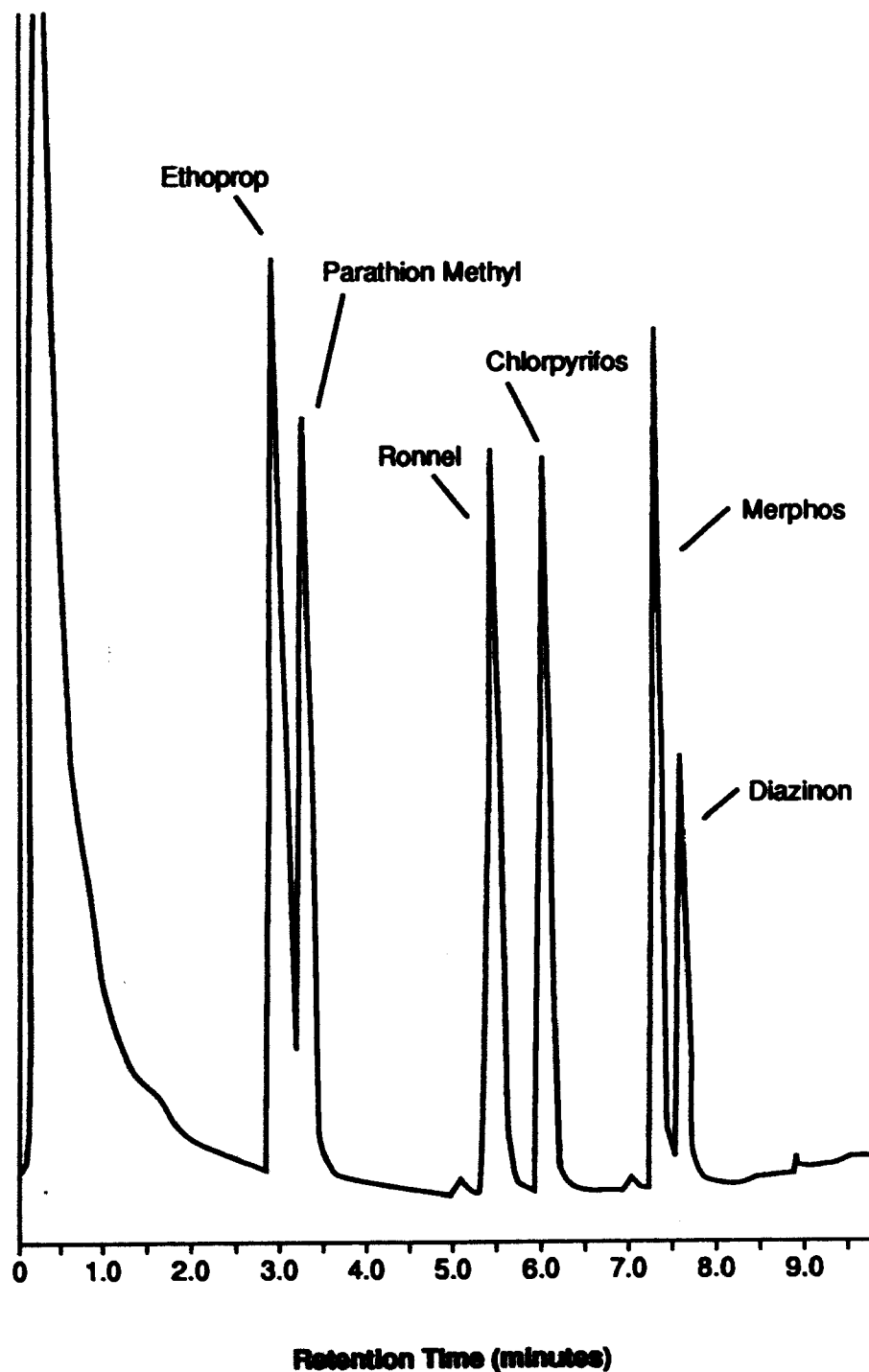
A82-002-53

Figure 1. Gas Chromatogram of Organophosphorus Pesticides on Column 1a.
For Conditions, See Table 1.



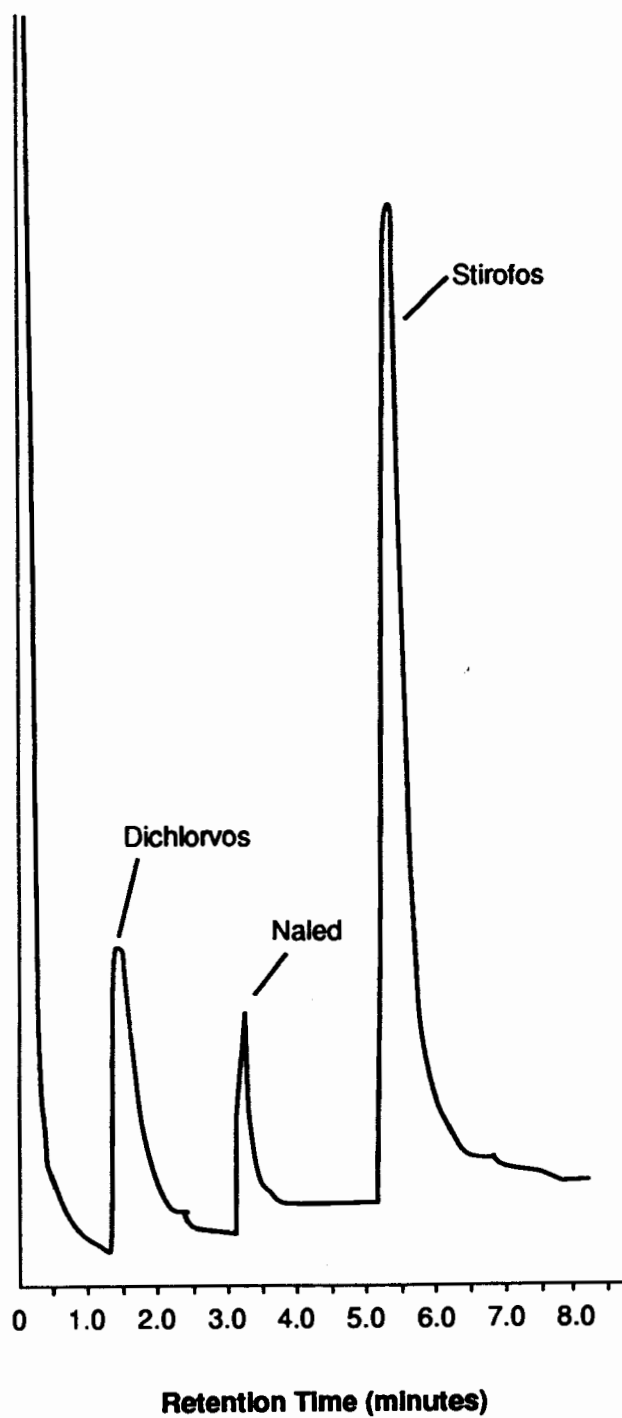
A52-002-52

Figure 2. Gas Chromatogram of Organophosphorous Pesticides on Column 1b.
For Conditions, See Table 1.



A62-002-54

Figure 3. Gas Chromatogram of Organophosphorus Pesticides on Column 2.
For Conditions, See Table 1.



A52-002-55

Figure 4. Gas Chromatogram of Organophosphorus Pesticides on Column 3. For Conditions, See Table 1.

Method 622.1
The Determination of
Thiophosphate Pesticides in
Municipal and Industrial
Wastewater

Method 622.1

The Determination of Thiophosphate Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1** This method covers the determination of certain thiophosphate pesticides. The following parameters can be determined by this method:

<i>Parameter</i>	<i>CAS No.</i>
Aspon	3244-90-4
Dichlofenthion	97-17-6
Famphur	52-85-7
Fenitrothion	122-14-5
Fonophos	944-22-9
Phosmet	732-11-6
Thionazin	297-97-2

- 1.2** This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3** The method detection limit (MDL, defined in Section 15) for each parameter is listed in Table 2. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4** The sample extraction and concentration steps in this method are essentially the same as in certain other 600-series methods. Thus, a single sample may be extracted to measure the compounds included in the scope of the methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures.
- 1.5** This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.6** When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Section 14 provides gas

chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. SUMMARY OF METHOD

- 2.1 A measured volume of sample, approximately 1 L, is extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated to 1.0 mL. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by alkali flame detector gas chromatography (GC/AFD).¹
- 2.2 This method provides a Florisil column cleanup procedure to aid in the elimination of interferences that may be encountered.

3. INTERFERENCES

- 3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.
 - 3.1.1 Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thoroughly rinsing with tap and reagent water. Drain dry and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric glassware. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.
 - 3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDLs listed in Table 2.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the

chemical analysis. Additional references to laboratory safety are available and have been identified³⁻⁵ for the information of the analyst.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab-sample bottle: Amber borosilicate or flint glass, 1-quart or 1-L volume, fitted with screw-caps lined with PTFE. Foil may be substituted for PTFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsing with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel: 2000-mL, with PTFE stopcock.

5.2.2 Drying column: Chromatographic column 400 mm long by 10 mm ID with coarse frit.

5.2.3 Chromatographic column: 400 mm long by 19 mm ID with 250-mL reservoir at the top and PTFE stopcock (Kontes K-420290 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. A ground-glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Snyder column, Kuderna-Danish: Two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.8 Vials: Amber glass, 10- to 15-mL capacity with PTFE-lined screw-cap.

5.2.9 Erlenmeyer flask: 250-mL.

5.2.10 Graduated cylinder: 1000-mL.

5.3 Boiling chips: Approximately 10/40 mesh carborundum. Heat at 400°C for 4 hours or extract in a Soxhlet with methylene chloride.

5.4 Water bath: Heated, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.

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

- 5.6** Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.
- 5.6.1** Column 1: 180 cm long by 2 mm ID glass, packed with 3% SP-2250 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 12.1.
- 5.6.2** Column 2: 180 cm long by 2 mm ID glass, packed with 3% SP-2100 on Supelcoport (100/120 mesh) or equivalent.
- 5.6.3** Detector: Alkali flame detector (AFD), sometimes referred to as a nitrogen-phosphorous detector (NPD) or a thermionic-specific detector (TSD). This detector has proven effective in the analysis of wastewaters for the compounds listed in the scope and was used to develop the method performance statements in Section 15.

6. REAGENTS

- 6.1** Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2** Methylene chloride, methanol, petroleum ether, anhydrous ethyl ether, and acetone: Distilled-in-glass quality or equivalent. Ethyl ether must be free of peroxides as indicated by EM Quant Test Strips (available from Scientific Products Co., Catalog No. P 1126-8 and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips.
- 6.3** Sodium sulfate: ACS, granular, anhydrous; heated in a muffle furnace at 400°C overnight.
- 6.4** Florisil: PR grade (60/100 mesh). Purchase activated at 675°C and store in a brown glass bottle. To prepare for use, place 150 g in a wide-mouth jar and heat overnight at 160 to 170°C. Seal tightly with PTFE or aluminum-foil-lined screw-cap and cool to room temperature.
- 6.5** 6N sodium hydroxide.
- 6.6** 6N sulfuric acid.
- 6.7** Stock standard solutions (1.00 µg/µL): Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.
- 6.7.1** Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in distilled-in-glass quality ethyl ether and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 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.
- 6.7.2** Transfer the stock standard solutions into PTFE-sealed screw-cap bottles. Store at 4°C and protect from light. Frequently check standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
- 6.7.3** Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1** Establish gas chromatographic operating parameters equivalent to those indicated in Table 2. The gas chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2** External standard calibration procedure:
- 7.2.1** For each compound of interest, prepare calibration standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with ethyl ether. One of the external standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
 - 7.2.2** Using injections of 1 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each compound at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.
 - 7.2.3** The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.
- 7.3** Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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.
- 7.3.1** Prepare calibration standards at a minimum of three concentration levels for each compound 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 ethyl ether. One of the standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

- 7.3.2** Using injections of 1 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_b)}{(A_b)(C_s)}$$

where

A_s = Response for the parameter to be measured

A_b = Response for the internal standard

C_b = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, 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_b , against RF.

- 7.3.3** The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.
- 7.4** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.
- 8.1.1** Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2** In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

- 8.1.3** The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2** To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
- 8.2.1** Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol, 1000 times more concentrated than the selected concentrations.
- 8.2.2** Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
- 8.2.3** Calculate the average percent recovery (R) and the standard deviation of the percent recovery (s) for the results. Wastewater background corrections must be made before R and s calculations are performed.
- 8.2.4** Using the appropriate data from Table 3, determine the recovery and single-operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.
- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
- 8.3.1** Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts that are useful in observing trends in performance.

- 8.3.2** The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁶
- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples

processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.¹

- 8.5 Before processing any samples, the analyst should demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.
- 8.6 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 monitor the precision of the sampling technique. 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 perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

- 9.1 Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3 Adjust the pH of the sample to 6 to 8 with 6N sodium hydroxide or 6N sulfuric acid immediately after sampling.

10. SAMPLE EXTRACTION

- 10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel. Check the pH of the sample with wide range pH paper and adjust to 6 to 8 with 6N sodium hydroxide or 6N sulfuric acid.
- 10.2 Add 60 mL of methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.
- 10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.
- 10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

- 10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with 30 to 40 mL of methylene chloride.
- 10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.7 Remove the macro-Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. Add one or two clean boiling chips and attach a two-ball micro-Snyder column to the concentrator tube. Prewet the micro-Snyder column with methylene chloride and concentrate the solvent extract as before. When an apparent volume of 0.5 mL is reached, or the solution stops boiling, remove the K-D apparatus and allow it to drain and cool for 10 minutes.
- 10.8 Remove the micro-Snyder column and adjust the volume of the extract to 1.0 mL with methylene chloride. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract is to be stored longer than 3 days, transfer the extract to a screw-capped vial with a PTFE-lined cap. If the sample extract requires no further cleanup, proceed with the gas chromatographic analysis in Section 12. If the sample requires cleanup, proceed to Section 11.
- 10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than that reported in Table 3.
- 11.2 The following Florisil column cleanup procedure has been demonstrated to be applicable to the seven thiophosphate pesticides listed in Table 1.
 - 11.2.1 Add 20 g of Florisil to 100 mL of ethyl ether and 400 μ L of reagent water in a 250-mL Erlenmeyer flask. Shake vigorously for 15 minutes. Transfer the slurry to a chromatographic column (Florisil may be retained with a plug of glass wool). Allow the solvent to elute from the column until the Florisil is almost exposed to the air. Wash the column with 25 mL of petroleum ether. Use a column flow of 2 to 2.5 mL/min throughout the wash and elution profiles. Add an additional 50 mL of petroleum ether to the head of the column.

- 11.2.2** Quantitatively add the sample extract from Section 10.8 to the head of the column. Allow the solvent to elute from the column until the Florisil is almost exposed to the air. Elute the column with 50 mL of 6% ethyl ether in petroleum ether. Discard this fraction.
- 11.2.3** Elute the column with 50 mL of 15% ethyl ether in petroleum ether (Fraction 1) and collect eluate in a K-D apparatus. Repeat process with 50 mL of 50% ethyl ether in petroleum ether (Fraction 2), 50 mL of 100% ethyl ether (Fraction 3), 50 mL 6% acetone in ethyl ether (Fraction 4), and 100 mL 15% acetone in ethyl ether (Fraction 5), collecting each fraction in a separate K-D apparatus. The elution patterns for the thiophosphates are shown in Table 1. Concentrate each fraction to 1 mL as described in Sections 10.6 and 10.7. Proceed with gas chromatographic analysis.
- 11.2.4** The above-mentioned fractions can be combined before concentration at the discretion of the analyst.

12. GAS CHROMATOGRAPHY

- 12.1** Table 2 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Examples of the separations achieved by Column 1 and Column 2 are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.
- 12.2** Calibrate the gas chromatographic system daily as described in Section 7.
- 12.3** If the internal standard approach is being used, the analyst must not add the internal standard to the sample extracts until immediately before injection into the instrument. Mix thoroughly.
- 12.4** Inject 1 to 5 μL of the sample extract using the solvent flush technique.² Record the volume injected to the nearest 0.05 μL , and record the resulting peak sizes in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.
- 12.5** The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention-time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6** If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7** If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_e)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_e = Volume of water extracted, in mL

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_e)}$$

where

A_s = Response for parameter to be measured

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_e = Volume of water extracted, in L

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

14. GC/MS CONFIRMATION

14.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The

instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 seconds per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all glass or glass-lined materials is recommended. When using a fused-silica capillary column, the column outlet should be threaded through the interface to within a few millimeters of the entrance to the source ionization chamber. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.

- 14.2** Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.¹⁰
- 14.3** At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all DFTPP performance criteria are achieved.⁹
- 14.4** To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
 - 14.4.1** The molecular ion and all other ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to $\pm 10\%$. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20 to 40%.
 - 14.4.2** The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.
 - 14.4.3** Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.
- 14.5** Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.
- 14.6** Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternative packed or capillary GC columns or additional cleanup (Section 11).

15. METHOD PERFORMANCE

- 15.1** 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.¹¹ The MDL concentrations listed in Table 2 were obtained using reagent water.¹ Similar results were achieved using representative wastewaters.
- 15.2** This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from $10 \times \text{MDL}$ to $1000 \times \text{MDL}$.
- 15.3** In a single laboratory, Battelle Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 3 were obtained after Florisil cleanup. Seven replicates of

each of two different wastewaters were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 3.¹

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Table 1. Elution Orders and Recoveries of Thiophosphates from Florisil

Compound	Recovery in Specified Fraction, % (a)							Total
	F1	F2	F3	F4	F5	F6	F7	
Aspon			94	2				96
Dichlofenthion			92					92
Famphur						6	103	109
Fenitrothion				51	55			106
Fonophos			84	6				90
Phosmet						37	69	106
Thionazine				2	93			95

(a) Results of single determination with 100 μ g of each compound. Elution solvents were 50 mL each of the following:

F1 = 2% methylene chloride in petroleum ether

F2 = 6% ethyl ether in petroleum ether

F3 = 15% ethyl ether in petroleum ether

F4 = 50% ethyl ether in petroleum ether

F5 = 100% ethyl ether

F6 = 6% acetone in ethyl ether

F7 = 15% acetone in ethyl ether

Table 2. Chromatographic Conditions and Estimated Method Detection Limits

<i>Parameter</i>	<i>Retention Time (Minutes)</i>		<i>MDL (µg/L)</i>
	<i>Column 1</i>	<i>Column 2</i>	
Thionazin	18.3	25.0	1
Fonophos	20.5	27.8	0.7
Dichlofenthion	21.4	29.4	0.7
Aspon	22.6	30.2	0.6
Fenitrothion	23.1	30.8	2
Famphur	28.1	34.8	19
Phosmet	30.0	36.2	1

Column 1 conditions: Supelcoport (100/120 mesh) coated with 3% SP-2250 packed in a 1.8 m long by 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. Column temperature is programmed from 80°C to 300°C at 8°C/min with a 4 minute hold at each extreme, injector temperature is 250°C and detector is 300°C. Alkali flame detector at bead voltage of 16 volts.

Column 2 conditions: Supelcoport (100/120 mesh) coated with 3% SP-2100 packed in a 1.8 m long by 2 mm ID glass columns with helium carrier gas at a flow rate of 30 mL/minute. Column temperature is programmed from 80°C to 300°C at 8°C/min with a 4 minute hold at each extreme, injector temperature is 250°C and detector is 300°C.

Table 3. Single-Laboratory Accuracy and Precision (a)

<i>Parameter</i>	<i>Sample Type (b)</i>	<i>Background (µg/L) (c)</i>	<i>Spike (µg/L)</i>	<i>Mean Recovery %</i>	<i>% Relative Standard Deviation</i>	<i>Number of Replicates</i>
Aspon	1	ND	50	83	7	7
	2	ND	500	87	3	7
Dichlofenthion	1	ND	50	83	7	7
	2	ND	500	84	4	7
Famphur	1	ND	50	86	6	7
	2	ND	500	86	4	7
Fenitrothion	1	ND	50	82	7	7
	2	ND	500	83	4	7
Fonophos	1	ND	50	84	7	7
	2	ND	500	86	4	7
Phosmet	1	ND	50	85	5	7
	2	ND	500	87	5	7
Thionizin	1	ND	50	84	7	7
	2	ND	500	89	5	7

(a) Column 1 conditions were used.

(b) 1 = Low-level relevant industrial effluent
2 = Municipal sewage influent

(c) ND = Not detected

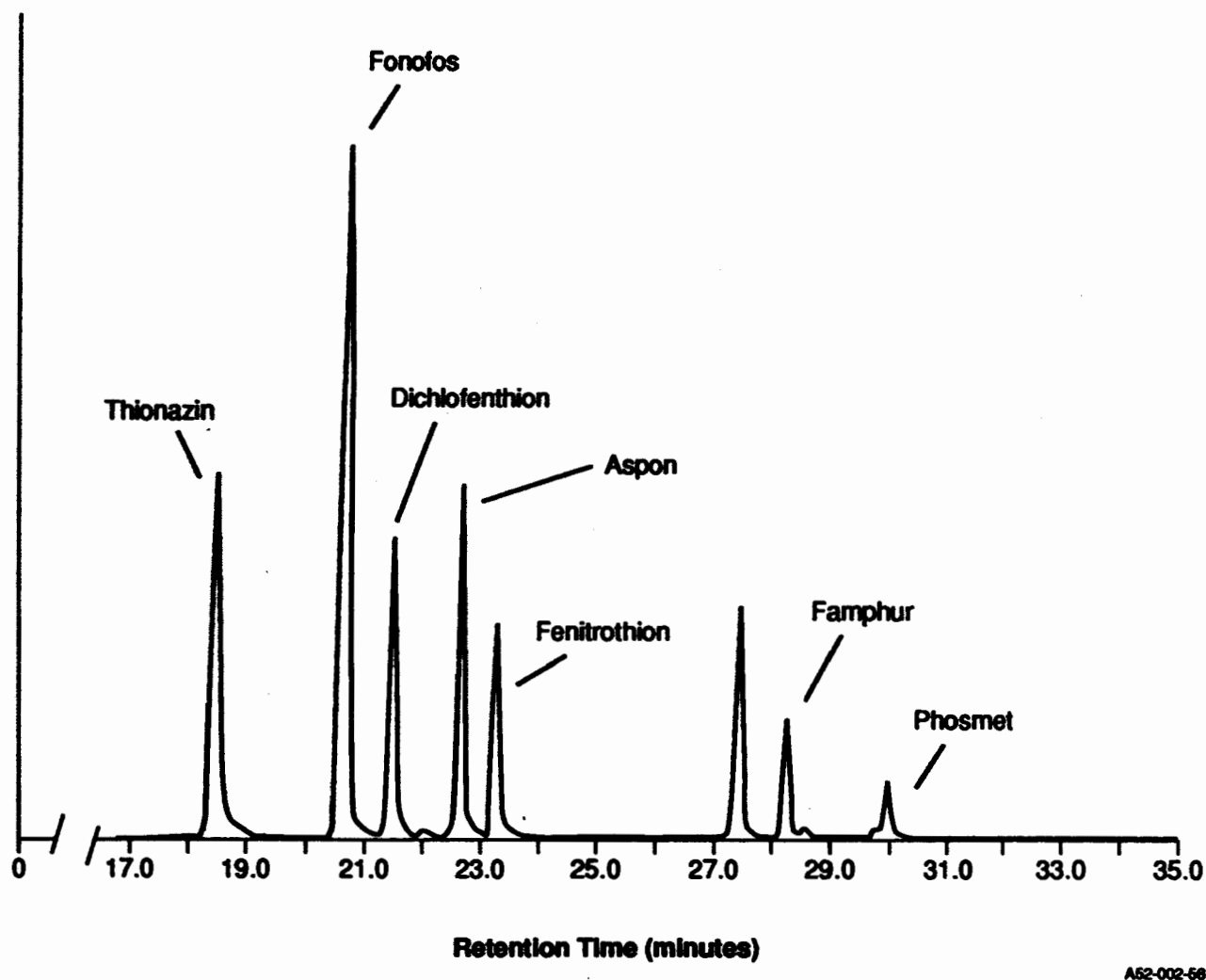
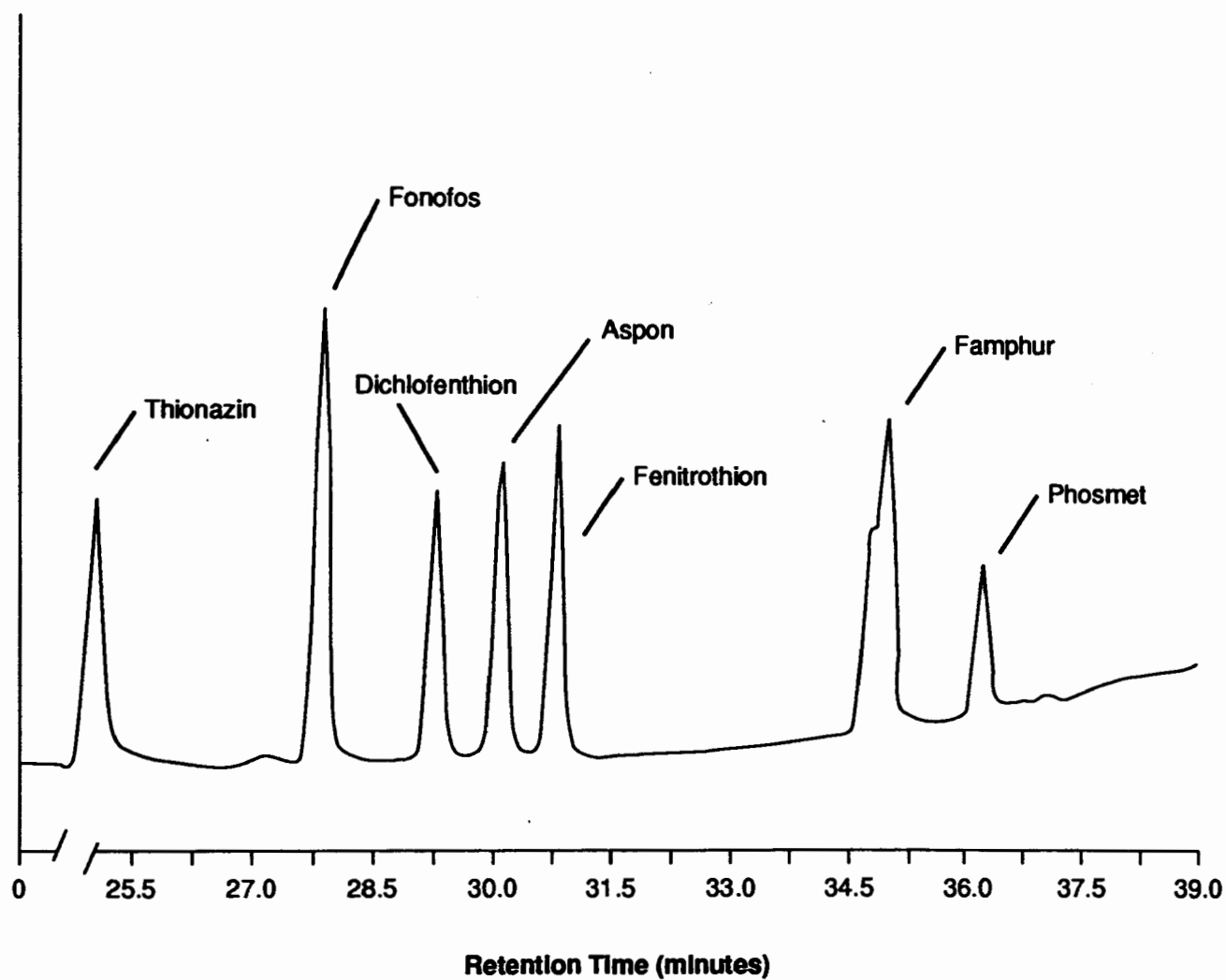


Figure 1. GC-AFD Chromatogram of 100 ng Each of Seven Thiophosphates (Column 1).



A52-002-57

Figure 2. GC-FID Chromatogram of 100 ng Each of Seven Thiophosphates (Column 2).

Method 627
The Determination of
Dinitroaniline Pesticides in
Municipal and Industrial
Wastewater

Method 627

The Determination of Dinitroaniline Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1** This method covers the determination of certain dinitroaniline pesticides. The following parameters can be determined by this method:

<i>Parameter</i>	<i>STORET No.</i>	<i>CAS No.</i>
Benfluralin	39002	1861-40-1
Ethalfuralin	—	55283-68-6
Isopropalin	—	33820-53-0
Profluralin	—	26399-36-0
Trifluralin	39030	1582-09-8

- 1.2** This method fails to distinguish between benfluralin, ethalfuralin, and trifluralin. When more than one of these materials may be present in a sample, the results are reported as trifluralin.
- 1.3** This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.4** The method detection limits (MDL, defined in Section 15) for four of the parameters are listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.5** The sample extraction and concentration steps in this method are essentially the same as several other the 600-series methods. Thus, a single sample may be extracted to measure the parameters included in the scope of each of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures. Under gas chromatography, the analyst is allowed the latitude to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters (see Section 12).
- 1.6** This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

- 1.7** When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column within the limitations described in Section 1.2. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. SUMMARY OF METHOD

- 2.1** A measured volume of sample, approximately 1 L, is extracted with 15% methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to hexane during concentration to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by electron capture (EC) gas chromatography.¹
- 2.2** This method provides an optional Florisil column cleanup procedure to aid in the elimination or reduction of interferences which may be encountered.

3. INTERFERENCES

- 3.1** Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

- 3.2** Interferences by phthalate esters can pose a major problem in pesticide analysis when the EC detector is used. These compounds generally appear in the chromatogram as large, late-eluting peaks. Common flexible plastics contain varying amounts of phthalates. These phthalates are easily extracted or leached from such materials during laboratory operations. Cross-contamination of clean glassware occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Interferences from phthalates can be minimized by avoiding the use of plastics in the laboratory. Exhaustive cleanup of reagents and glassware may be required to eliminate background phthalate contamination.^{3,4}

- 3.3** Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. Unique samples may require special cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

- 4.1** The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified⁵⁻⁷ for the information of the analyst.

5. APPARATUS AND MATERIALS

- 5.1** Sampling equipment, for discrete or composite sampling.
- 5.1.1** Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.
- 5.1.2** Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.
- 5.2** Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)
- 5.2.1** Separatory funnel: 2000-mL, with TFE-fluorocarbon stopcock, ground-glass or TFE stopper.
- 5.2.2** Drying column: Chromatographic column 400 mm long by 19 mm ID with coarse-fritted disc.
- 5.2.3** Concentrator tube, Kuderna-Danish: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground-glass stopper is used to prevent evaporation of extracts.
- 5.2.4** Evaporative flask, Kuderna-Danish: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.
- 5.2.5** Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).

- 5.2.6 Vials: Amber glass, 10- to 15-mL capacity with TFE-fluorocarbon-lined screw-cap.
- 5.3 Boiling chips: approximately 10/40 mesh. Heat at 400°C for 30 minutes or perform a Soxhlet extraction with methylene chloride.
- 5.4 Water bath: Heated, with concentric ring cover, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.
- 5.5 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6 Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.
 - 5.6.1 Column 1: 180 cm long by 2 mm ID glass, packed with 1.5% OV-17/1.95% OV-210 on Gas Chrom Q (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 12.1.
 - 5.6.2 Column 2: 180 cm long by 2 mm ID glass, packed with Ultrabond 20M (100/120 mesh) or equivalent.
 - 5.6.3 Detector: Electron capture. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. REAGENTS

- 6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2 Acetone, hexane, methylene chloride: Pesticide-quality or equivalent.
- 6.3 Sodium sulfate: ACS granular, anhydrous. Condition by heat in a shallow tray at 400°C for a minimum of 4 hours to remove phthalates and other interfering organic substances. Alternatively, heat 16 hours at 450 to 500°C in a shallow tray or perform a Soxhlet extraction with methylene chloride for 48 hours.
- 6.4 Stock standard solutions (1.00 $\mu\text{g}/\mu\text{L}$): Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.
 - 6.4.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in pesticide-quality hexane and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.
 - 6.4.2 Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw-cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

- 6.4.3** Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1** Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. These parameters do not adequately resolve benfluralin, ethalfluralin, and trifluralin. When more than one of these compounds may be present in a sample, the instrument must be calibrated with trifluralin. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2** External standard calibration procedure.
- 7.2.1** For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with hexane. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
- 7.2.2** Using injections of 1 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.
- 7.2.3** The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.
- 7.3** Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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.
- 7.3.1** Prepare calibration standards at a minimum of three concentration levels for each parameter 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 hexane. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

- 7.3.2** Using injections of 1 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} , against RF.

- 7.3.3** The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.
- 7.4** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.
- 8.1.1** Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2** In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
- 8.1.3** The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

- 8.2** To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
- 8.2.1** Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone, 1000 times more concentrated than the selected concentrations.
 - 8.2.2** Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
 - 8.2.3** Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.
 - 8.2.4** Table 2 provides single-operator recovery and precision for isopropalin, profluralin and trifluralin. Similar results should be expected for benfluralin and ethalfluralin. Compare these results to the values calculated in Section 8.2.3.
- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
- 8.3.1** Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁸ that are useful in observing trends in performance.

- 8.3.2** The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁸
- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

- 8.5** Before processing any samples, the analyst must demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.
- 8.6** 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 monitor the precision of the sampling technique. 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 perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

- 9.1** Grab samples must be collected in glass containers. Conventional sampling practices⁹ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2** The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3** All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

10. SAMPLE EXTRACTION

- 10.1** Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel.
- 10.2** Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.
- 10.3** Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.
- 10.4** Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.5** Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

- 10.6** Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.7** Increase the temperature of the hot water bath to about 80°C. Momentarily remove the Snyder column, add 50 mL of hexane and a new boiling chip, and reattach the Snyder column. Pour about 1 mL of hexane into the top of the Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 minutes. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.8** Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to TFE-fluorocarbon-sealed screw-cap vials. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis. If the sample requires cleanup, proceed to Section 11.
- 10.9** Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1** Cleanup procedures may not be necessary for a relatively clean sample matrix. If particular circumstances demand the use of a cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

12. GAS CHROMATOGRAPHY

- 12.1** Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. An example of the separations achieved by Column 1 is shown in Figure 1. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.
- 12.2** Calibrate the system daily as described in Section 7. Since the gas chromatographic conditions provided do not adequately separate benfluralin, ethalfluralin, and trifluralin, calibrate with trifluralin if more than one of these materials may be present in a sample.

- 12.3 If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.
- 12.4 Inject 1 to 5 μL of the sample extract using the solvent-flush technique.¹⁰ Record the volume injected to the nearest 0.05 μL , and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.
- 12.5 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

- 13.1 Determine the concentration of individual compounds in the sample.

- 13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_e)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_e = Volume of water extracted, in mL

- 13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where

A_s = Response for parameter to be measured

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_o = Volume of water extracted, in L

- 13.2** Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results. Results for benfluralin and ethalfluralin must be reported as trifluralin unless the sample has been characterized beyond the capabilities provided in this method.
- 13.3** For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. GC/MS CONFIRMATION

- 14.1** It is recommended that GC/MS techniques be judiciously employed to support qualitative compound identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 seconds per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.
- 14.2** Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.¹¹
- 14.3** At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.¹²
- 14.4** To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
- 14.4.1** All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to $\pm 10\%$. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20 to 40%.

14.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.

14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.

14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.

14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. METHOD PERFORMANCE

15.1 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.¹³ The MDL concentrations listed in Table 1 were obtained using reagent water.¹

15.2 In a single laboratory (West Cost Technical Services, Inc.) using reagent water and effluents from pesticide manufacturers and the average recoveries presented in Table 2 were obtained.¹ The standard deviations of the percent recoveries of these measurements are also included in Table 2.

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Table 1. Gas Chromatography and Method Detection Limits of Dinitroanilines

<i>Parameter</i>	<i>Retention Time Minimum</i>		<i>Method Detection Limit (µg/L)</i>
	<i>Column 1</i>	<i>Column 2</i>	
Trifluralin	1.6	2.2	0.03
Benfluralin	1.6	2.3	ND
Ethalfuralin	1.6	2.3	ND
Profluralin	2.3	3.4	0.14
Isopropalin	6.4	6.3	0.02

ND = Not determined.

Column 1 conditions: Gas Chrom Q (100/200 mesh) coated with 1.5% OV-17/1.95% OV-210 packed in a 1.8 m long by 2 mm ID glass column with 95% argon/5% methane carrier gas at a flow rate of 30 mL/min. Column temperature: isothermal at 190°C.

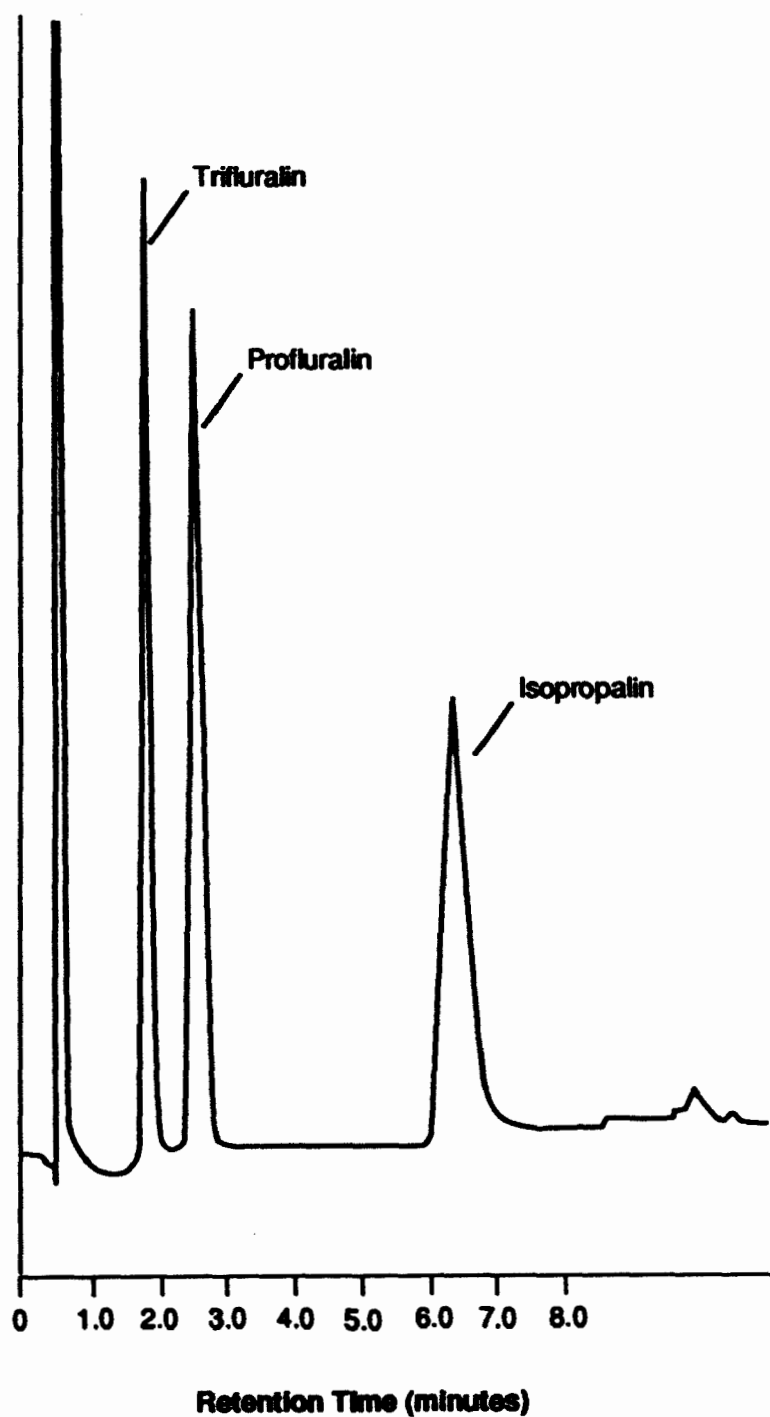
Column 2 conditions: Ultrabond 20M (100/120 mesh) packed in a 1.8 m long by 2 mm ID glass column with nitrogen carrier gas at a flow rate of 30 mL/min. Column temperature: held at 160°C for 2 minutes, then programmed to 200°C at 10°C/min.

Table 2. Single-Operator Accuracy and Precision

<i>Parameter</i>	<i>Sample Type</i>	<i>Spike Range (µg/L)</i>	<i>Number of Replicates</i>	<i>Average Percent Recovery</i>	<i>Standard Deviation %</i>
Benfluralin	IW	2.00	2	93	—
Isopropalin	DW	0.50	7	93	1.1
	IW	2.20	7	88	13.2
Profluralin	DW	0.50	7	99	9.0
	IW	2.04	7	73	5.8
Trifluralin	DW	0.50	7	97	1.8
	IW	2.08	7	77	20.0

IW = Industrial wastewater, pesticide manufacturing

DW = Reagent water



A52-002-56

Figure 1. Gas Chromatogram of Dinitroaniline Pesticides on Column 1.
For Conditions, See Table 1.

Method 629
The Determination of Cyanazine
in Municipal and Industrial
Wastewater

Method 629

The Determination of Cyanazine in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of cyanazine. The following parameter can be determined by this method:

<i>Parameter</i>	<i>STORET No.</i>	<i>CAS No.</i>
Cyanazine	--	21725-46-2

- 1.2 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compound listed above in industrial and municipal discharges as provided under 40 CFR 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 CFR 136.4 and 136.5.
- 1.3 The estimated method detection limit (MDL, defined in Section 15) for cyanazine is 6 µg/L. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.5 When this method is used to analyze unfamiliar samples for cyanazine, compound identifications should be supported by at least one additional qualitative technique.

2. SUMMARY OF METHOD

- 2.1 A measured volume of sample, approximately 1 L, is extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to methanol during concentration to a volume of 10 mL or less. HPLC conditions are described which permit the separation and measurement of cyanazine in the extract by HPLC with a UV detector.¹
- 2.2 This method provides an optional Florisil column cleanup procedure to aid in the elimination or reduction of interferences which may be encountered.

3. INTERFERENCES

- 3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified³⁻⁵ for the information of the analyst.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)

- 5.2.1 Separatory funnel: 2000-mL, with TFE-fluorocarbon stopcock, ground-glass or TFE stopper.
- 5.2.2 Drying column: Chromatographic column 400 mm long by 19 mm ID with coarse-fritted disc.
- 5.2.3 Chromatographic column: 400 mm long by 19 mm ID with coarse-fritted disc at bottom and TFE-fluorocarbon stopcock (Kontes K-420540-0224 or equivalent).
- 5.2.4 Concentrator tube, Kuderna-Danish: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground-glass stopper is used to prevent evaporation of extracts.
- 5.2.5 Evaporative flask, Kuderna-Danish: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.
- 5.2.6 Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).
- 5.2.7 Vials: Amber glass, 10- to 15-mL capacity with TFE-fluorocarbon-lined screw-cap.
- 5.3 Boiling chips: Approximately 10/40 mesh. Heat at 400°C for 30 minutes or perform a Soxhlet extraction with methylene chloride.
- 5.4 Water bath: Heated, with concentric ring cover, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.
- 5.5 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6 Filtration apparatus: As needed to filter chromatographic solvents prior to HPLC.
- 5.7 Liquid chromatograph: High performance analytical system complete with high pressure syringes or sample injection loop, analytical columns, detector, and strip-chart recorder. A guard column is recommended for all applications.
 - 5.7.1 Gradient pumping system, constant flow.
 - 5.7.2 Column: 25 cm long by 2.6 mm ID stainless steel packed with Spherisorb ODS (10 μm) or equivalent. This column was used to develop the method performance statements in Section 14. Alternative columns may be used in accordance with the provisions described in Section 12.1.
 - 5.7.3 Detector: Ultraviolet, 254 nm. This detector has proven effective in the analysis of wastewaters for cyanazine and was used to develop the method performance statements in Section 14. Alternative detectors may be used in accordance with the provisions described in Section 12.1.

6. REAGENTS

- 6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2 Acetone, hexane, methylene chloride: Pesticide-quality or equivalent.

- 6.3 Ethyl ether: Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Cat. No. P1126-8, and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.
- 6.4 Methanol: HPLC/UV quality.
- 6.5 Sodium sulfate: ACS, granular, anhydrous. Condition by heating in a shallow tray at 400°C for a minimum of 4 hours to remove phthalates and other interfering organic substances. Alternatively, heat 16 hours at 450 to 500°C in a shallow tray or perform a Soxhlet extraction with methylene chloride for 48 hours.
- 6.6 Florisil: PR grade (60/100 mesh). Purchase activated at 675°C and store in dark in glass container with ground-glass stopper or foil-lined screw-cap. Before use, activate each batch at least 16 hours at 130°C in a foil-covered glass container.
- 6.7 Stock standard solution (1.00 µg/µL): A stock standard solution may be prepared from pure standard material or purchased as a certified solution.
 - 6.7.1 Prepare a stock standard solution by accurately weighing approximately 0.0100 g of cyanazine. Dissolve the material in UV quality methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.
 - 6.7.2 Transfer the stock standard solution into a TFE-fluorocarbon-sealed screw-cap vial. Store at 4°C and protect from light. Frequently check the stock standard solution for signs of degradation or evaporation, especially just prior to preparing calibration standards from it.
 - 6.7.3 The stock standard solution must be replaced after 6 months, or sooner if comparison with a check standard indicates a problem.

7. CALIBRATION

- 7.1 Establish HPLC operating parameters equivalent to those indicated in Table 1. The HPLC system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2 External standard calibration procedure:
 - 7.2.1 Prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of stock standard to volumetric flasks and diluting to volume with methanol. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
 - 7.2.2 Using injections of 10 µg/L of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for cyanazine. Alternatively, the ratio of the response to the mass injected, defined

as the calibration factor (CF), may be calculated at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared.

7.3 Internal standard calibration procedure: To use this approach, the analyst must select an internal standard similar in analytical behavior to cyanazine. 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.

7.3.1 Prepare calibration standards at a minimum of three concentration levels by adding volumes of stock standard to volumetric flasks. To each calibration standard, add a known constant amount of internal standard, and dilute to volume with methanol. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

7.3.2 Using injections of 10 $\mu\text{g/L}$ of each calibration standard, tabulate the peak height or area responses against the concentration for both cyanazine and internal standard. Calculate response factors (RF) as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared.

- 7.4** The cleanup procedure in Section 11 utilizes Florisil chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil which is used, the use of the lauric acid value is suggested. This procedure⁶ determines the adsorption from hexane solution of lauric acid, in milligrams per gram of Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.
- 7.5** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.
- 8.1.1** Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2** In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
- 8.1.3** The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2** To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
- 8.2.1** Select a representative spike concentration. Using stock standard, prepare a quality control check sample concentrate in methanol, 1000 times more concentrated than the selected concentrations.
- 8.2.2** Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels; and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
- 8.2.3** Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.
- 8.2.4** Using the appropriate data from Table 2, determine the recovery and single-operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁷ that are useful in observing trends in performance.

- 8.3.2** The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁷

- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery of cyanazine does not fall within the control limits for method performance, the results reported for cyanazine in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.
- 8.5** Before processing any samples, the analyst must demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.
- 8.6** 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 monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram as cyanazine, confirmatory techniques, such as chromatography with a dissimilar column or ratio of absorbance at two or more wavelengths, must be used. Whenever possible, the laboratory should perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

- 9.1** Grab samples must be collected in glass containers. Conventional sampling practices⁸ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

- 9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

10. SAMPLE EXTRACTION

- 10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel.
- 10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.
- 10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.
- 10.4 It is necessary to exchange the extract solvent to hexane if the Florisil cleanup procedure is to be used. For direct HPLC analysis, the extract solvent must be changed to methanol. The analyst should only exchange a portion of the extract to methanol if there is a possibility that cleanup may be necessary.
- 10.5 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.6 Pour a measured fraction or all of the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.
- 10.7 Add 1 or 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.
- 10.8 Increase the temperature of the hot water bath to about 80°C. Momentarily remove the Snyder column, add 50 mL of hexane or methanol and a new boiling chip, and reattach the Snyder column. Pour about 1 mL of solvent into the top of the Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 minutes. When the

apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

- 10.9** Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane or methanol and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than 2 days, they should be transferred to TFE-fluorocarbon-sealed screw-cap vials. If the sample extract requires no further cleanup, proceed with HPLC analysis. If the sample requires cleanup, proceed to Section 11.
- 10.10** Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1** Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various industrial and municipal effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of cyanazine for the cleanup procedure is no less than 85%.
- 11.2** The following Florisil column cleanup procedure has been demonstrated to be applicable to cyanazine.
- 11.2.1** Add a weight of Florisil (nominally 20 g) predetermined by calibration (Section 7.4 and 7.5) to a chromatographic column. Settle the Florisil by tapping the column. Add anhydrous sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatography column. Discard the eluate.
- 11.2.2** Adjust the sample extract volume to 10 mL with hexane and transfer it from the K-D concentrator tube to the Florisil column. Rinse the tube twice with 1 to 2 mL hexane, adding each rinse to the column.
- 11.2.3** Drain the column until the sodium sulfate layer is nearly exposed. Elute the column with 200 mL of 6% ethyl ether in hexane (V/V) (Fraction 1) and with 200 mL of 15% ethyl ether in hexane (V/V) (Fraction 2) using a drip rate of about 5 mL/min. These fractions may be discarded. Place a 500-mL K-D flask and clean concentrator tube under the chromatography column. Elute the column with 200 mL of 50% ethyl ether in hexane (V/V) (Fraction 3) into the K-D flask. Cyanazine elutes quantitatively in Fraction 3.
- 11.2.4** Concentrate the eluate by standard K-D techniques (Section 10.7), exchanging the solvent to methanol. Adjust final volume to 10 mL with methanol. Analyze by HPLC.

12. LIQUID CHROMATOGRAPHY

- 12.1** Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are the estimated retention time and method detection limit that can be achieved by this method. An example of the separations achieved by this column is shown in

Figure 1. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.

- 12.2 Calibrate the system daily as described in Section 7.
- 12.3 If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.
- 12.4 Inject 10 µg/L of the sample extract. Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units.
- 12.5 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

- 13.1 Determine the concentration of cyanazine in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_s)}$$

where

A = Amount of cyanazine injected, in nanograms.

V_i = Volume of extract injected, in µg/L

V_t = Volume of total extract, in µg/L

V_s = Volume of water extracted, in mL

- 13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for cyanazine

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_o = Volume of water extracted, in liters

- 13.2** Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 13.3** For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for cyanazine must be labeled as suspect.

14. METHOD PERFORMANCE

- 14.1** 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.⁹ The MDL concentration listed in Table 1 was estimated from the response of a 254 nm UV detector to the compound. The estimate is based upon the amount of material required to yield a signal 5 times the HPLC background noise, assuming a 10- μg injection from a 10-mL final extract of a 1-L sample.
- 14.2** In a single laboratory (West Cost Technical Services, Inc.), using effluents from pesticide manufacturers and publicly owned treatment works (POTW), the average recoveries presented in Table 2 were obtained.¹ The standard deviations of the percent recoveries of these measurements are also included in Table 2.

References

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5. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
6. ASTM Annual Book of Standards, Part 31, D3086, Appendix X3, "Standardization of Florisil Column by Weight Adjustment Based on Adsorption of Lauric Acid," American Society for Testing and Materials, Philadelphia, PA, p. 765, 1980.
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8. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.
9. Glaser, J.A. et.al, "Trace Analysis for Wastewaters," *Environmental Science & Technology*, 15, 1426 (1981).

Table 1. Chromatographic Conditions and Estimated Detection Limit

<i>Parameter</i>	<i>Retention Time (min)</i>	<i>Estimated MDL (µg/L)</i>
Cyanazine	10.0	6

Column conditions: Spherisorb ODS (10 µm) packed in a 25 cm long by 2.6 mm ID stainless steel column with a mobile phase flow rate of 1.0 mL/min. Mobile phase: Linear gradient from 50% solvent B to 100% solvent B in 2 min, where solvent A is 25% methanol in water and solvent B is 50% methanol in water.

Table 2. Single-Operator Accuracy and Precision

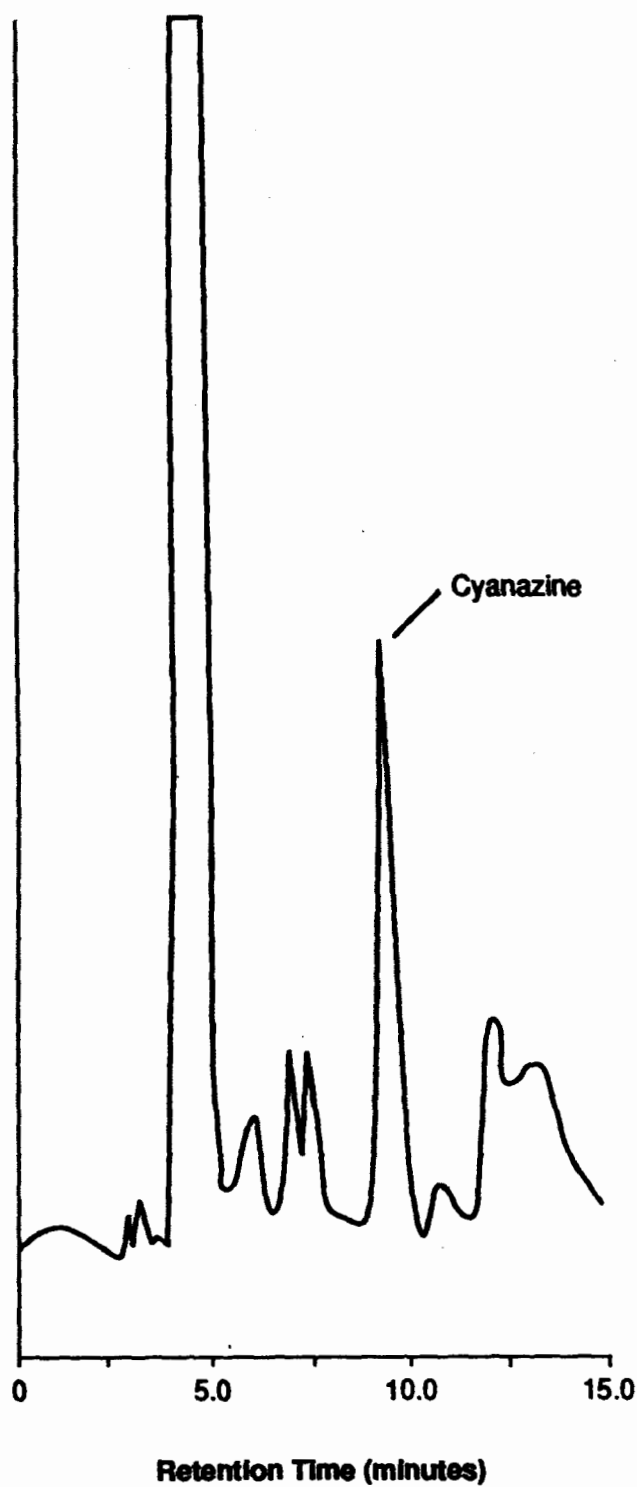
<i>Parameter</i>	<i>Sample Type</i>	<i>Spike (µg/L)</i>	<i>No. of Replicates</i>	<i>Average Percent Recovery</i>	<i>Standard Deviation (%)</i>
Cyanazine	DW	121	7	100.0	8.9
	MW	60.8	7	85.5	3.9
	PW	10,100	3	94.3	-
	IW	10,100	2	78.0	-

DW = Reagent water

MW = Municipal wastewater

PW = Process water, pesticide manufacturing

IW = Industrial wastewater, pesticide manufacturing



A52-002-50

Figure 1. Liquid Chromatogram of Cyanazine in Process Water Extract on Column 1. For Conditions, See Table 1.

Method 630
The Determination
of Dithiocarbamate
Pesticides in Municipal and
Industrial Wastewater

Method 630

The Determination of Dithiocarbamate Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of dithiocarbamate pesticides. The following parameters can be determined by this method:

<i>Parameter</i>	<i>STORET No.</i>	<i>CAS. No.</i>
Amoban		3566-10-7
AOP		—
Busan 40		51026-28-9
Busan 85		128-03-0
Ferbam		14484-64-1
KN Methyl		137-41-7
Mancozeb		8018-01-7
Maneb		12427-38-1
Metham		137-42-8
Nabam		142-59-6
Niacide		8011-66-3
Polynam		9006-42-2
Sodium dimethyldithiocarbamate		128-04-1
Thiram		137-26-8
ZAC		—
Zineb		12122-67-7
Ziram		137-30-4

- 1.2 This method fails to distinguish between the individual dithiocarbamates. The compounds above are reduced to carbon disulfide and the total dithiocarbamate concentration is measured. Unless the sample can be otherwise characterized, all results are reported as Ziram. Carbon disulfide is a known interferent.
- 1.3 This is a colorimetric method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.4 The method detection limit (MDL, defined in Section 12) for maneb, metham and ziram are listed in Table 1. The MDL for a specific dithiocarbamate or wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.5 This method is restricted to use by or under the supervision of analysts experienced in trace organic analyses. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. SUMMARY OF METHOD

- 2.1** A measured volume of sample, approximately 1 L, is digested with acid to yield carbon disulfide by hydrolysis of the dithiocarbamate moiety. The evolved CS₂ is purged from the sample and absorbed by a color reagent. The absorbance of the solution is measured at 380 and 435 nm using a UV-visible spectrophotometer.¹

3. INTERFERENCES

- 3.1** Method interferences may be caused by contaminants in reagents, glassware, and other sample processing hardware that lead to high blank values and biased results. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.² After each use, rinse the decomposition flask and condenser with 4N NaOH and reagent water. Overnight soaking in 4N NaOH may be necessary. Clean the H₂S scrubber between each use with 0.1N HCl in methanol, rinse three times with methanol, and bake at 200°C for 15 minutes. Rinse the CS₂ trap with methanol three times between each use and follow by heating for 15 minutes at 200°C. Should it become difficult to force the color reagent through the glass frit of the CS₂ trap, clean in the same manner as the H₂S scrubber. After cooling, store glassware sealed to prevent any accumulation of dust or other contaminants.

3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems.

- 3.2** Carbon disulfide may be a significant direct interferent in wastewaters. Its elimination or control is not addressed in this method. If correction for background carbon disulfide is required, the CS₂ should be measured by an independent procedure, such as direct aqueous injection gas chromatography.

- 3.3** Additional matrix interferences may be caused by contaminants that are codistilled from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup provided by the H₂S trap will eliminate or reduce some of these interferences, but unique samples may require additional clean-up approaches to achieve the MDL listed in Table 1.

4. SAFETY

- 4.1** The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified³⁻⁵ for the information of the analyst.

5. APPARATUS AND MATERIALS.

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Dithiocarbamate hydrolysis apparatus (Figure 1): (Available from Southern Scientific Inc., Box 83, Micanopy, Florida 32267). Apparatus includes the following or equivalent components:

5.2.1 Hot plate with magnetic stirrer.

5.2.2 Hydrolysis flask: 2-L, flat bottom with ground-glass joints, 2 necks.

5.2.3 Condenser: Low internal volume, ground-glass joints, Liebig (Kontes K-447000, 100 mm or equivalent).

5.2.4 Gas-washing bottles: 125-mL, with extra-coarse porosity (Kontes K-657750 or equivalent).

5.2.5 Addition funnel: 60-mL, ground-glass joint to fit hydrolysis flask, with long stem to reach at least 2 cm below the liquid level in the hydrolysis flask.

5.2.6 Dust trap (adapter): To fit top of addition funnel (Kontes K-174000 or equivalent).

5.2.7 Vacuum source: Stable pressure with needle valve for control.

5.3 UV-visible spectrophotometer: Double beam with extended cell path length capability of 1.0 and 4.0 cm cells.

5.4 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g. The preparation of calibration standards for some dithiocarbamates (e.g., metham) requires the use of a balance capable of weighing 10 µg.

6. REAGENTS

6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest. Prepare by boiling distilled water 15 minutes immediately before use.

6.2 Acetonitrile, diethanolamine, methanol: ACS grade.

6.3 Ethanol: 95%.

6.4 Cupric acetate: Monohydrate, ACS grade.

6.5 Hydrochloric acid: Concentrated.

- 6.6 Hydrochloric acid, 0.1N in methanol: Slowly add 8.3 mL concentrated HCl to methanol and dilute to 100 mL.
- 6.7 Sodium hydroxide, 4N: Dissolve 16 g ACS grade NaOH pellets in reagent water and dilute to 100 mL.
- 6.8 Stannous chloride: $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, ACS grade.
- 6.9 Zinc acetate solution, 20%: Dissolve 20 g ACS grade $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2) \cdot 2\text{H}_2\text{O}$ in reagent water and dilute to 100 mL.
- 6.10 Color reagent: Add 0.012 g cupric acetate monohydrate to 25 g diethanolamine. Mix thoroughly while diluting to 250 mL with ethanol. Store in amber bottle with TFE-fluorocarbon-lined cap.
- 6.11 Decomposition reagent: Dissolve 9.5 g stannous chloride in 300 mL concentrated hydrochloric acid. Prepare fresh daily.
- 6.12 Stock standard solutions (1.00 $\mu\text{g}/\mu\text{L}$): Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.
 - 6.12.1 Prepare a stock standard solution for ziram by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in acetonitrile and dilute to volume in a 1-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.
 - 6.12.2 Transfer the stock standard solution into a TFE-fluorocarbon-sealed screw-cap vial. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
 - 6.12.3 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.
 - 6.12.4 When using other dithiocarbamates for calibration, such as maneb or metham, it may be necessary to weigh microgram amounts of the pure material into small aluminum foil boats and place them directly in the hydrolysis flask.

7. CALIBRATION

- 7.1 Use ziram as the standard for total dithiocarbamates when a mixture of dithiocarbamates is likely to be present. Use the specific dithiocarbamate as a standard when only one pesticide is present and its identity has been established.
- 7.2 With the apparatus assembled and reagents in place, Section 10, pour 1,500 mL of reagent water into each decomposition flask, add 30 mL of decomposition reagent, and start aspiration.
- 7.3 Spike the water in each flask with an accurately known weight of dithiocarbamate standard. Use a series of weights equivalent to 5 to 200 μg of CS_2 . Follow the procedure outlined Section 10.
- 7.4 Prepare calibration curves at a minimum of three concentrations by plotting absorbance vs. weight of dithiocarbamate. A separate curve is prepared from readings taken at 435 nm and at 380 nm for each cell path length used. Normally the 435 nm curve is used for calibration above 30 μg

ziram (4 cm cell), and the 380 nm curve is used for calibration below 30 µg ziram. The choice of which curve to use is left to the discretion of the analyst. It is recommended that the curves be transformed into mathematical equations using linear least squares fit for the data from 435 nm and quadratic least squares fit for data from the 380 nm.

- 7.5** The working calibration curve must be verified on each working shift by the measurement of one or more calibration standards. If the response varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared.

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.
- 8.1.1** Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2** The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2** To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
- 8.2.1** Select a representative spike concentration for each compound to be measured.
- 8.2.2** Add the known amount of dithiocarbamate standard to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
- 8.2.3** Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.
- 8.2.4** Using the appropriate data from Table 1, determine the recovery and single-operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.
- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
- 8.3.1** Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁶ that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁶

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 11.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.

8.6 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 monitor the precision of the sampling technique. Whenever possible, the laboratory should perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

9.1 Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 All samples must be analyzed within 7 days of collection.

10. SAMPLE ANALYSIS

10.1 Assemble the hydrolysis apparatus as follows (see Figure 1):

10.1.1 Place the hydrolysis flask on the hot plate.

10.1.2 Place the addition funnel in one of the necks of the hydrolysis flask and the dust trap in the top of the funnel.

- 10.1.3** Place the condenser in the other neck and attach two gas-washing bottles in succession to the condenser outlet.
- 10.1.4** Attach a vacuum line with a flow valve to the second scrubber .
- 10.2** Allow the sample to warm to room temperature. Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into the 2-L hydrolysis flask. Rinse the bottle four times with 100-mL aliquots of reagent water, adding the washes to the hydrolysis flask. Bring the volume in the hydrolysis flask to approximately 1,500 mL with reagent water.
- 10.3** Place 5.0 mL of color reagent into the CS₂ trap (second gas-washing bottle). Place 9 mL of zinc acetate solution into the H₂S scrubber (first gas washing bottle). Add 2 mL of ethanol to the H₂S scrubber. Place a magnetic stirring bar in the hydrolysis flask and place the flask on the hotplate/magnetic stirrer (ambient at this time). Assemble the apparatus providing adequate support for all glassware. The addition funnel stem opening must be below the water level. Ground-glass joints may be slightly coated with silicone grease.
- 10.4** Start the stirrer, begin water flow through the condenser, and turn on hot plate and begin heating the flask. Open the needle valve slightly and start the aspirator. By closing the needle valve, adjust the airflow through the absorption train until the proper flow is attained. (The column of bubbles extends to the bottom of the spherical expansion chamber at the top of the CS₂ trap.) Add 30 mL of decomposition reagent to the flask.

NOTE: The analyst must ensure that the sample pH is less than 2 during hydrolysis.

- 10.5** Bring the liquid in the flask to a gentle boil. Continue the boiling for 60 minutes, then remove the heat. Continue aspiration until boiling ceases.
- 10.6** Transfer the contents of the CS₂ trap into a 25.0-mL volumetric flask by forcing the liquid through the glass frit and out of the inlet arm with pressure from a large pipette bulb. Ensure quantitative transfer by rinsing the trap three times with ethanol. Bring the colored solution to volume with ethanol. Mix thoroughly and allow the color to develop for at least 15 minutes but not more than 2 hours before determining the absorbance.
- 10.7** Determine the absorbance of the sample at 435 nm and 380 nm using a 1-cm cell or a 4-cm cell as necessary. Determine the weight of dithiocarbamate from the appropriate calibration curve prepared in Section 7.4.
- 10.8** Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1,000-mL graduated cylinder. Record the sample volume to the nearest 5 mL. If a smaller measured aliquot of sample was used to remain within the range of the color reagent, this step may be omitted.

11. CALCULATIONS

- 11.1** Determine the concentration of total dithiocarbamates in the sample as ziram directly from the calibration curve. When a specific dithiocarbamate is being measured, quantitate in terms of the selected pesticide.
- 11.2** Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 11.3** For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

12. METHOD PERFORMANCE

- 12.1** 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.⁸ The MDL concentrations listed in Table 1 were determined using wastewater, and are expressed in concentration units of the spiked materials.¹
- 12.2** In a single laboratory, Environmental Science and Engineering, using spiked wastewater samples, the average recoveries presented in Table 1 were obtained. The percent standard deviation of the recovery is also included in Table 1.¹ All recoveries are based on calibrations using the specific dithiocarbamate instead of ziram.

References

1. "Pesticides Methods Development," Report for EPA Contract 68-03-2897 (In preparation).
2. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.
3. "Carcinogens - Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.
4. "OSHA Safety and Health Standards, General Industry," (29 *CFR* 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).
5. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
6. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U. S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory - Cincinnati, Ohio 45268, March 1979.
7. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.
8. Glaser, J.A. et.al, "Trace Analysis for Wastewaters," *Environmental Science & Technology*, 15, 1426 (1981).

Table 1. Method Performance

<i>Parameter</i>	<i>Method Detection Limit ($\mu\text{g/L}$)</i>	<i>Sample Type*</i>	<i>Number of Replicates</i>	<i>Spike ($\mu\text{g/L}$)</i>	<i>Mean Recovery (%)</i>	<i>Standard Deviation (%)</i>
Maneb	15.3	1	7	31.5	97.1	15.5
Metham	3.7	2	7	20.1	94.5	5.9
		3	7	250.0	65.2	2.8
Ziram	1.9	4	8	32.2	100.0	2.0
		5	8	1050.0	96.2	10.0

*Sample type:

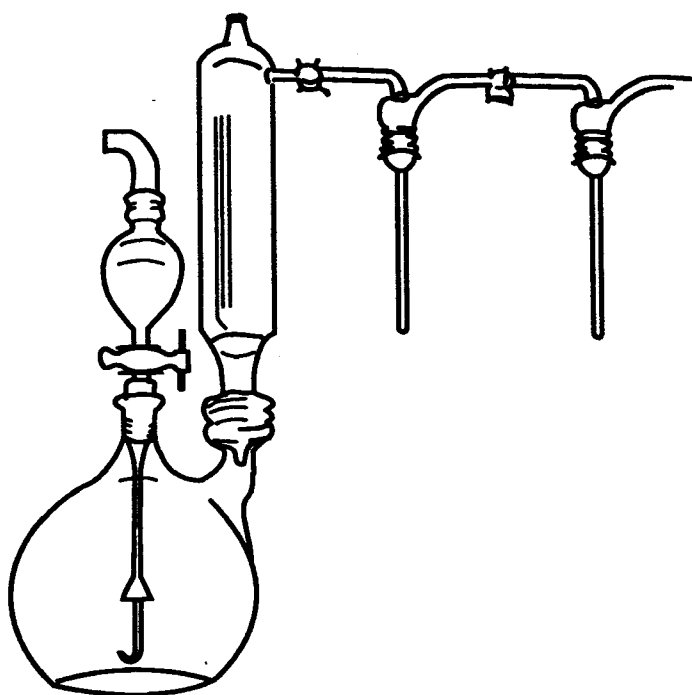
1 = Municipal wastewater

2 = Mixture of 13% industrial (pesticide manufacturing) wastewater and 87% municipal wastewater

3 = Industrial wastewater, pesticide manufacturing

4 = Mixture of 40% industrial and 60% municipal wastewater

5 = 7% industrial process water, 7% industrial wastewater, 86% municipal wastewater



A52-002-60

Figure 1. Dithiocarbamate Hydrolysis Apparatus.

Method 630.1
The Determination of
Dithiocarbamates Pesticides in
Municipal and Industrial
Wastewater

Method 630.1

The Determination of Dithiocarbamates Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of certain dithiocarbamates pesticides after conversion to carbon disulfide. The following parameters can be determined by this method:

<i>Parameter</i>	<i>CAS. No.</i>
Amobam	3566-10-7
Busan 40	51026-28-9
Busan 85	128-03-0
EXD	502-55-6
Ferbam	14484-64-1
KN Methyl	137-41-7
Metham	137-42-8
Nabam	142-59-6
Nabonate	138-93-2
Sodium dimethyldithiocarbamate	128-04-1
Thiram	137-26-8
Zineb	12122-67-7
Ziram	137-30-4

- 1.2 The compounds are decomposed to form carbon disulfide (CS₂) and the total dithiocarbamate concentration is measured from the amount of CS₂ produced by acid hydrolysis. Unless the sample can be otherwise characterized, all results are reported as ziram.
- 1.3 This is a total-residue gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.4 The method detection limits (MDLs, defined in Section 14) for the parameters listed in Section 1.1 are listed in Table 1. The MDLs for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. SUMMARY OF METHOD¹

- 2.1** A measured 5-mL volume of sample is digested with acid to yield CS₂ by hydrolysis of the dithiocarbamate moiety. The evolved CS₂ is extracted from water into hexane. Gas chromatographic conditions are described which permit the separation and measurement of CS₂ in the extract by gas chromatography with a Hall detector in the sulfur mode.
- 2.2** This method provides a cleanup procedure involving purging of any indigenous CS₂ from the sample at pH 12 to 13. This procedure is performed using a vortex evaporator.

3. INTERFERENCES

- 3.1** Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.
 - 3.1.1** Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water, drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.
 - 3.1.2** The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.2** Carbon disulfide may be a direct interferent in wastewaters. This method includes procedures to purge CS₂ from the wastewater prior to acid hydrolysis of the sample. A vortex evaporator is used for CS₂ removal.
- 3.3** Additional matrix interferences may be caused by contaminants that are coextracted from the sample and from other CS₂ generating compounds. The extent of matrix interferences will vary considerably from source to source, depending upon the nature of the sample.

4. SAFETY

- 4.1** The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified³⁻⁵ for the information of the analyst.
- 4.2** Nabam (ethylene bis (dithiocarbamate)) has been identified as having substantial evidence of carcinogenicity and should be handled according to OSHA regulations.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Sample containers: 40-mL screw-cap vials (Pierce No. 13075 or equivalent): each equipped with a polytetrafluoroethylene (PTFE)-faced silicone septum (Pierce No. 12722 or equivalent). Prior to use, wash vials and septa with detergent and rinse with tap and distilled water. Allow the vials and septa to air dry at room temperature, place in a 105°C oven for 1 hour, then remove and allow to cool in an area known to be free of organics.

5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Glassware.

5.2.1 Centrifuge tube: 15-mL, conical, with PTFE-lined screw-cap.

5.2.2 Volumetric flask: 250-mL with glass stopper.

5.2.3 Bottles: 100- to 200-mL capacity with PTFE-lined screw-caps.

5.3 Vortex Evaporator: Buchler 3-2200, equipped with sample block to hold 36 15-mL conical-bottom centrifuge tubes and appropriate vacuum cover.

5.4 Water bath: Heated, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.

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

5.6 Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column: 180 cm by 2 mm ID glass, packed with 0.1% SP-1000 on Carbowax C (80/100 mesh) or equivalent. This column was used to develop the method performance statements in Section 14. Alternative columns may be used in accordance with the provisions described in Section 11.1.

5.6.2 Detector: Hall detector operated in the sulfur mode. This detector has proven effective in the analysis of wastewaters for the compounds listed in the scope and was used to develop the method performance statements in Section 14.

6. REAGENTS

6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the MDL of each parameter of interest.

6.2 Hexane: Distilled-in-glass quality or equivalent.

6.3 Sulfuric acid, 12N: Slowly add 100 mL concentrated sulfuric acid to 200 mL reagent water.

- 6.4 Sodium phosphate, tribasic, dodeca-hydrate: Baker reagent grade or equivalent.
- 6.5 Tribasic sodium phosphate, 0.1M: Dissolve 38 g of tribasic sodium phosphate in reagent water and dilute to 1000 mL with reagent water.
- 6.6 Stannous chloride: $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, ACS grade.
- 6.7 Stannous chloride reagent: Dissolve 1.5 g stannous chloride in 100 mL 12N sulfuric acid. Prepare fresh daily.
- 6.8 Sodium chloride: Heated at 45°C for 8 hours.
- 6.9 Stock standard solutions (0.1 $\mu\text{g}/\mu\text{L}$): Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.
 - 6.9.1 Prepare dithiocarbamate spiking solutions by accurately weighing about 0.025 g of pure material. Dissolve the material in 0.1M Na_3PO_4 and dilute to volume in a 250-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 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.
 - 6.9.2 (0.1 $\mu\text{g}/\mu\text{L}$) Prepare CS_2 stock standard solution by adding 7.9 μL of CS_2 to hexane and diluting to volume in a 100-mL volumetric flask.
 - 6.9.3 Transfer the stock standard solutions into PTFE-sealed screw-cap bottles. Store at 4°C. Frequently check standard solutions for signs of degradation or evaporation.
 - 6.9.4 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1 Use ziram as the standard for total dithiocarbamates when a mixture of dithiocarbamates is likely to be present. Use the specific dithiocarbamate as a standard when only one pesticide is present and its identity has been established.
- 7.2 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system can be calibrated using the external standard technique (Section 7.3).
- 7.3 External standard calibration procedure:
 - 7.3.1 Prepare calibration standards at a minimum of three concentration levels by adding volumes of the CS_2 stock standard to a volumetric flask and diluting to volume with hexane. One of the external standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
 - 7.3.2 Using injections of 1 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for CS_2 . Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated at each standard concentration. If the

relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.3.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for CS₂ varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate the absence of interferences from the reagents.

8. QUALITY CONTROL

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

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

8.2.1 Select a representative spike concentration for each compound to be measured.

8.2.2 Add a known amount of an individual dithiocarbamate standard to a minimum of four 5-mL aliquots of 0.1M tribasic sodium phosphate. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 2, determine the recovery and single-operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁶ that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁶

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples processed as part of the same set must be qualified as described in Section 12.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a 5-mL aliquot of 0.1M tribasic sodium phosphate that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 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 monitor the precision of the sampling technique. 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 perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. SAMPLES COLLECTION, PRESERVATION, AND HANDLING

9.1 Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 Add 15.2 g of tribasic sodium phosphate per 40 mL of sample to the sample to adjust pH to 12 to 13 at time of collection.

10. SAMPLE CLEANUP AND EXTRACTION

- 10.1 Place 5 mL of sample in a 15-mL conical centrifuge tube.
- 10.2 Add 0.75 g of NaCl and shake tube to dissolve salt.
- 10.3 Add 2 mL of MTBE and process in a vortex evaporator for 10 minutes with the temperature at 30°C, a vacuum of 30 inches Hg, and the vortex speed control set at 4.5.
- 10.4 Repeat step in Section 10.3 twice.
- 10.5 Add 0.75 mL of hexane and 2.5 mL of SnCl₂ reagent to the aqueous layer. Cap tube tightly and invert in a water bath at 50°C for 30 minutes.
- 10.6 Remove tube from water bath and let cool inverted to room temperature.
- 10.7 Shake tube for 1 minute without venting. Analyze the hexane layer by GC with a Hall detector in the sulfur mode. If CS₂ levels are outside of the GC calibration range, the sample can be diluted a known amount with hexane and reanalyzed.

11. GAS CHROMATOGRAPHY

- 11.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention time and MDLs that can be achieved by this method. An example of the chromatography achieved from Column 1 is shown in Figure 1. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.
- 11.2 Calibrate the gas chromatographic system daily as described in Section 7.
- 11.3 Inject 1 to 5 µL of the sample extract using the solvent flush technique.⁸ Record the volume injected to the nearest 0.05 µL, and the resulting peak sizes in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.
- 11.4 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 11.5 If the response for the peak exceeds the working range of the system, dilute the extract with hexane and reanalyze.
- 11.6 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

12. CALCULATIONS

- 12.1 Determine the concentration of carbon disulfide in the sample.
 - 12.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in

Section 7.2.2. The concentration of dithiocarbamate in the sample can be calculated as follows:

Equation 1

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)(M_c)}{(V_t)(V_e)(76)(C)}$$

where

A = Amount of CS_2 injected, in ng

V_i = Volume of extract injected, in $\mu\text{g/L}$

V_t = Volume of total extract, in μL

V_e = Volume of water extracted, in mL

M_c = Molecular weight of dithiocarbamate

C = Theoretical number of moles of CS_2 formed per mole of dithiocarbamate

- 12.2 Determine the concentration of total dithiocarbamates in the sample as ziram. When a specific dithiocarbamate is being measured, quantitate in terms of the selected pesticide.
- 12.3 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 12.4 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

13. METHOD PERFORMANCE

- 13.1 The 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.⁹ The MDL concentrations listed in Table 1 were obtained using spiked reagent water samples.¹
- 13.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from 10 $\mu\text{g/L}$ to 1000 $\mu\text{g/L}$.
- 13.3 In a single laboratory, Battelle Columbus Laboratories, using spiked wastewater samples, the average recoveries of the parameters listed in Section 1.1 presented in Table 2 were obtained. Seven replicates of the wastewater were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 2.¹

References

1. "Determination of Pesticides and Priority Pollutants in Industrial and Municipal Wastewaters," EPA Contract Report 68-03-1760, Work Assignment 4 (in preparation).
2. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, Pennsylvania, p. 679, 1980.
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Table 1. Chromatographic Conditions and Method Detection Limits

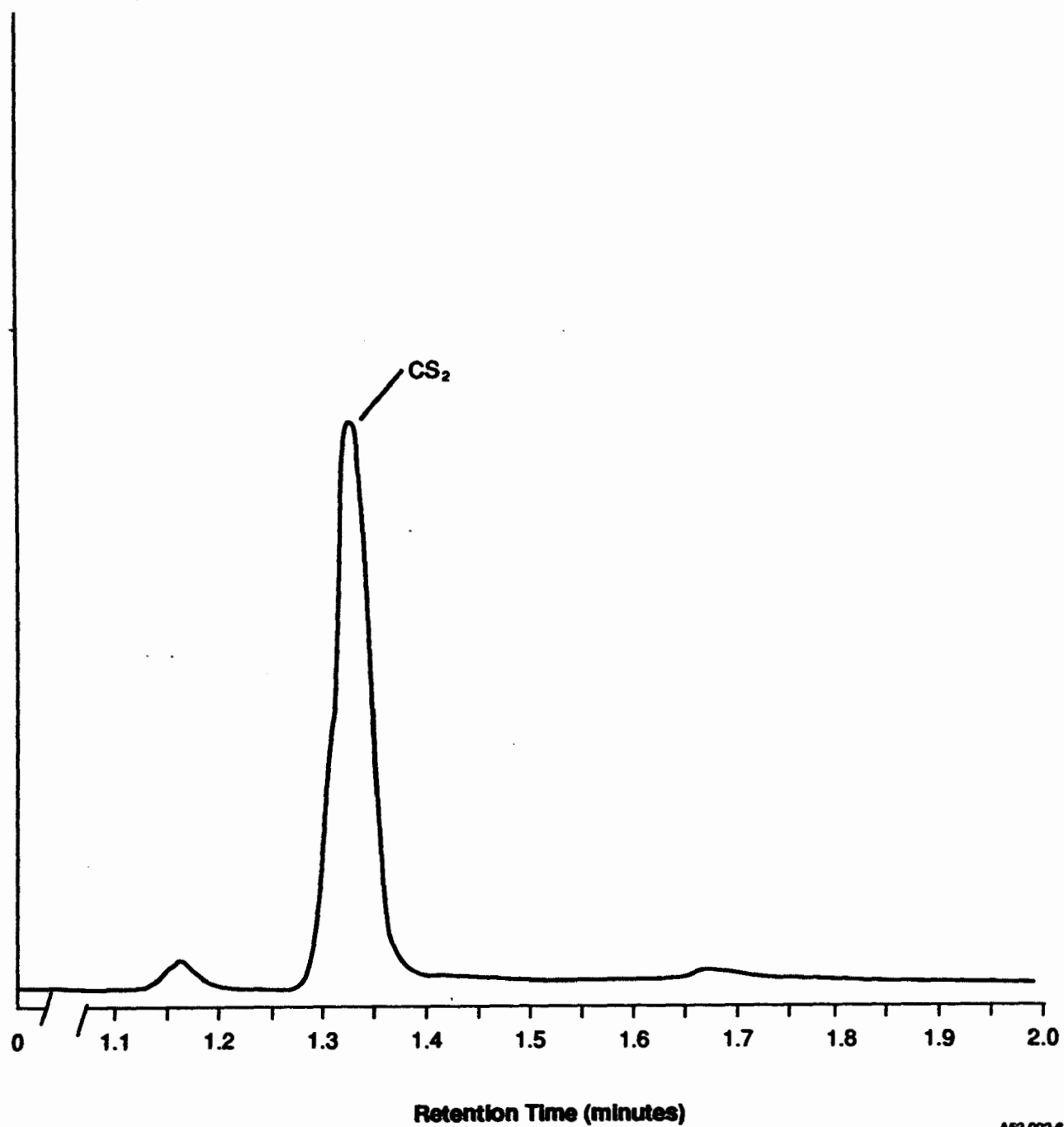
<i>Parameter</i>	<i>Retention Time (min.)(a)</i>	<i>MDL (µg/L)</i>
Amobam	1.3	1.1
Busan 40	1.3	4.4
Busan 85	1.3	1.3
EXD	1.3	5.2
Ferbam	1.3	2.9
KN Methyl	1.3	2.7
Metham	1.3	3.1
Nabam	1.3	1.6
Nabonate	1.3	0.9
Na DMDTC	1.3	2.8
Thiram	1.3	2.2
Zineb	1.3	4.1
Ziram	1.3	4.6

(a) Retention time of CS₂ under the following conditions: Carbopack C (80/100 mesh) coated with 0.1% Sp-1000 packed in a 180 cm long by 2 mm ID glass column with helium carrier gas at a flow rate of 25 mL/min. Column temperature held at 7°C for 3 minutes, programmed at 20°C/min to 120°C, and then held at 120°C for 5 minutes. Column effluent is vented from the Hall detector after elution of CS₂ from the column. Injector temperature and detector temperatures are 200°C. The Hall detector is operated in the sulfur mode following manufacturer's specifications.

Table 2. Single-Laboratory Accuracy and Precision

<i>Parameter</i>	<i>Sample Type(a)</i>	<i>Background (µg/L)</i>	<i>Spike (µg/L)</i>	<i>Mean Recovery (%)</i>	<i>Standard Deviation</i>	<i>Number of Replicates</i>
Amobam	1	4.6	50	90	7.8	7
	1	4.6	500	93	8.7	7
Busan 40	1	6.6	50	110	7.2	7
	1	6.6	500	100	6.1	7
Busan 85	1	5.9	50	110	5.5	7
	1	5.9	500	100	2.0	7
END	1	4.5	50	71	7.5	7
	1	4.5	500	76	2.4	7
Ferbam	1	5.2	50	94	4.8	7
	1	5.2	500	110	1.8	7
KN Methyl	1	5.4	50	90	6.1	7
	1	5.4	500	89	2.5	7
Methan	1	6.2	50	110	5.2	7
	1	6.2	500	84	5.9	7
Nabam	1	4.8	50	62	6.6	7
	1	4.8	500	65	13	7
Nabonate	1	6.1	50	66	11	7
	1	6.1	500	56	12	7
Na DMDTC	1	5.4	50	110	2.5	7
	1	5.4	500	110	4.2	7
Thiram	1	4.5	50	89	2.9	7
	1	4.5	500	82	3.4	7
Zineb	1	5.2	50	87	3.4	7
	1	5.2	500	86	9.4	7
Ziram	1	5.7	50	100	12	7
	1	5.7	500	95	19	7

(a) 1 = Wastewater from a manufacturer of a dithiocarbamate diluted 1000:1 with Columbus POTW secondary effluent.



A52-002-61

Figure 1. GC-HALL Chromatogram of 0.1 ng of CS₂.

Method 631
***The Determination of Benomyl
and Carbendazim in Municipal
and Industrial Wastewater***

Method 631

The Determination of Benomyl and Carbendazim in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1** This method covers the determination of benomyl and carbendazim. The following parameters can be determined by this method:

<i>Parameter</i>	<i>Storet No.</i>	<i>CAS No.</i>
Benomyl	--	17804-35-2
Carbendazim	--	10605-21-7

- 1.2** Benomyl cannot be determined directly by this method. Benomyl is hydrolyzed to carbendazim, and both compounds are measured and reported as carbendazim.
- 1.3** This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 CFR 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.
- 1.4** The method detection limit (MDL, defined in Section 15) for each parameter is 8.7 µg/L. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.5** This method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.6** When this method is used to analyze unfamiliar samples for either of the compounds above, compound identifications should be supported by at least one additional qualitative technique.

2. SUMMARY OF METHOD

- 2.1** A measured volume of sample, approximately 1 L, is acidified if necessary to hydrolyze benomyl to carbendazim. The total carbendazim is extracted with methylene chloride using a separatory funnel. The extract is dried and exchanged to methanol during concentration to a volume of 10 mL or less. HPLC conditions are described which permit the separation and measurement of total carbendazim in the extract by HPLC with a UV detector.^{1,2}

3. INTERFERENCES

- 3.1** Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in liquid chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences

under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.³ Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. Unique samples may require cleanup approaches to achieve the MDL listed in Section 1.

4. SAFETY

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified^{4,6} for the information of the analyst.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

- 5.2** Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)
- 5.2.1** Separatory funnel: 250-mL, with TFE-fluorocarbon stopcock, ground-glass or TFE stopper.
 - 5.2.2** Drying column: Chromatographic column 400 mm long by 19 mm ID with coarse-fritted disc.
 - 5.2.3** Concentrator tube, Kuderna-Danish: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground-glass stopper is used to prevent evaporation of extracts.
 - 5.2.4** Evaporative flask, Kuderna-Danish: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.
 - 5.2.5** Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).
 - 5.2.6** Vials: Amber glass, 10- to 15-mL capacity with TFE-fluorocarbon-lined screw-cap.
- 5.3** Boiling chips: Approximately 10/40 mesh. Heat at 400°C for 30 minutes or perform a Soxhlet extraction with methylene chloride.
- 5.4** Water bath: Heated, with concentric ring cover, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.
- 5.5** Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6** Liquid chromatograph: High performance analytical system complete with high pressure syringes or sample injection loop, analytical columns, detector and strip-chart recorder. A guard column is recommended for all applications.
- 5.6.1** Column: 30 cm long by 4 mm ID stainless steel, packed with μ Bondapak C_{18} (10 μ) or equivalent. This column was used to develop the method performance statements in Section 14. Alternative columns may be used in accordance with the provisions described in Section 12.1.
 - 5.6.2** Detector: Ultraviolet, 254 nm. This detector has proven effective in the analysis of wastewaters and was used to develop the method performance statements in Section 14. Alternative detectors may be used in accordance with the provisions described in Section 12.1.

6. REAGENTS

- 6.1** Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2** Methylene chloride, methanol: Pesticide-quality or equivalent.
- 6.3** Sodium sulfate: ACS, granular, anhydrous. Condition by heating in a shallow tray at 400°C for a minimum of 4 hours to remove phthalates and other interfering organic substances. Alternatively, heat 16 hours at 450 to 500°C in a shallow tray or perform a Soxhlet extraction with methylene chloride for 48 hours.
- 6.4** Sodium hydroxide solution (10N): Dissolve 40g NaOH in reagent water and dilute to 100 mL.
- 6.5** Sulfuric acid solution (1 + 1): Slowly add 50 mL H_2SO_4 (sp. gr. 1.84) to 50 mL of reagent water.

- 6.6** Mobile phase: Methanol/water (1+1). Mix equal volumes of HPLC/UV quality methanol and reagent water.
- 6.7** Stock standard solution (1.00 $\mu\text{g}/\mu\text{L}$): The stock standard solution may be prepared from a pure standard material or purchased as a certified solution.
- 6.7.1** Prepare the stock standard solution by accurately weighing approximately 0.0100 g of pure carbendazim. Dissolve the material in HPLC/UV quality methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.
- 6.7.2** Transfer the stock standard solution into a TFE-fluorocarbon-sealed screw-cap vial. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
- 6.7.3** The stock standard solution must be replaced after 6 months, or sooner if comparison with a check standard indicates a problem.

7. CALIBRATION

- 7.1** Establish HPLC operating parameters equivalent to those indicated in Table 1. The HPLC system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2** External standard calibration procedure.
- 7.2.1** Prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of carbendazim stock standard to volumetric flasks and diluting to volume with methanol. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
- 7.2.2** Using injections of 10 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for carbendazim. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for carbendazim at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.
- 7.2.3** The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared.

7.3 Internal standard calibration procedure: To use this approach, the analyst must select an internal standard similar to carbendazim in analytical behavior. 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.

7.3.1 Prepare calibration standards at a minimum of three concentration levels of carbendazim by adding volumes of stock standard to volumetric flasks. To each calibration standard, add a known constant amount of internal standard, and dilute to volume with methanol. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

7.3.2 Using injections of 10 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} , against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for carbendazim varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. QUALITY CONTROL

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

- 8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
- 8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
 - 8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate of either benomyl or carbendazim in methanol, 1000 times more concentrated than the selected concentrations.
 - 8.2.2 Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
 - 8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.
 - 8.2.4 Using the appropriate data from Table 2, determine the recovery and single-operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.
- 8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
 - 8.3.1 Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁷ that are useful in observing trends in performance.

- 8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁷

- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for benomyl or carbendazim does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.
- 8.5** Before processing any samples, the analyst must demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.
- 8.6** 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 monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram as carbendazim, confirmatory techniques such as chromatography with a dissimilar column, or ratio of absorbance at two or more wavelengths may be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. *SAMPLE COLLECTION, PRESERVATION, AND HANDLING*

- 9.1** Grab samples must be collected in glass containers. Conventional sampling practices⁸ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2** The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3** All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

10. *SAMPLE EXTRACTION*

- 10.1** Using a 250-mL graduated cylinder, measure 150 mL of well-mixed sample into a 250-mL Erlenmeyer flask. If benomyl is a potentiality in the sample, continue with Section 10.2. If only carbendazim is to be measured, proceed directly to Section 10.3.
- 10.2** Carefully add 2 mL of 1+1 sulfuric acid and a TFE-fluorocarbon covered magnetic stirring bar to the sample. Check the sample with wide-range pH paper to insure that the pH is less than 1.0. Stir at room temperature for 16 to 24 hours.
- 10.3** Adjust the sample pH to within the range of 6 to 8 with sodium hydroxide. Pour the entire sample into a 250-mL separatory funnel.
- 10.4** Add 60 mL methylene chloride to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between

layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

- 10.5 Add a second 60-mL volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.
- 10.6 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.7 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.
- 10.8 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.9 Increase the temperature of the hot water bath to 85 to 90°C. Momentarily remove the Snyder column, add 50 mL of methanol and a new boiling chip and reattach the Snyder column. Pour about 1 mL of methanol into the top of the Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 minutes. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.10 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methanol and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than 2 days, they should be transferred to TFE-fluorocarbon-sealed screw-cap vials. Proceed with HPLC analysis.

11. CLEANUP AND SEPARATION

- 11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. If particular circumstances demand the use of a cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

12. LIQUID CHROMATOGRAPHY

- 12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are the estimated retention time and method detection limit that can be achieved by this method. An example of the separation achieved by this column is shown in Figure 1. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.
- 12.2 Calibrate the system daily as described in Section 7.
- 12.3 If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.
- 12.4 Inject 10 μL of the sample extract. Record the volume injected to the nearest 0.05 μL , and the resulting peak size in area or peak height units.
- 12.5 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

- 13.1 Determine the concentration of carbendazim in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_s)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_s = Volume of water extracted, in mL

- 13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where

A_s = Response for parameter to be measured

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_o = Volume of water extracted, in L

- 13.2** If the sample was treated to hydrolyze benomyl, report the results as benomyl (measured as carbendazim). If the hydrolysis step was omitted, report results as carbendazim. Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 13.3** For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. METHOD PERFORMANCE

- 14.1** 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.⁹ The MDL concentrations listed in Table 1 were determined by extracting 1000-mL aliquots of reagent water with three 350 mL volumes of methylene chloride.¹
- 14.2** In a single laboratory (West Cost Technical Services, Inc.), using reagent water and effluents from publicly owned treatment works (POTW), the average recoveries presented in Table 2 were obtained.¹ The standard deviations of the percent recoveries of these measurements are also included in Table 2. All results were obtained using the same experimental scale described in Section 14.1.

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Table 1. Chromatographic Conditions and Method Detection Limits

<i>Parameter</i>	<i>Retention Time (min)</i>	<i>Method Detection Limit (µg/L)</i>
Benomyl (as carbendazim)	—	25.0
Carbendazim	8.1	8.7

Column conditions: μ Bondapak C₁₈ (10 μ m) packed in a 30 cm long by 4 mm ID stainless steel column with a mobile phase flow rate of 2.0 mL/min at ambient temperature.

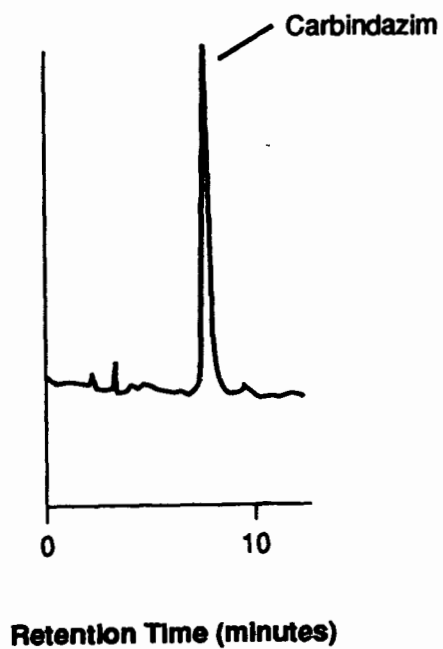
Mobile phase: methanol/water (1+1).

Table 2. Single-Operator Accuracy and Precision

<i>Parameter</i>	<i>Sample Type</i>	<i>Number of Replicates</i>	<i>Spike (µg/L)</i>	<i>Average Percent Recovery</i>	<i>Standard Deviation (%)</i>
Benomyl (as carbendazim)	DW	7	51.5	70	15.5
	MW	7	51.5	78	8.8
	MW	7	103	99	6.4
Carbendazim	DW	7	50	106	5.5
	MW	7	50	117	18.5
	MW	7	100	108	11.3

DW = Reagent water

MW = Municipal wastewater



A52-002-62

Figure 1. Liquid Chromatogram of Carbendazim on Column 1.
For Conditions, See Table 1.

Method 632
The Determination of
Carbamate and Urea Pesticides
in Municipal and Industrial
Wastewater

Method 632

The Determination of Carbamate and Urea Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of certain carbamate and urea pesticides. The following parameters can be determined by this method:

<i>Parameter</i>	<i>STORET No.</i>	<i>CAS No.</i>
Aminocarb	—	2032-59-9
Barban	—	101-27-9
Carbaryl	39750	63-25-2
Carbofuran	81405	1563-66-2
Chlorpropham	—	101-21-3
Diuron	39650	330-54-1
Fenuron	—	101-42-8
Fenuron-TCA	—	4482-55-7
Fluometuron	—	2164-17-2
Linuron	—	330-55-2
Methiocarb	—	2032-65-7
Methomyl	39051	16752-77-5
Mexacarbate	—	315-18-4
Monuron	—	150-68-5
Monuron-TCA	—	140-41-0
Neburon	—	555-37-3
Oxamyl	—	23135-22-0
Propham	39052	122-42-9
Propoxur	—	114-26-1
Siduron	—	1982-49-6
Swep	—	1918-18-9

- 1.2 This method cannot distinguish monuron from monuron-TCA and fenuron from fenuron-TCA. Results for the paired parameters are reported as monuron and fenuron respectively.
- 1.3 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.4 The method detection limit (MDL, defined in Section 15) for many of the parameters are listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

- 1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique.

2. SUMMARY OF METHOD

- 2.1 A measured volume of sample, approximately 1 L, is extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated to a volume of 10 mL or less. HPLC chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by HPLC with a UV detector.^{1,2}
- 2.2 This method provides an optional Florisil column cleanup procedure to aid in the elimination or reduction of interferences which may be encountered.

3. INTERFERENCES

- 3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in liquid chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.
 - 3.1.1 Glassware must be scrupulously cleaned.³ Clean all glassware, as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.
 - 3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever

means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified^{4,6} for the information of the analyst.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grap-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel 2000-mL, with TFE-fluorocarbon stopcock, ground-glass or TFE stopper.

5.2.2 Drying column: Chromatographic column 400 mm long by 19 mm ID with coarse-fritted disc.

5.2.3 Chromatographic column: 400 mm long by 19 mm ID with coarse-fritted disc at bottom and TFE-fluorocarbon stopcock (Kontes K-420540-0224 or equivalent).

5.2.4 Flask: round-bottom 500-mL, with standard taper to fit rotary evaporator.

5.2.5 Vials: Amber glass, 10- to 15-mL capacity with TFE-fluorocarbon-lined screw-cap.

5.3 Rotary evaporator.

5.4 Water bath: Heated, with concentric ring cover, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.

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

5.6 Filtration apparatus: As needed to filter chromatographic solvents prior to HPLC.

5.7 Liquid chromatograph: High performance analytical system complete with high pressure syringes or sample injection loop, analytical columns, detector, and strip-chart recorder. A guard column is recommended for all applications.

5.7.1 Gradient pumping system, constant flow.

5.7.2 Column: 30 cm long by 4 mm ID stainless steel packed with μ Bondapak C₁₈ (10 μm) or equivalent. This column was used to develop the method performance statements in

Section 14. Alternative columns may be used in accordance with the provisions described in Section 12.1.

- 5.7.3** Detector: Ultraviolet, capable of monitoring at 254 nm and 280 nm. This detector has proven effective in the analysis of wastewaters and was used to develop the method performance statements in Section 14. Alternative detectors may be used in accordance with the provisions described in Section 12.1.

6. REAGENTS

- 6.1** Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2** Acetone, acetonitrile, hexane, methylene chloride, methanol: Pesticide-quality or equivalent.
- 6.3** Ethyl ether: Nanograde, redistilled in glass if necessary. Must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Cat. No. P1126-8, and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.
- 6.4** Sodium sulfate: ACS, granular, anhydrous. Condition by heating in a shallow tray at 400°C for a minimum of 4 hours to remove phthalates and other interfering organic substances. Alternatively, heat 16 hours at 450 to 500°C in a shallow tray or perform a Soxhlet extraction with methylene chloride for 48 hours.
- 6.5** Florisil: PR grade (60/100 mesh). Purchase activated at 675°C and store in dark in glass container with ground-glass stopper or foil-lined screw-cap. Before use activate each batch at least 16 hours at 130°C in a foil-covered glass container.
- 6.6** Acetic acid: Glacial.
- 6.7** Stock standard solutions (1.00 µg/µL): Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.
- 6.7.1** Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in pesticide-quality acetonitrile or methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.
- 6.7.2** Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw-cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
- 6.7.3** Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1** Establish HPLC operating parameters equivalent to those indicated in Table 1. The HPLC system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2** External standard calibration procedure.
- 7.2.1** For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with acetonitrile or methanol. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
- 7.2.2** Using injections of 10 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.
- 7.2.3** The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.
- 7.3** Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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.
- 7.3.1** Prepare calibration standards at a minimum of three concentration levels for each parameter 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 acetonitrile or methanol. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.
- 7.3.2** Using injections of 10 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} against RF.

- 7.3.3** The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.
- 7.4** The cleanup procedure in Section 11 utilizes Florisil chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil which is used, the use of the lauric acid value is suggested. This procedure determines the adsorption from hexane solution of the lauric acid, in milligrams per gram of Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.
- 7.5** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.
- 8.1.1** Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2** In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
- 8.1.3** The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

- 8.2** To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
- 8.2.1** Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetonitrile or methanol, 1000 times more concentrated than the selected concentrations.
- 8.2.2** Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
- 8.2.3** Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.
- 8.2.4** Table 2 provides single-operator recovery and precision for most of the carbamate and urea pesticides. Similar results should be expected from reagent water for all compounds listed in the method. Compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.
- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
- 8.3.1** Calculate upper and lower control limits for method performance as follows:

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

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁸ that are useful in observing trends in performance.

- 8.3.2** The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁸
- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

- 8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.
- 8.6 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 monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques, such as chromatography with a dissimilar column, or ratio of absorbance at two or more wavelengths, may be used. Whenever possible, the laboratory should perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

- 9.1 Grab-samples must be collected in glass containers. Conventional sampling practices⁹ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

10. SAMPLE EXTRACTION

- 10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel.
- 10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.
- 10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.
- 10.4 It is necessary to exchange the extract solvent to hexane if the Florisil clean up procedure is to be used. For direct HPLC analysis the extract solvent must be exchanged to a solvent (either methanol or acetonitrile) that is compatible with the mobile phase. The analyst should only exchange a portion of the extract to HPLC solvent if there is a possibility that cleanup may be necessary.

- 10.5 Pass a measured fraction or all of the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate and collect the extract in a 500-mL round-bottom flask. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.
- 10.6 Attach the 500-mL round-bottom flask containing the extract to the rotary evaporator and partially immerse in the 50°C water bath.
- 10.7 Concentrate the extract to approximately 5 mL in the rotary evaporator at a temperature of 50°C. Other concentration techniques may be used if the requirements of Section 8.2 are met.
- 10.8 Add 50 mL of hexane, methanol, or acetonitrile to the round-bottom flask and concentrate the solvent extract as before. When the apparent volume of liquid reaches approximately 5 mL remove the 500-mL round-bottom flask from the rotary evaporator and transfer the concentrated extract to a 10-mL volumetric flask, quantitatively washing with 2 mL of solvent. Adjust the volume to 10 mL.
- 10.9 Stopper the volumetric flask and store refrigerated at 4°C if further processing will not be performed immediately. If the extracts will be stored longer than 2 days, they should be transferred to TFE-fluorocarbon-sealed screw-cap bottles.
- 10.10 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various industrial and municipal effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.
- 11.2 The following Florisil column cleanup procedure has been demonstrated to be applicable to the five pesticides listed in Table 3. It should also be applicable to the cleanup of extracts for the other carbamate and urea pesticides listed in the scope of this method.
 - 11.2.1 Add a weight of Florisil (nominally 20 g) predetermined by calibration (Section 7.4 and 7.5), to a chromatographic column. Settle the Florisil by tapping the column. Add anhydrous sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatography column. Discard the eluate.
 - 11.2.2 Adjust the sample extract volume to 10 mL with hexane and transfer it from the volumetric flask to the Florisil column. Rinse the flask twice with 1 to 2 mL hexane, adding each rinse to the column.
 - 11.2.3 Drain the column until the sodium sulfate layer is nearly exposed. Elute the column with 200 mL of 20% ethyl ether in hexane (V/V) (Fraction 1) using a drip rate of about 5 mL/min. Place a 500-mL round-bottom flask under the chromatography column. Elute the column again, using 200 mL of 6% acetone in hexane (V/V) (Fraction 2), into a second flask. Perform a third elution using 200 mL of 15% acetone in hexane (V/V)

(Fraction 3), and a final elution with 200 mL of 50% acetone in hexane (V/V) (Fraction 4), into separate flasks. The elution patterns for five of the pesticides are shown in Table 3.

- 11.2.4 Concentrate the eluates to 10 mL with a rotary evaporator as described in Section 10.7, exchanging the solvent to acetonitrile or methanol as required.

12. LIQUID CHROMATOGRAPHY

- 12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are estimated retention-times and method detection limits that can be achieved by this method. An example of the separations achieved by this column is shown in Figure 1. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.
- 12.2 Calibrate the system daily as described in Section 7. The standards and extracts must be in the solvent (acetonitrile or methanol) compatible with the mobile phase.
- 12.3 If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.
- 12.4 Inject 10 μ L of the sample extract. Record the volume injected to the nearest 0.05 μ L, and the resulting peak size in area or peak height units.
- 12.5 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

- 13.1 Determine the concentration of individual compounds in the sample.
- 13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_e)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_e = Volume of water extracted, in mL

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_e)}$$

where

A_s = Response for parameter to be measured

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_e = Volume of water extracted, in L

- 13.2** Calculate and report fenuron-TCA as fenuron and monuron-TCA as monuron. Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 13.3** For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. METHOD PERFORMANCE

- 14.1** 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.¹⁰ The MDL concentrations listed in Table 1 were obtained using reagent water or river water.^{2,11}
- 14.2** In a single laboratory, the average recoveries presented in Table 2 were obtained using this method.^{2,11} The standard deviations of the percent recoveries of these measurements are also included in Table 2.

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Table 1. Chromatographic Conditions and Method Detection Limits

<i>Parameter</i>	<i>Mobile Phase*</i>	<i>Retention Time (Min)</i>	<i>UV Wavelength (nm)</i>	<i>Method Detection Limit (µg/L)</i>
Mexacarbate	A	8.7	254	0.52
Propoxur	A	14.3	280	0.11
Monuron	A	14.4	254	0.003
Carbaryl	A	17.0	280	0.02
Propham	A	17.2	254	0.07
Diuron	A	19.5	254	0.009
Linuron	A	21.0	254	0.009
Methiocarb	A	21.4	254	0.02
Chlorpropham	A	21.8	254	0.03
Barban	A	22.3	254	0.05
Neburon	A	24.3	254	0.012
Propoxur	B	2.0	280	0.11
Methomyl	B	6.5	254	8.9
Carbaryl	B	14.1	280	0.02
Diuron	B	15.5	254	0.009
Linuron	B	17.9	254	0.009
Propoxur	C	1.7	280	0.11
Carbofuran	C	3.5	280	3.2
Fluorometuron	C	3.6	254	11.1
Oxamyl	D	3.2	254	9.2

*Mobile Phase:

- A Methanol/1% acetic acid, programmed linearly from 5 to 95% methanol at 2.0 mL/min flow rate and at ambient temperature.
- B Acetonitrile/water, programmed linearly from 10% to 100% acetonitrile in 30 minutes at a flow rate of 2.0 mL/min.
- C 50% acetonitrile in water at a flow rate of 2.0 mL/min.
- D 35% methanol in water at a flow rate of 2.0 mL/min.

Column: μ Bondapak C₁₈ (10 μ m) packed in a 30 cm long by 4 mm ID stainless steel column, with a Whatmann Co. PELL ODS (30-38 μ m) guard column, 7 cm long by 4 mm ID.

Table 2. Single-Operator Accuracy and Precision

<i>Parameter</i>	<i>Sample Type *</i>	<i>Spike (µg/L)</i>	<i>No. of Analyses</i>	<i>Average Percent Recovery</i>	<i>Standard Deviation %</i>
Fluorometuron	1	50	7	93.9	7.0
	2	50	7	80.0	7.2
	4	1724	7	99	11.6
Propoxur	1	550	7	94.5	1.7
	3	2200	3	105	3.0
	4	550	7	87.2	7.3
	5	0.5	5	93	6.0
Oxamyl	1	100	7	87	8.4
	2	53	7	84.9	5.5
	2	1080	7	89.8	2.7
Methomyl	1	100	4	74.4	2.4
	3	30660	4	48.2	2.8
	2	100	7	91.8	2.8
	2	1960	7	94.4	1.9
Diuron	1	10	4	89.8	1.0
	3	500	4	56.1	5.0
	2	10	7	90.0	2.5
	2	400	7	95.7	3.2
	5	0.05	5	98	4.7
Linuron	1	10	4	95.0	3.4
	3	4000	4	72.2	5.1
	2	10	7	93.0	1.5
	2	210	7	103	4.6
	5	0.05	5	99	4.7
Carbofuran	1	37	7	87.8	2.7
	4	148	7	99.3	1.4
Barban	5	0.3	5	98	4.1
Carbaryl	5	0.1	5	101	4.1
Chlorpropham	5	0.2	5	95	3.9
Methiocarb	5	0.2	5	95	2.6
Mexacarbate	5	4.0	5	96	3.5
Monuron	5	0.05	5	97	1.7
Neburon	5	0.05	5	96	6.6
Propham	5	0.3	5	88	5.9

* Sample Type

- 1 = Reagent Water
- 2 = Municipal wastewater
- 3 = Industrial process water, pesticide manufacturing
- 4 = Industrial wastewater, pesticide manufacturing
- 5 = River Water

Table 3. Florisil Fractionation Patterns

<i>Parameter</i>	<i>Percent Recovery by Fraction</i>			
	<i>No. 1</i>	<i>No. 2</i>	<i>No. 3</i>	<i>No. 4</i>
Diuron	0	0	24	58
Linuron	0	13	82	0
Methomyl	0	0	0	84
Oxamyl	0	0	92	0
Propachlor	0	94	0	0

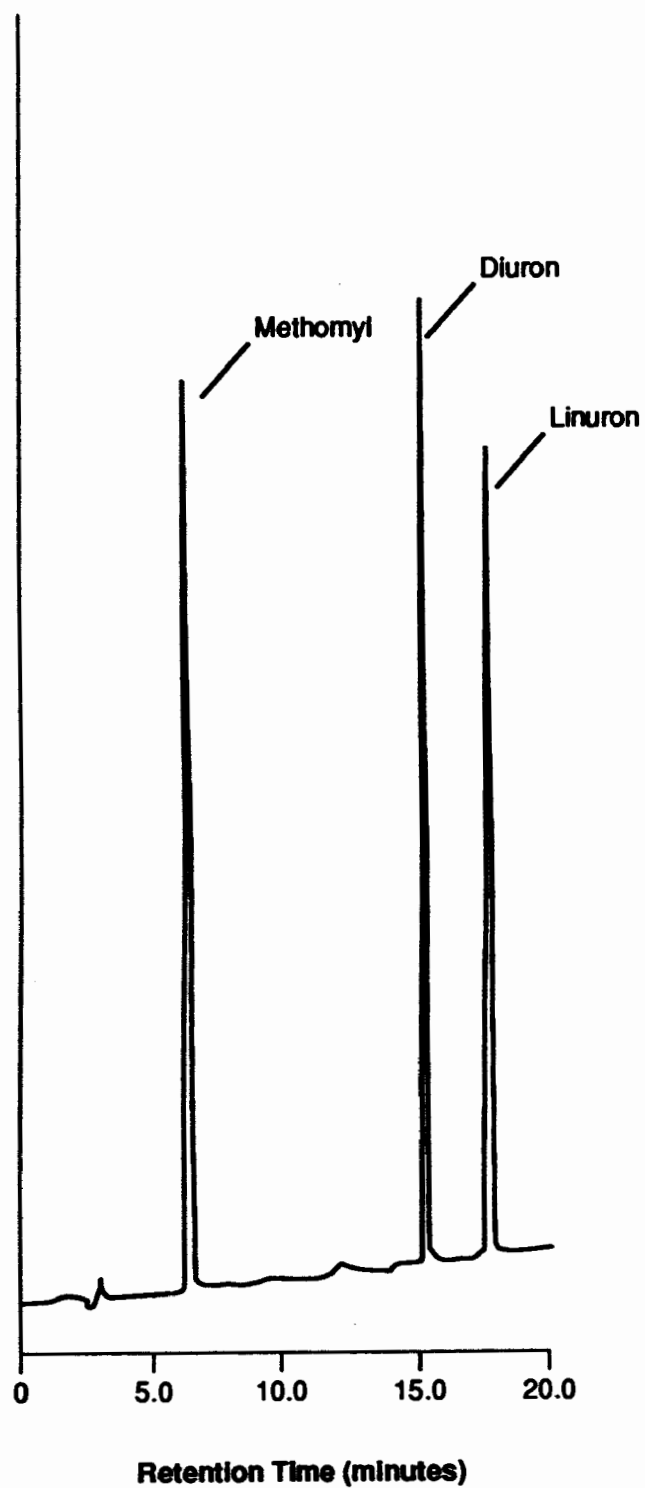
Florisil eluate composition by fraction:

Fraction 1 - 200 mL of 20% ethyl ether in hexane

Fraction 2 - 200 mL of 6% acetone in hexane

Fraction 3 - 200 mL of 15% acetone in hexane

Fraction 4 - 200 mL of 50% acetone in hexane



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Figure 1. Liquid Chromatogram of Diuron, Linuron and Methomyl on Column 1.
For Conditions, See Table 1.

Method 632.1
The Determination of
Carbamate and Amide
Pesticides in Municipal and
Industrial Wastewater

Method 632.1

The Determination of Carbamate and Amide Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of certain carbamate/amide pesticides in municipal and industrial wastewater. The following parameters may be determined by this method.

<i>Parameter</i>	<i>CAS No.</i>
Napropamide	15299-99-1
Propanil	709-98-8
Vacor	53558-25-1

- 1.2 The estimated detection limits (EDLs) for the parameters above are listed in Table 1. The EDL was calculated from the minimum detectable response being equal to five times the background noise using a 10-mL final extract volume of a 1-L sample and an injection volume of 100 μ L. The EDL for a specific wastewater may be different depending on the nature of interferences in the sample matrix.
- 1.3 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compounds listed above in municipal and industrial discharges. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identification should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second HPLC column that can be used to confirm measurements made with the primary column.
- 1.4 This method is restricted to use by or under the supervision of analysts experienced in the operation of liquid chromatographs and in the interpretation of liquid chromatograms.

2. SUMMARY OF METHOD

- 2.1 The carbamate/amide pesticides are removed from the sample matrix by extraction with methylene chloride. The extract is dried, exchanged to HPLC mobile phase and analyzed by liquid chromatography with ultraviolet (UV) detection.

3. INTERFERENCES

- 3.1 Solvent, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of liquid chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 9.1.

- 3.1.1 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.1.2 Glassware must be scrupulously cleaned¹. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap water and reagent water. It should then be drained dry and heated in a muffle furnace at 400°C for 15 to 30 minutes. Solvent rinses with acetone and pesticide-quality hexane may be substituted for the heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store the glassware inverted or capped with aluminum foil.
- 3.2 Matrix interferences may be caused by UV-active contaminants that are coextracted from the samples. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. Unique samples may require cleanup approaches to achieve the detection limits listed in Table 1.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified^{2,4} for the information of the analyst.

5. APPARATUS AND EQUIPMENT

- 5.1 Sample Containers: Narrow-mouth glass bottles, 1-L or 1-quart volume, equipped with polytetrafluoroethylene (PTFE)-lined screw-caps. Wide-mouth glass bottles, 1-quart volume, equipped with PTFE-lined screw-caps may also be used. Prior to use, wash bottles and cap liners with detergent and rinse with tap and distilled water. Allow the bottles and cap liners to air dry, then muffle the bottles at 400°C for 1 hour. After cooling, rinse the bottle and cap liners with hexane, seal the bottles, and store in a dust-free environment.
 - 5.1.1 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.
- 5.2 Rotary evaporator: With 24/40 joints and associated water bath and vacuum for operation at reduced pressure (Servo Instruments VE-1000-B or equivalent).

- 5.3** High performance liquid chromatography (HPLC) Apparatus: Analytical system complete with liquid chromatograph and all required accessories including syringes, analytical columns, and mobile phases. The system must be compatible with the specified detectors and strip-chart recorder. A data system is recommended for measuring peak areas.
- 5.3.1** Gradient pumping system.
- 5.3.2** Injector valve (Rheodyne 7125 or equivalent) with 100- μ L loop.
- 5.3.3** Column 1: 250 mm long by 4.0 mm ID, stainless steel, packed with reverse-phase Ultrasphere ODS, 5 μ , or equivalent.
- 5.3.4** Column 2: 250 mm long by 4.6 mm ID, packed with reverse phase Dupont Zorbax ODS, 10 μ , or equivalent.
- 5.3.5** Ultraviolet detector, variable wavelength, capable of monitoring at 254 nm.
- 5.3.6** Strip-chart recorder compatible with detector, 250-mm. (A data system for measuring peak areas is recommended.)
- 5.4** Boiling flask: 250-mL, flat-bottom, 24/40 joint.
- 5.5** Drying column: Approximately 400 mm long by 20 mm ID borosilicate glass, equipped with coarse-fritted bottom plate.
- 5.6** Miscellaneous.
- 5.6.1** Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6.2** Separatory funnels: 2-L, equipped with PTFE stopcocks.
- 5.6.3** Boiling chips: Approximately 10/40 mesh. Heat to 400°C for 30 minutes or perform a Soxhlet extraction with methylene chloride for 2 hours.
- 5.6.4** Standard solution storage containers: 15-mL bottles with PTFE-lined screw-caps.
- 5.6.5** Volumetric flasks: 5- and 10-mL, Class A.
- 5.6.6** Pasteur pipettes with bulbs.

6. REAGENTS AND CONSUMABLE MATERIALS

- 6.1** Reagents.
- 6.1.1** Acetone, acetonitrile, hexane, and methylene chloride: Demonstrated to be free of analytes and interferences.
- 6.1.2** Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.1.3** Sodium sulfate: Granular, anhydrous. Condition by heating at 400°C for 4 hours in a shallow tray.
- 6.1.4** HPLC Mobile Phase, Column 1: Add 400 mL of acetonitrile to a 1-L volumetric flask and dilute to volume with reagent water.
- 6.1.5** HPLC Mobile Phase, Column 2: Add 550 mL of acetonitrile to a 1-L volumetric flask and dilute to volume with reagent water.
- 6.1.6** Sodium hydroxide solution (1.0N): Dissolve 40 g of NaOH in reagent water and dilute to 1,000 mL.

- 6.1.7 Sodium chloride: ACS, crystals.
- 6.1.8 Sodium thiosulfate: ACS, granular.
- 6.1.9 Sulfuric acid solution (1+1): Slowly add 50 mL of H₂SO₄ (specific gravity 1.84) to 50 mL of reagent water.
- 6.2 Standard stock solutions (1.00 µg/µL): These solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedures.
 - 6.2.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide-quality (9:1) acetonitrile/acetone and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is certified at 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.
 - 6.2.2 Transfer the stock standards to PTFE-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standards should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
 - 6.2.3 Stock standards must be replaced after 6 months, or when comparison with quality control check samples indicates a problem.

7. **SAMPLE COLLECTION, PRESERVATION, AND STORAGE**

- 7.1 Collect all samples in duplicate. Grab samples must be collected in glass containers. Conventional sampling practices⁵ should be followed, except that the bottle must not be prewashed with sample before collection.
- 7.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction. Chemical preservatives should not be used in the field unless more than 24 hours will elapse before delivery to the laboratory. If the samples will not be extracted within 48 hours of collection, the sample should be adjusted to a pH of 2.0 to 4.0 with sulfuric acid, and add 35 mg of sodium thiosulfate per liter of sample for each part per million of free chlorine.
- 7.3 All samples must be extracted within 7 days and completely analyzed within 30 days of extraction.

8. **CALIBRATION**

- 8.1 Establish liquid chromatographic operating parameters equivalent to those indicated in Table 1. The chromatographic system can be calibrated using the external standard technique (Section 8.2).
- 8.2 External standard calibration procedure.
 - 8.2.1 Prepare calibration standards at a minimum of three concentration levels of the analytes by adding volumes of the stock standard to a volumetric flask and diluting to volume with HPLC mobile phase. One of the standards should be at a concentration near, but greater than, the EDL, and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.
 - 8.2.2 Using injections of 100 µL of each calibration standard, tabulate peak height or area response against the mass injected. The results are used to prepare a calibration curve

for the analytes. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (< 10% relative standard deviation, RSD), linearity of the calibration curve can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

- 8.2.3** The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any analyte varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or factor must be prepared.

9. QUALITY CONTROL

9.1 Monitoring for interferences.

- 9.1.1** Analyze a laboratory reagent blank each time a set of samples is extracted. A laboratory reagent blank is an aliquot of reagent water. If the reagent blank contains a reportable level of the analytes, immediately check the entire analytical system to locate and correct for possible interferences and repeat the test.

9.2 Assessing accuracy.

- 9.2.1** After every ten samples, and preferably in the middle of each day, analyze a laboratory control standard. Calibration standards may not be used for accuracy assessments and the laboratory control standard may not be used for calibration of the analytical system.
- 9.2.1.1** Laboratory control standard concentrate: From stock standards prepared as described in Section 6.2, prepare a laboratory control standard concentrate that contains the analytes at a concentration of 10 $\mu\text{g/mL}$ in acetonitrile.⁶
- 9.2.1.2** Laboratory control standard: Using a pipette, add 1.0 mL of the laboratory control standard concentrate to a 1-L aliquot of reagent water.
- 9.2.1.3** Analyze the laboratory control standard as described in Section 10. Calculate the percent recovery (P_i) with the equation:

Equation 1

$$P_i = \frac{100S_i}{T_i}$$

where

S_i = The analytical results from the laboratory control standard, in $\mu\text{g/L}$

T_i = The known concentration of the spike, in $\mu\text{g/L}$

- 9.2.2** At least annually, the laboratory should participate in formal performance evaluation studies, where solutions of unknown concentrations are analyzed and the performance of all participants is compared.

9.3 Assessing precision.

9.3.1 Precision assessments for this method are based upon the analysis of field duplicates (Section 7.1). Analyze both aliquots for at least 10% of all samples. To the extent practical, the samples for duplication should contain reportable levels of the analytes.

9.3.2 Calculate the relative range⁶ (RR_i) with the equation:

Equation 2

$$RR_i = \frac{100R_i}{X_i}$$

where

R_i = The absolute difference between the duplicate measurements X_1 and X_2 , in $\mu\text{g/L}$

X_i = The average concentration found $\left[\frac{X_1 + X_2}{2} \right]$, in $\mu\text{g/L}$

9.3.3 Individual relative range measurements are pooled to determine average relative range or to develop an expression of relative range as a function of concentration.

10. PROCEDURE

10.1 Sample extraction.

10.1.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel. Check the pH of the sample with wide-range pH paper and adjust to within the range of 6.5 to 7.5 with sodium hydroxide or sulfuric acid by slow addition and thorough mixing. Add 200 g of sodium chloride, and mix to dissolve.

10.1.2 Add 60 mL of methylene chloride to the sample bottle and shake for 30 seconds to rinse the walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release vapor pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends on the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation. Collect the extract in a 250-mL Erlenmeyer flask.

10.1.3 Add an additional 60-mL volume of methylene chloride to the sample bottle and complete the extraction procedure a second time, combining the extracts in the Erlenmeyer flask.

10.1.4 Perform a third extraction in the same manner. Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, collecting the extract in a 250-mL flat-bottom boiling flask. Rinse the Erlenmeyer flask and column with about 30 mL of methylene chloride to complete the transfer.

- 10.1.5 Concentrate the combined methylene chloride extracts to about 1 mL on a rotary evaporator with bath temperature between 35 and 40°C. Add 15 mL of acetonitrile, and reconcentrate to about 1 mL. Transfer the extract to a 10-mL volumetric flask. Rinse the boiling flask with about 1 mL of acetonitrile, and transfer to the volumetric flask. A 5-mL syringe is recommended for this operation. Rinse the boiling flask further with a 1-mL portion of acetonitrile, and transfer to the volumetric flask.
- 10.1.6 Add exactly 5.0 mL of HPLC-grade water to the flask, and dilute to 10 mL with acetonitrile. If the extracts will be stored longer than 2 days, they should be transferred to PTFE-sealed screw-cap bottles. If the sample extract requires no cleanup, proceed with chromatographic analysis. If the sample requires cleanup, proceed to Section 10.2.
- 10.1.7 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1,000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

10.2 Cleanup and separation.

- 10.2.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. If particular circumstances demand the use of a cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.

- 10.2.2 Proceed with liquid chromatography as described in Section 10.3.

10.3 Liquid chromatography analysis.

- 10.3.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are the estimated retention times and estimated detection limits that can be achieved by this method. An example of the separation achieved by the primary column of the analytes is shown in Figures 1 and 2. Other columns, chromatographic conditions, or detectors may be used if data quality comparable to Table 2 is achieved.
- 10.3.2 Calibrate the system daily as described in Section 8.
- 10.3.3 Inject 100 μ L of the sample extract. Monitor the column eluent at 254 nm. Record the resulting peak size in area or peak height units.
- 10.3.4 The retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 10.3.5 If the response for the peak exceeds the working range of the system, dilute the sample with mobile phase and reanalyze.
- 10.3.6 If the measurement of the peak response is prevented by the presence of interferences, cleanup is required.

11. CALCULATIONS

- 11.1 Determine the concentration of analytes in the sample.

- 11.1.1** Calculate the amount of analytes injected from the peak response using the calibration curve or calibration factor in Section 8.2.2. The concentration in the sample can be calculated from the equation:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_w)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_w = Volume of water extracted, in mL

- 11.2** Report results in milligrams per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

12. METHOD PERFORMANCE

- 12.1** The EDLs and associated chromatographic conditions for the analytes are listed in Table 1.⁷ The EDL is defined as the minimum response being equal to 5 times the background noise, assuming a 10-mL final extract volume of a 1-L sample and an HPLC injection volume of 100 μL .
- 12.2** Single-operator accuracy and precision studies were conducted by Environmental Science and Engineering, Inc., in the designated matrix. The results of these studies are presented in Table 2.

References

1. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, Pennsylvania, p. 679, 1986.
2. "Carcinogens - Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.
3. "OSHA Safety and Health Standards, General Industry" (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976).
4. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
5. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, Pennsylvania, p. 76, 1986.
6. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, March 1979.
7. "Evaluation of Ten Pesticides," U.S. Environmental Protection Agency, Contract 68-03-1760, Task No. 11, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio (In preparation.).

Table 1. Chromatographic Conditions and Estimated Detection Limits

<i>Parameter</i>	<i>Retention Time (Minutes)</i>		<i>Estimated Detection Limit (µg/L)</i>
	<i>Column 1</i>	<i>Column 2</i>	
Vacor (RH 787)	6.0	3.8	0.20
Propanil	12.4	6.9	0.85
Napropamide	15.2	9.5	0.31

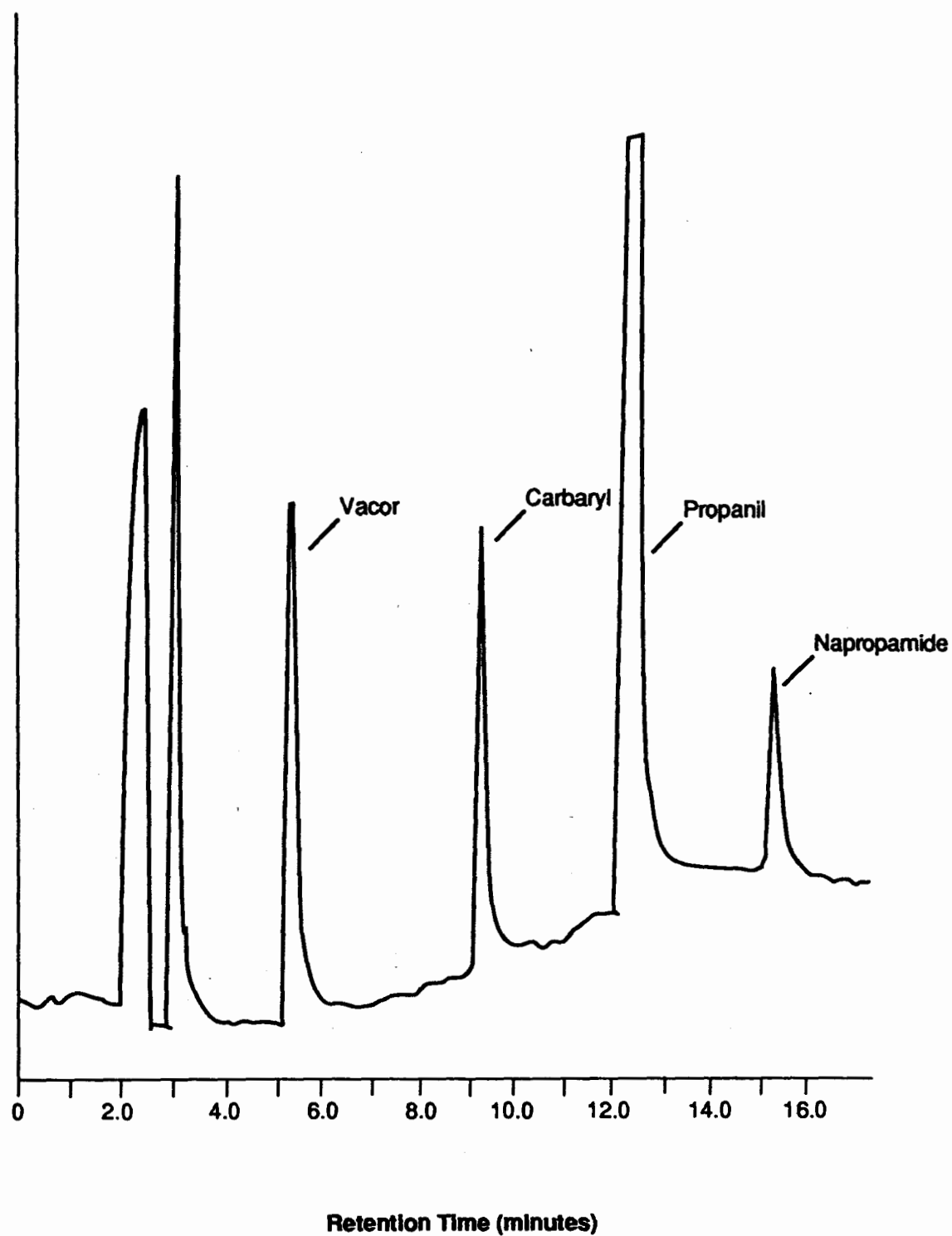
Column 1: 25 cm long by 4 mm ID, stainless steel, packed with Ultrasphere ODS (particle size 5 µ); mobile phase: 40% acetonitrile/HPLC water programmed to 65% acetonitrile/HPLC water over 10 minutes at a flow rate of 1.0 mL/min at ambient temperature.

Column 2: 25 cm long by 4.6 mm ID, stainless steel, packed with Zorbax ODS (DuPont); mobile phase: Isocratic elution with 55% acetonitrile/HPLC water at a flow rate of 1.0 mL/min for 6 minutes then linear flow gradient to 1.5 mL/min over 3 minutes at ambient temperature.

Table 2. Single-Laboratory Accuracy and Precision

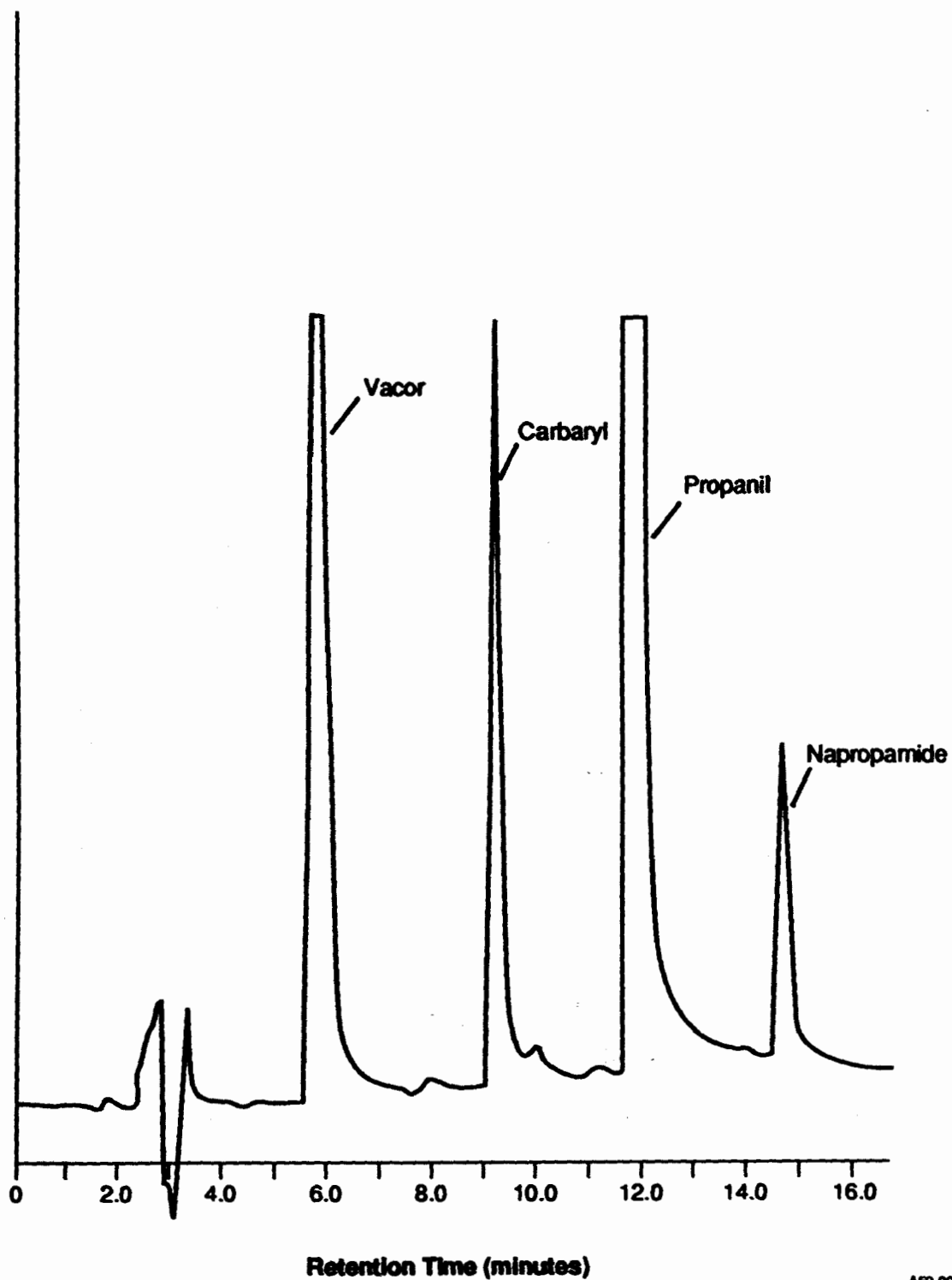
<i>Parameter</i>	<i>Matrix Type*</i>	<i>Spike Range (µg/L)</i>	<i>Number of Replicates</i>	<i>Average Percent Recovery</i>	<i>Standard Deviation (%)</i>
Napropamide	1	11.5	7	113.8	15.7
	1	597.0	7	104.0	16.0
Propanil	1	14.0	7	99.8	12.4
	1	676.0	7	96.4	7.6
Vacor (RH787)	1	12.9	7	98.2	17.5
	1	655.0	7	111.2	5.2

* 1 = Spiked municipal wastewater



A52-002-64

Figure 1. HPLC Chromatogram of Carbamates/Amides on Column 1.



A52-002-65

Figure 2. HPLC Chromatogram of Carbamate/Amides in Wastewater Extract on Column 1.

Method 633
The Determination of
Organonitrogen Pesticides in
Municipal and Industrial
Wastewater

Method 633

The Determination of Organonitrogen Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of certain organonitrogen pesticides. The following parameters can be determined by this method:

<i>Parameter</i>	<i>STORET No.</i>	<i>CAS No.</i>
Bromacil	—	314-40-9
Deet	—	134-62-3
Hexazinone	—	51235-04-2
Metribuzin	81408	21087-64-9
Terbacil	—	5902-51-2
Triadimefon	—	43121-43-3
Tricyclazole	—	41814-78-2

- 1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3 The method detection limit (MDL, defined in Section 15) for five of the parameters are listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.5 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. SUMMARY OF METHOD

- 2.1 A measured volume of sample, approximately 1 L, is extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to acetone during

concentration to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by gas chromatography with a thermionic bead detector.¹

3. INTERFERENCES

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. Unique samples may require special cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified³⁻⁵ for the information of the analyst.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with TFE-fluorocarbon. Aluminum foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from

light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

- 5.1.2** Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.
- 5.2** Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)
- 5.2.1** Separatory funnel: 2000-mL, with TFE-fluorocarbon stopcock, ground-glass or TFE stopper.
- 5.2.2** Drying column: Chromatographic column 400 mm long by 19 mm ID with coarse-fritted disc.
- 5.2.3** Concentrator tube, Kuderna-Danish: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground-glass stopper is used to prevent evaporation of extracts.
- 5.2.4** Evaporative flask, Kuderna-Danish: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.
- 5.2.5** Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).
- 5.2.6** Vials: Amber glass, 10- to 15-mL capacity with TFE-fluorocarbon-lined screw-cap.
- 5.3** Boiling chips: Approximately 10/40 mesh. Heat at 400°C for 30 minutes or performed Soxhlet extraction with methylene chloride.
- 5.4** Water bath: Heated, with concentric ring cover, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.
- 5.5** Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6** Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.
- 5.6.1** Column 1: 180 cm long by 2 mm ID glass, packed with 3% SP-2250DB on Supelcoport (100/120 mesh) or equivalent. Operation of this column at high temperatures will seriously reduce its useful period of performance. This column was used to develop the method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 12.1.
- 5.6.2** Column 2: 180 cm long by 2 mm ID glass, packed with 3% SP-2401 on Supelcoport (100/120 mesh) or equivalent.
- 5.6.3** Detector: Thermionic bead in the nitrogen mode. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. REAGENTS

- 6.1 Reagent water:** Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2 Acetone, methylene chloride:** Pesticide-quality or equivalent.
- 6.3 Sodium sulfate:** ACS, granular, anhydrous. Condition by heating in a shallow tray at 400°C for a minimum of 4 hours to remove phthalates and other interfering organic substances. Alternatively, heat 16 hours at 450 to 500°C in a shallow tray or perform a Soxhlet extraction with methylene chloride for 48 hours.
- 6.4 Stock standard solutions (1.00 µg/µL):** Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.
- 6.4.1** Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in pesticide-quality acetone and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.
- 6.4.2** Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw-cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
- 6.4.3** Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1** Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2 External standard calibration procedure.**
- 7.2.1** For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with acetone. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
- 7.2.2** Using injections of 1 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less

than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter 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 acetone. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

7.3.2 Using injections of 1 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s/A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

- 7.4** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

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

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone, 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 2, determine the recovery and single-operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

Where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁶ that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁶

- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.
- 8.5** Before processing any samples, the analyst must demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagent, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.
- 8.6** 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 monitor the precision of the sampling technique. 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 perform analysis of quality control materials and participate in relevant performance evaluation studies.

9. *SAMPLE COLLECTION, PRESERVATION, AND HANDLING*

- 9.1** Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2** The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3** All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

10. *SAMPLE EXTRACTION*

- 10.1** Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel.

- 10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.
- 10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.
- 10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.
- 10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.7 Increase the temperature of the hot water bath to about 70°C. Momentarily remove the Snyder column, add 50 mL of acetone and a new boiling chip and reattach the Snyder column. Pour about 1 mL of acetone into the top of the Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 minutes. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.8 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than 2 days, they should be transferred to TFE-fluorocarbon-sealed screw-cap vials. Analyze by gas chromatography.
- 10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. If particular circumstances demand the use of a cleanup procedure, the analyst must determine the elution

profile and demonstrate that the recovery of each compound of interest for the cleanup procedure is no less than 85%.

12. GAS CHROMATOGRAPHY

- 12.1** Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. An example of the separations achieved by Column 1 is shown in Figure 1. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.
- 12.2** Calibrate the system daily as described in Section 7.
- 12.3** If the internal standard approach is being used, add the internal standard to sample extracts immediately before injection into the instrument. Mix thoroughly.
- 12.4** Inject 1 to 5 μL of the sample extract using the solvent-flush technique.⁸ Record the volume injected to the nearest 0.05 μL , and the resulting peak size in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.
- 12.5** The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6** If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7** If the measurement of the peak response is prevented by the presence of interferences, cleanup is required.

13. CALCULATIONS

- 13.1** Determine the concentration of individual compounds in the sample.
- 13.1.1** If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_e)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_e = Volume of water extracted, in mL

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(\text{RF})(V_e)}$$

where

A_s = Response for parameter to be measured

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_e = Volume of water extracted, in L

- 13.2** Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 13.3** For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. GC/MS CONFIRMATION

- 14.1** It is recommended that GC/MS techniques be judiciously employed to support qualitative compound identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 seconds per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.
- 14.2** Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.⁹

- 14.3** At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all decafluorotriphenyl phosphine (DFTPP) performance criteria are achieved.¹⁰
- 14.4** To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
- 14.4.1** All ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to $\pm 10\%$. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20 to 40%.
- 14.4.2** The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.
- 14.4.3** Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.
- 14.5** Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.
- 14.6** Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).

15. METHOD PERFORMANCE

- 15.1** 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.¹¹ The MDL concentrations listed in Table 1 were obtained using reagent water.¹
- 15.2** In a single laboratory (West Cost Technical Services, Inc.), using effluents from pesticide manufacturers and publicly owned treatment works (POTW), the average recoveries presented in Table 2 were obtained.¹ The standard deviations of the percent recoveries of these measurements are also included in Table 2.

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Table 1. Chromatographic Conditions and Method Detection Limits

<i>Parameter</i>	<i>GC Column</i>	<i>Retention Time (Min)</i>	<i>Method Detection Limit (µg/L)</i>
Terbacil	1a	2.1	ND
Bromacil	1a	3.7	2.38
Hexazinone	1a	7.6	0.72
Tricyclazole	1b	3.5	ND
Metribuzin	2a	2.4	0.46
Triadimefon	2a	4.1	0.78
Deet	2b	4.6	3.39

ND = Not determined

Column 1a conditions: Supelcoport (100/120 mesh) coated with 3% SP-2250DB packed in a 180 cm long by 2 mm ID glass column with nitrogen carrier gas at a flow rate of 30 mL/min. Column temperature, programmed: initial 210°C, hold for 1 minute, then program at 10°C to 250°C and hold. A thermionic bead detector in the nitrogen mode was used to calculate the MDL.

Column 1b conditions: Same as Column 1a, except column temperature isothermal at 240°C.

Column 2a conditions: Supelcoport (100/120 mesh) coated with 3% SP-2401 packed in a 180 cm long by 2 mm ID glass column with nitrogen carrier gas at a flow rate of 30 mL/min. Column temperature, programmed: initial 160°C, programmed at injection at 10°C/min to 230°C.

Column 2b conditions: Same as Column 2a, except temperature programmed: initial 130°C, hold for 1 minute, then program at 12°C/min to 200°C.

Table 2. Single-Operator Accuracy and Precision

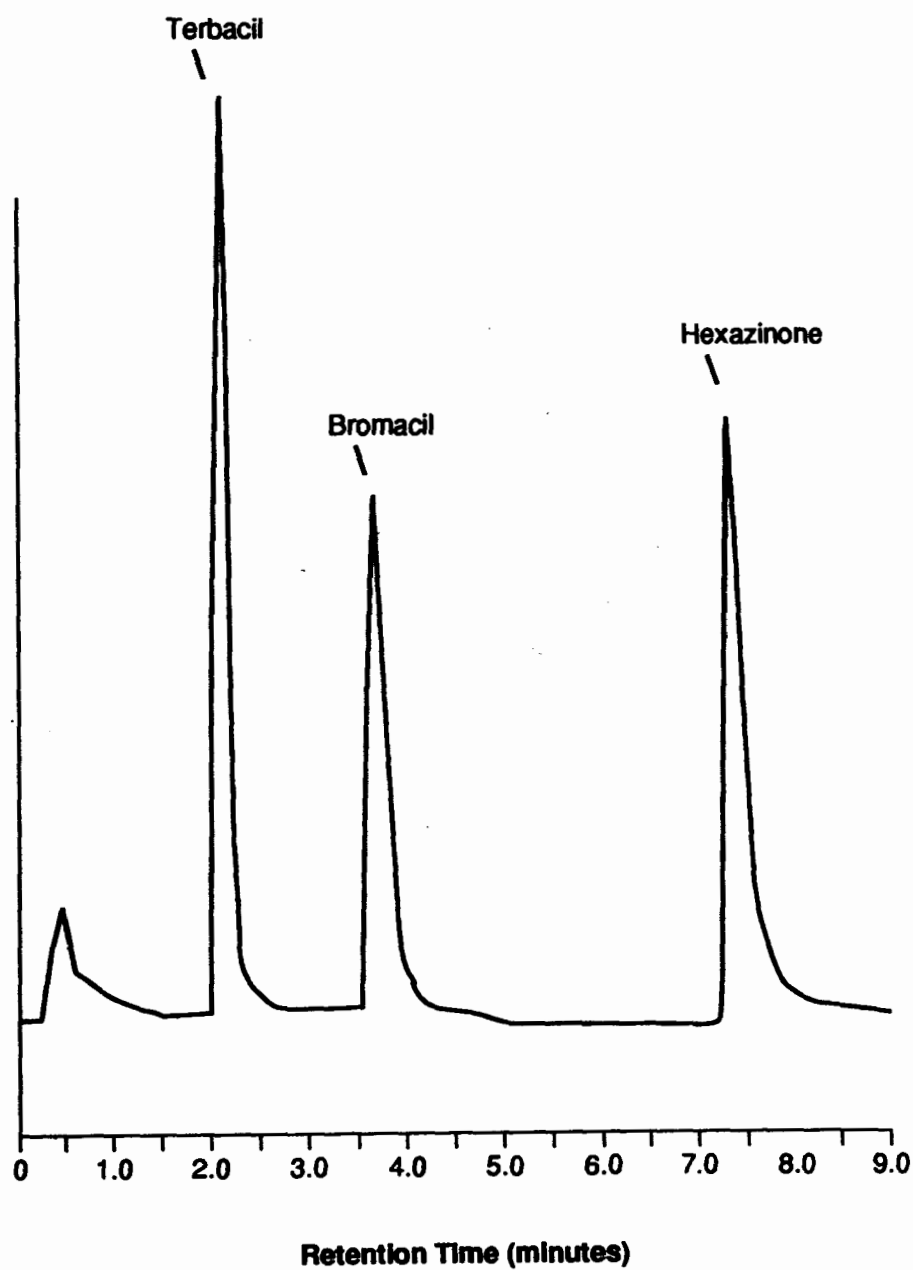
<i>Parameter</i>	<i>Sample Type</i>	<i>Spike ($\mu\text{g/L}$)</i>	<i>Number of Replicates</i>	<i>Mean Recovery (%)</i>	<i>Standard Deviation (%)</i>
Bromacil	DW	5.0	7	92.2	13.9
	MW	11.1	7	89.0	3.9
	MW	333.0	7	95.0	0.8
Deet	DW	5.8	7	99.1	18.4
	MW	5.2	7	92.6	5.9
	MW	515.0	7	94.2	2.2
Hexazinon	DW	4.9	7	86.6	4.1
	MW	10.1	7	92.2	5.3
	MW	369.0	7	94.0	1.9
Metribuzin	DW	5.2	6	98.2	2.7
Terbacil	MW	32.8	7	106.7	3.6
	MW	656.0	7	101.0	1.2
Triadmefon	DW	5.2	6	126.0	6.0
	PW	515.0	4	71.8	4.5
	IW	154.5	7	70.4	3.8
Tricyclazole	MW	12.3	7	69.0	1.9
	MW	303.0	7	98.0	1.2

DW = Reagent water

MW = Municipal wastewater

PW = Process water, pesticide manufacturing

IW = Industrial wastewater, pesticide manufacturing



A52-002-88

Figure 1. Gas Chromatogram of Organonitrogen Pesticides on Column 1.
For Conditions, See Table 1.

Method 633.1
***The Determination of Neutral
Nitrogen-Containing Pesticides
in Municipal and Industrial
Wastewaters***

Method 633.1

The Determination of Neutral Nitrogen-Containing Pesticides in Municipal and Industrial Wastewaters

1. SCOPE AND APPLICATION

- 1.1** This method covers the determination of certain neutral nitrogen containing pesticides. The following parameters can be determined by this method:

<i>Parameter</i>	<i>CAS No.</i>
Fenarimol	60168-88-9
MGK 264-A	113-48-4
MGK 264-B	113-48-4
MGK 326	136-45-8
Pronamide	23950-58-5

- 1.2** This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3** The method detection limit (MDL, defined in Section 15) for each compound is listed in Table 2. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4** The sample extraction and concentration steps in this method are similar to those in other 600-series methods. Thus, a single sample may be extracted to measure the compounds included in the scope of the methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures.
- 1.5** This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.6** When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. SUMMARY OF METHOD

- 2.1** A measured volume of sample, approximately 1 L, is extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated to 1.0 mL. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by alkali flame detector gas chromatography (GC/AFD).¹
- 2.2** This method provides an optional Florisil column cleanup procedure to aid in the elimination of interferences which may be encountered.

3. INTERFERENCES

- 3.1** Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.
 - 3.1.1** Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. Follow by rinsing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.
 - 3.1.2** The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.2** Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 2.

4. SAFETY

- 4.1** The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of materials data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified³⁻⁵ for the information of the analyst.

5. APPARATUS AND MATERIALS

- 5.1 Sampling equipment, for discrete or composite sampling.**
- 5.1.1** Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with PTFE. Foil may be substituted for PTFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.
 - 5.1.2** Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.
- 5.2 Glassware.** (All specifications are suggested. Catalog numbers are included for illustration only.)
- 5.2.1** Separatory funnel: 2000-mL, with PTFE stopcock.
 - 5.2.2** Drying column: Chromatographic column 400 mm long by 10 mm ID with coarse frit.
 - 5.2.3** Chromatographic column: 400 mm long by 19 mm ID with 250-mL reservoir at the top and PTFE stopcock (Kontes K-420290 or equivalent).
 - 5.2.4** Concentrator tube, Kuderna-Danish: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. A ground-glass stopper is used to prevent evaporation of extracts.
 - 5.2.5** Evaporative flask, Kuderna-Danish 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.
 - 5.2.6** Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).
 - 5.2.7** Snyder column, Kuderna-Danish: Two-ball micro (Kontes K-569001-0219 or equivalent).
 - 5.2.8** Vials: Amber glass, 10- to 15-mL capacity with PTFE-lined screw-cap.
 - 5.2.9** Erlenmeyer flask: 250-mL.
 - 5.2.10** Graduated cylinder: 1000-mL.
 - 5.2.11** Beaker: 250-mL.
- 5.3** Boiling chips: Approximately 10/40 mesh carborundum. Heat to 400°C for 4 hours or perform a Soxhlet extraction with methylene chloride.
- 5.4** Water bath: Heated, capable of temperature control ($\pm 20^\circ\text{C}$). The bath should be used in a hood.
- 5.5** Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6** Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

- 5.6.1 Column 1: 180 cm long by 2 mm ID glass, packed with 3% SP-2250 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 15. Alternative columns may be used in accordance with the provisions described in Section 12.1.
- 5.6.2 Column 2: 180 cm long by 2 mm ID glass, packed with 3% SP-2100 on Supelcoport (100/120 mesh) or equivalent.
- 5.6.3 Detector: Alkali flame detector (AFD), sometimes referred to as a nitrogen-phosphorous detector (NPD) or a thermionic-specific detector (TSD). This detector has proven effective in the analysis of wastewaters for the compounds listed in the scope and was used to develop the method performance statements in Section 15.

6. REAGENTS

- 6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2 Methylene chloride, methanol, petroleum ether, ethyl ether, acetone: Distilled-in-glass quality or equivalent. Ethyl ether must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Catalog No. P1126-8 and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips.
- 6.3 6N sodium hydroxide: Dissolve 24.0 g NaOH in 100 mL of reagent water.
- 6.4 6N sulfuric acid: Slowly add 16.7 mL of concentrated H₂SO₄ (94%) to about 50 mL of reagent water. Dilute to 100 mL with reagent water.
- 6.5 Sodium sulfate: (ACS), granular, anhydrous; heated in a muffle furnace at 400°C overnight.
- 6.6 Florisil: PR grade (60/100 mesh). Purchase activated at 675°C and store in brown glass bottle. To prepare for use, place 150 g in a wide-mouth jar and heat overnight at 160 to 170°C. Seal tightly with PTFE or aluminum-foil-lined screw-cap and cool to room temperature.
- 6.7 Stock standard solutions (1.00 µg/µL): Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.
 - 6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in distilled-in-glass quality methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 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.
 - 6.7.2 Transfer the stock standard solutions into PTFE-sealed screw-cap bottles. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
 - 6.7.3 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1** Establish gas chromatographic operating parameters equivalent to those indicated in Table 2. The gas chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2** External standard calibration procedure.
- 7.2.1** For each compound of interest, prepare calibration standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with acetone. One of the external standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
- 7.2.2** Using injections of 1 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each compound at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.
- 7.2.3** The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.
- 7.3** Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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.
- 7.3.1** Prepare calibration standards at a minimum of three concentration levels for each compound 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 acetone. One of the standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.
- 7.3.2** Using injections of 1 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, 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} against RF.

- 7.3.3** The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.
- 7.4** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.
- 8.1.1** Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2** In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
- 8.1.3** The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2** To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
- 8.2.1** Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol, 1000 times more concentrated than the selected concentrations.

- 8.2.2** Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
- 8.2.3** Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.
- 8.2.4** Using the appropriate data from Table 3, determine the recovery and single-operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.
- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
- 8.3.1** Calculate upper and lower control limits for method performance as follows:

$$\text{Upper control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁶ that are useful in observing trends in performance.

- 8.3.2** The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁶
- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.
- 8.5** Before processing any samples, the analyst should demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.
- 8.6** 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 monitor the precision of the

sampling technique. 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 perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

- 9.1 Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3 Adjust the pH of the sample to 6 to 8 with 6N sodium hydroxide or 6N sulfuric acid immediately after sampling.

10. SAMPLE EXTRACTION

- 10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel. Check the pH of the sample with wide range pH paper and adjust to 6 to 8 with 6N sodium hydroxide or 6N sulfuric acid.
- 10.2 Add 60 mL of methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.
- 10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.
- 10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with 30 to 40 mL of methylene chloride.
- 10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the macro Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the

concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

- 10.7 Remove the macro-Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. Add one or two clean boiling chips and attach a two-ball micro-Snyder column to the concentrator tube. Prewet the micro-Snyder column with methylene chloride and concentrate the solvent extract as before. When an apparent volume of 0.5 mL is reached, or the solution stops boiling, remove the K-D apparatus and allow it to drain and cool for 10 minutes.
- 10.8 Remove the micro-Snyder column and adjust the volume of the extract to 1.0 mL with methylene chloride. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract is to be stored longer than 2 days, transfer the extract to a screw-capped vial with a PTFE-lined cap. If the sample extract requires no further cleanup, proceed with solvent exchange to acetone as described in Section 10.9. If the sample requires cleanup, proceed to Section 11.
- 10.9 Add one or two clean boiling chips to the concentrator tube along with 10 mL of acetone. Attach the two-ball macro Snyder column and prewet the column with about 1 mL of acetone. Adjust the temperature of the water bath to 85 to 95°C. Concentrate the solvent extract as before to an apparent volume of 0.5 mL and allow it to drain and cool for 10 minutes. Add a second 10 mL of acetone to the concentrator tube and repeat the concentration procedure a second time. Adjust the final volume of the extract to 1.0 mL with acetone.
- 10.10 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.
- 11.2 The following Florisil cleanup procedure has been demonstrated to be applicable to the four neutral nitrogen pesticides listed in Table 1.
 - 11.2.1 Slurry 20 g of Florisil in 100 mL of ethyl ether and 400 μ L of reagent water. Transfer the slurry to a chromatographic column (Florisil may be retained with a plug of glass wool). Allow the solvent to elute from the column until the Florisil is almost exposed to the air. Wash the column with 25 mL of petroleum ether. Use a column flow rate of 2 to 2.5 mL/min throughout the wash and elution profiles. Add an additional 50 mL of petroleum ether to the head of the column.
 - 11.2.2 Quantitatively transfer the sample from Section 10.8 to the petroleum ether suspended over the column. Allow the solvent to elute from the column until the Florisil is almost exposed to the air. Elute the column with 50 mL of 50% ethyl ether in petroleum ether. Discard this fraction.

11.2.3 Elute the column with 50 mL of 100% ethyl ether (Fraction 1) and collect in a K-D apparatus. Repeat procedure with 50 mL 6% acetone in ethyl ether (Fraction 2), 50 mL 15% acetone in ethyl ether (Fraction 3), 50 mL 50% acetone in ethyl ether (Fraction 4), and 100 mL 100% acetone (Fraction 5), collecting each in a separate K-D apparatus. The elution patterns for the neutral nitrogen compounds are shown in Table 1. Concentrate each fraction to 1 mL as described in Section 10.6 and 10.7. The fractions may be combined before concentration at the discretion of the analyst. Solvent exchange Fraction 1 to acetone as described in Section 10.9 if the fractions are not combined.

11.2.4 Proceed with gas chromatographic analysis.

12. GAS CHROMATOGRAPHY

12.1 Table 2 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Examples of the separations achieved by Column 1 and Column 2 are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the gas chromatographic system daily as described in Section 7.

12.3 If the internal standard approach is being used, the analyst must not add the internal standard to the sample extracts until immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 1 to 5 μL of the sample extract using the solvent flush technique.⁸ Record the volume injected to the nearest 0.05 μL , and the resulting peak sizes in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.

12.5 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_e)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_e = Volume of water extracted, in mL

- 13.1.2** If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where

A_s = Response for parameter to be measured

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_o = Volume of water extracted, in L

- 13.2** Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 13.3** For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

14. GC/MS CONFIRMATION

- 14.1** It is recommended that GC/MS techniques be judiciously employed to support qualitative identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 seconds per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all glass or glass-lined materials is recommended. When using a fused-silica capillary column, the column outlet should be threaded through the interface to within a few mm of the entrance to the source ionization chamber. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.

- 14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.¹⁰
- 14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all DFTPP performance criteria are achieved.⁹
- 14.4 To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 50 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
 - 14.4.1 The molecular ion and all other ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to $\pm 10\%$. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20 to 40%.
 - 14.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.
 - 14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.
- 14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.
- 14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternative packed or capillary GC columns or additional cleanup (Section 11).

15. METHOD PERFORMANCE

- 15.1 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.¹¹ The MDL concentrations listed in Table 2 were obtained using reagent water.¹ Similar results were achieved using representative wastewaters.
- 15.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from $10 \times \text{MDL}$ to $1000 \times \text{MDL}$.
- 15.3 In a single laboratory, Battelle's Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 3 were obtained after Florisil cleanup. Seven replicates of each of two different wastewaters were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 3.¹

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Table 1. Elution Characteristics of the Neutral Nitrogen Compounds on 2% Deactivated Florisil

<i>Parameter</i>	<i>Elution in Specified Fraction(a)</i>				
	<i>F1</i>	<i>F2</i>	<i>F3</i>	<i>F4</i>	<i>F5</i>
Fenarimol		X	X		
MGK 264		X	X		
MGK326				X	X
Pronamide	X				

(a) Elution solvents are 50 mL each of the following:

F1 = 100% ethyl ether

F2 = 6% acetone in ethyl ether

F3 = 15% acetone in ethyl ether

F4 = 50% acetone in ethyl ether

F5 = 100% acetone (100 mL)

Table 2. Chromatographic Conditions and Method Detection Limits

<i>Parameter</i>	<i>Retention Time (min.)</i>		<i>MDL (µg/L)</i>
	<i>Column 1</i>	<i>Column 2</i>	
Pronamide	19.9	22.0	4
MGK 326	21.9	23.8	6
MGK 264	23.0 and 23.5(a)	25.5 and 27.5(a)	2
Fenarimol	30.6	32.2	4

Column 1 conditions: Supelcoport (100/120 mesh) coated with 3% SP-2250 packed in a 1.8 m long by 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. Column temperature is programmed from 80°C to 300°C at 8°C/min with a 4 minute hold at each extreme, injector temperature is 250°C and detector is 300°C. Alkali flame detector at bead voltage of 16 V.

Column 2 conditions: Supelcoport (100/120 mesh) coated with 3% SP-2100 packed in a 1.8 m long by 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. All other conditions as for Column 1.

(a) Two isomers of MGK 264 are resolved from each other.

Table 3. Single-Laboratory Accuracy and Precision(a)

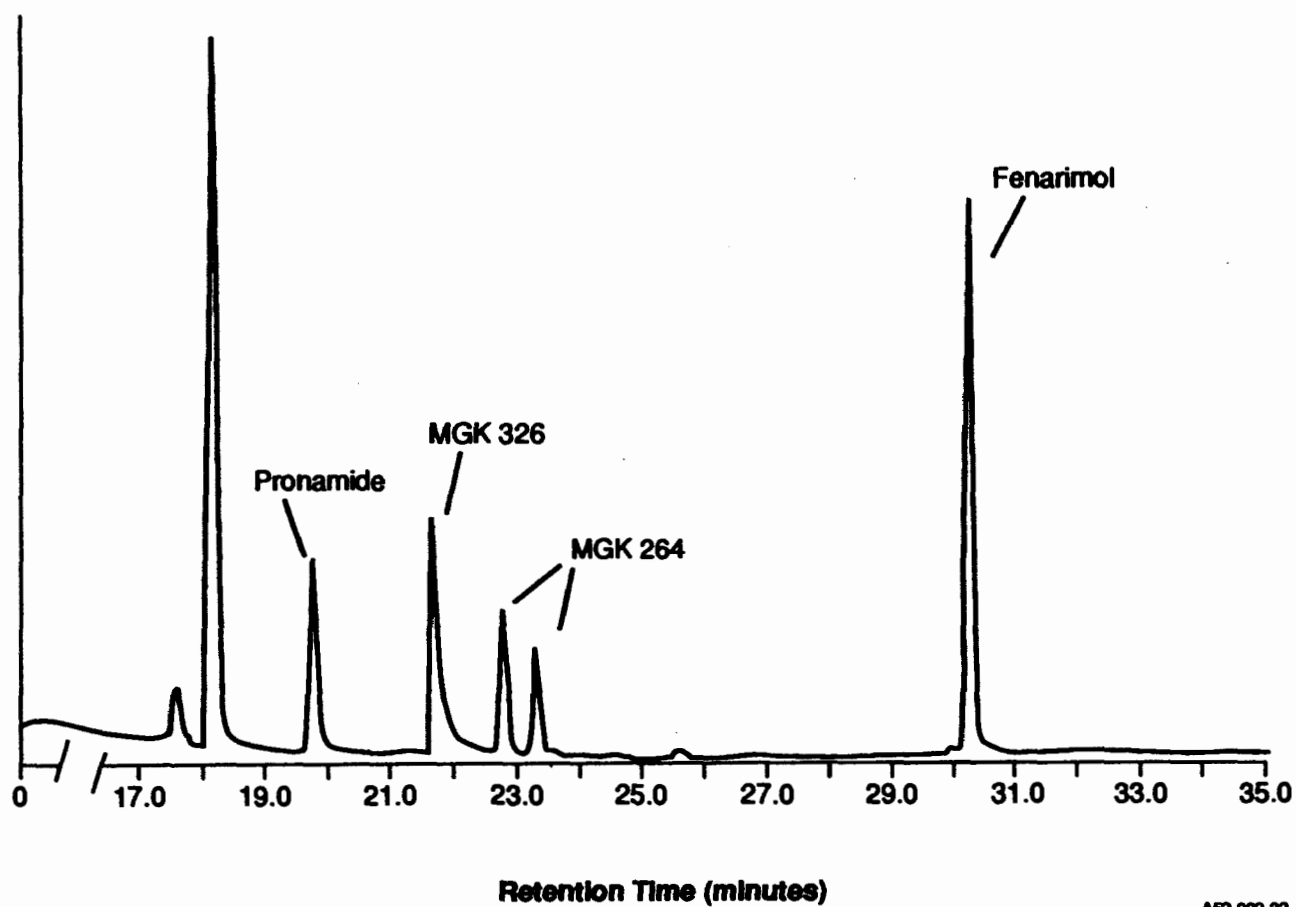
<i>Parameter</i>	<i>Sample Type(b)</i>	<i>Background, $\mu\text{g/L(c)}$</i>	<i>Spike Level, $\mu\text{g/L}$</i>	<i>Mean Recovery, %</i>	<i>Standard Deviation, %</i>	<i>Number of Replicates</i>
Fenarimol	1	1.8	20	98	4	7
	2	ND	500	96	4	7
MGK 264	1	ND	20	96	23	7
	2	ND	500	74	4	7
MGK 326	1	ND	20	108	7	7
	2	ND	500	95	4	7
Pronamide	1	ND	20	102	5	7
	2	210	500	86	3	7

(a) Column 1 conditions were used.

(b) 1 = Low level relevant industrial effluent

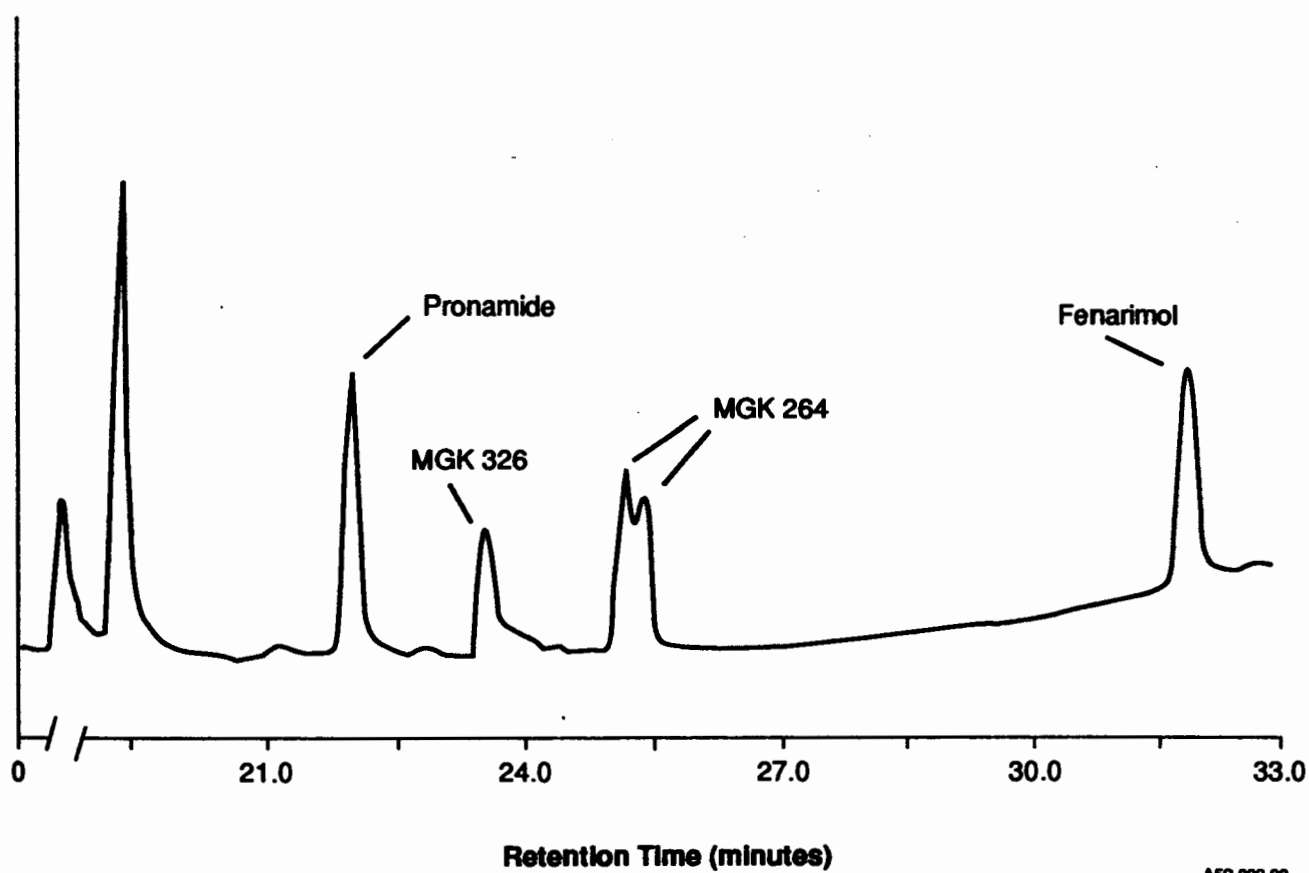
2 = High level relevant industrial effluent

(c) ND = Not detected



A52-002-32

Figure 1. GC-AFD Chromatogram of 100 ng Each of the Neutral Nitrogen Compounds (Column 1).



A52-002-33

Figure 2. GC-AFD Chromatogram of 200 ng Each of the Neutral Nitrogen Compounds (Column 2).

Method 634
***The Determination of
Thiocarbamate Pesticides in
Municipal and Industrial
Wastewaters***

Method 634

The Determination of Thiocarbamate Pesticides in Municipal and Industrial Wastewaters

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of certain thiocarbamate pesticides. The following parameters can be determined by this method:

<i>Parameter</i>	<i>CAS No.</i>
Butylate	2008-41-5
Cycloate	1134-23-2
EPTC	759-94-4
Molinate	2212-67-1
Pebulate	1114-71-2
Vernolate	1929-77-7

- 1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3 The method detection limit (MDL, defined in Section 15) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4 The sample extraction and concentration steps in this method are similar to other 600-series methods. Thus, a single sample may be extracted to measure the compounds included in the scope of the methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures.
- 1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Section 14 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. SUMMARY OF METHOD

- 2.1** A measured volume of sample, approximately 1 L, is extracted with methylene chloride using a continuous extractor. The methylene chloride extract is dried and concentrated to 5.0 mL. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by alkali flame detector (AFD) gas chromatography.¹
- 2.2** This method provides an optional silica gel column cleanup procedure to aid in the elimination of interferences which may be encountered.

3. INTERFERENCES

- 3.1** Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.
 - 3.1.1** Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.
 - 3.1.1** The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.2** Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

- 4.1** The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified³⁻⁵ for the information of the analyst.

5. APPARATUS AND MATERIALS

- 5.1** Sampling equipment, for discrete or composite sampling.

- 5.1.1 Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with PTFE. Aluminum foil may be substituted for PTFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.
- 5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.
- 5.2 Glassware. (All Specifications are suggested. Catalog numbers are included for illustration only.)
 - 5.2.1 Continuous extractor: 2000-mL, Hirschberg-Wolf, (Paxton Woods Glass Shop #1029 or equivalent).
 - 5.2.2 Drying column: Chromatographic column 400 mm long by 10 mm ID.
 - 5.2.3 Chromatographic column: 400 mm long by 19 mm ID with 250-mL reservoir at the top and PTFE stopcock (Kontes K-420290 or equivalent).
 - 5.2.4 Concentrator tube, Kuderna-Danish: 25-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. A ground-glass stopper is used to prevent evaporation of extracts.
 - 5.2.5 Evaporative flask, Kuderna-Danish: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.
 - 5.2.6 Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).
 - 5.2.7 Snyder column, Kuderna-Danish: Two-ball micro (Kontes K-569001-0219 or equivalent).
 - 5.2.8 Vials: Amber glass, 10- to 15-mL capacity with PTFE-lined screw-cap.
 - 5.2.9 Volumetric flask: 5-mL with glass stopper.
 - 5.2.10 Volumetric flask: 10-mL with glass stopper.
 - 5.2.11 Graduated cylinder: 1000-mL.
- 5.3 Boiling chips: Approximately 10/40 mesh carborundum. Heat to 400°C for 4 hours or extract in a Soxhlet extractor with methylene chloride.
- 5.4 Water bath: Heated, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.
- 5.5 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6 Gas chromatograph: Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.
 - 5.6.1 Column 1: 180 cm long by 2 mm ID glass, packed with 3% SP-2250 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance

statements in Section 15. Guidelines for the use of alternative columns are provided in Section 12.1.

5.6.2 Column 2: 180 cm long by 2 mm ID glass, packed with 3% SP-1000 on Supelcoport (100/120 mesh) or equivalent.

5.6.3 Detector: Alkali flame detector (AFD), sometimes referred to as a nitrogen-phosphorus detector (NPD) or a thermionic-specific detector (TSD). This detector has proven effective in the analysis of wastewaters for the compounds listed in the scope and was used to develop the method performance statements in Section 15. Alternative detectors, including a mass spectrometer, may be used in accordance with the provisions described in Section 12.1.

6. REAGENTS

6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Methylene chloride, methanol, petroleum ether, ethyl ether, toluene: distilled-in-glass quality or equivalent. Ethyl ether must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Catalog No. P1126-8 and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips.

6.3 Sodium sulfate: ACS, granular, anhydrous; heated in a muffle furnace at 400°C overnight.

6.4 Silica gel: Davison Grade 923, 100/200 mesh; activated by heating for 24 hours at 150°C.

6.5 6N sodium hydroxide: Dissolve 24.0 grams NaOH in 100 mL distilled water.

6.6 6N Sulfuric acid. Slowly add 16.6 mL concentrated H₂SO₄ to 50 mL distilled water and dilute to 100 mL with distilled water.

6.7 Stock standard solutions (1.00 µg/µl): Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in distilled-in-glass quality methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 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.

6.7.2 Transfer the stock standard solutions into PTFE-sealed screw-cap bottles. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.7.3 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1** Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2** External standard calibration procedure.
- 7.2.1** For each compound of interest, prepare calibration standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with toluene. 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.
- 7.2.2** Using injections of 2 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each compound at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.
- 7.2.3** The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.
- 7.3** Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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, although carbazole has been used successfully in some instances.
- 7.3.1** Prepare calibration standards at a minimum of three concentration levels for each parameter 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 toluene. 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.
- 7.3.2** Using injections of 2 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, 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} against RF.

- 7.3.3** The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.
- 7.4** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that are generated.
- 8.1.1** Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2** In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2
- 8.1.3** The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2** To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
- 8.2.1** Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol, 1000 times more concentrated than the selected concentrations.

- 8.2.2** Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
- 8.2.3** Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.
- 8.2.4** Using the appropriate data from Table 2, determine the recovery and single-operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.
- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
- 8.3.1** Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁶ that are useful in observing trends in performance.

- 8.3.2** The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation R and s . Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁶
- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.
- 8.5** Before processing any samples, the analyst should demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.
- 8.6** 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 monitor the precision of the

sampling technique. 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 perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. SAMPLES COLLECTION, PRESERVATION AND HANDLING

- 9.1** Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2** The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3** Adjust the pH of the sample to 6 to 8 with 6N sodium hydroxide or 6N sulfuric acid immediately after sampling.

10. SAMPLE EXTRACTION

- 10.1** Assemble continuous extraction apparatus by placing 5 to 10 carborundum chips into the 500-mL round-bottom flask and attaching to the extraction flask.
- 10.2** Add 400 mL methylene chloride to the extraction flask. Some methylene chloride should displace into the round-bottom flask.
- 10.3** Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into the extraction flask and add sufficient distilled water to fill extraction flask (2 L total volume aqueous phase).
- 10.4** Check the pH of the sample with wide range pH paper and adjust to 6 to 8 with 6N sodium hydroxide or 6N sulfuric acid.
- 10.5** Connect the stirring apparatus to the extraction flask without the frit touching the sample. Heat the methylene chloride in the round-bottom flask to continuous reflux and continue heating for 30 minutes to 1 hour until frit is thoroughly wetted with methylene chloride.
- 10.6** Lower frit until it just touches the sample and start the stirring apparatus rotating.
- 10.7** Continuously extract sample for 18 to 24 hours.
- 10.8** Assemble a Kuderna-Danish (K-D) concentrator by attaching a 25-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.9** Pour the extract from the round-bottom flask through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with 30 to 40 mL of methylene chloride.
- 10.10** Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube

is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

- 10.11** Remove the Snyder column and flask and adjust the volume of the extract to 5.0 mL with methylene chloride. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract is to be stored longer than 2 days, transfer the extract to a screw-capped vial with a PTFE-lined cap. If the sample extract requires no further cleanup, proceed with solvent exchange to toluene as described in Section 10.12, and then to gas chromatographic analysis as described in Section 12. If the sample requires cleanup, proceed to Section 11.
- 10.12** Add 2.5 mL of toluene and one or two clean boiling chips to the extract in the 25-mL concentrator tube and attach a two ball micro-Snyder column. Place the K-D apparatus in a hot water bath, 70 to 75°C. when the apparent volume of liquid reaches 2 to 2.5 mL. Remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Transfer the sample to a 5 mL volumetric flask and dilute to 5-mL with toluene. Proceed with gas chromatographic analysis.
- 10.13** Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 100-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1** Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.
- 11.2** Add 20 g of silica gel to a mixture of 100 mL of acetone and 1.2 mL of reagent water and stir for 30 minutes on a stirring plate. Transfer the slurry to a chromatographic column (silica gel may be retained with a plug of glass wool). Wash the column with 20 mL of methylene chloride followed by 30 mL petroleum ether. Allow the solvent to elute from the column until the silica gel is almost exposed to the air. Discard washings. Use a column flow rate of 2 to 2.5 mL/min throughout the wash and elution profiles. Add an additional 50 mL of petroleum ether to the head of the column.
- 11.3** Add the extract from Section 10.12 to the petroleum ether suspended above column. Allow the solvent to elute from the column until the silica gel is almost exposed to the air. Elute the column with 25 mL of petroleum ether (F1). Discard this fraction.
- 11.4** Elute the column with 100 mL of 50% ethyl ether in petroleum ether and collect in a K-D apparatus. Alternatively, separate fractions may be collected or combined at the discretion of the analyst. The elution profile of these compounds from silica gel is given in Table 3.

- 11.5** Concentrate the fraction to less than 5-mL after addition of 2.5 mL toluene as described in Section 10.12. Transfer sample to a 5-mL volumetric flask and dilute to 5 mL with toluene. Proceed with gas chromatographic analysis.

12. GAS CHROMATOGRAPHY

- 12.1** Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. An example of the separations achieved by Column 1 and Column 2 are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.
- 12.2** Calibrate the gas chromatographic system daily as described in Section 7.
- 12.3** If an internal standard approach is being used, the analyst must not add the internal standard to sample extracts until immediately before injection into the instrument. Mix thoroughly.
- 12.4** Inject 2 to 5 μL of the sample extract using the solvent-flush technique.^a Record the volume injected to the nearest 0.05 μL , and the resulting peak sizes in area or peak height units.
- 12.5** The width of the retention time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention-time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6** If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7** If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

- 13.1** Determine the concentration of individual compounds in the sample.
- 13.1.1** If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_e)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_e = Volume of water extracted, in mL

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(\text{RF})(V_o)}$$

where

A_s = Response for parameter to be measured

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_o = Volume of water extracted, in L

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

14. GC/MS CONFIRMATION

14.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 seconds per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all glass or glass-lined materials is recommended. When using a fused-silica capillary column, the column outlet should be threaded through the interface to within a few millimeters of the entrance to the source ionization chamber. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.

14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating

practices. Chromatographic tailing factors of less than 5.0 must be achieved. The calculation of tailing factors is illustrated in Method 625.

- 14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all DFTPP performance criteria are achieved.⁹
- 14.4 To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 50 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
 - 14.4.1 The molecular ion and all other ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to $\pm 10\%$. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20 to 40%.
 - 14.4.2 The retention time of the compound in the sample must be within 30 seconds of the same compound in the standard solution.
 - 14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC/MS only on the basis of retention-time data.
- 14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.
- 14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternative packed or capillary GC columns or additional cleanup (Section 11).

15. METHOD PERFORMANCE

- 15.1 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 Table 1 were obtained using reagent water.¹
- 15.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from $10 \times \text{MDL}$ to $1000 \times \text{MDL}$.
- 15.3 In a single laboratory, Battelle's Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 2 were obtained. Seven replicates of each of two different wastewaters were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 2.¹

References

1. "Development of Methods for Pesticides in Wastewaters". EPA Contract Report 68-03-2956 (In preparation).
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Table 1. Chromatographic Conditions and Method Detection Limits

<i>Parameter</i>	<i>Retention Time (minutes)</i>		<i>Method Detection Limit ($\mu\text{g/L}$)</i>
	<i>Column 1</i>	<i>Column 2</i>	
EPTC	12.8	17.9	0.9
Butylate	13.5	18.2	0.6
Vernolate	14.2	19.6	1.1
Pebulate	14.5	20.2	0.8
Molinate	16.6	23.8	0.6
Cycloate	17.5	24.2	1.6

Column 1 conditions: Supelcoport (100/120 mesh) coated with 3% SP-2250 packed in a 1.8 m long by 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. Column temperature is held at 80°C for 4 minutes, programmed from 80°C to 300°C at 8°C/min and held at 300°C for 4 minutes.

Column 2 conditions: Supelcoport (100/120 mesh) coated with 3% SP-2100 packed in a 1.8 m long by 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. Column temperature is held at 80°C for 10 minutes, programmed from 80°C to 250°C at 8°C/min and held at 250°C for 10 minutes.

Table 2. Single Laboratory Accuracy and Precision(a)

<i>Parameter</i>	<i>Average Percent Recovery</i>	<i>Relative Standard Deviation %</i>	<i>Spike Level (µg/ L)</i>	<i>Number of Analyses</i>	<i>Matrix Type(b)</i>
Butylate	80	18	5.0	7	1
	95	7.2	50	7	1
Cycloate	93	16	5.0	7	1
	95	7.3	50	7	1
EPTC	100	18	5.0	7	1
	100	4.8	50	7	1
Molinate	87	17	5.0	7	1
	93	8.4	50	7	1
Pebulate	97	26	5.0	7	1
	98	5.7	50	7	1
Vernolate	93	18	5.0	7	1
	96	10	50	7	1

(a) Column 1 conditions were used.

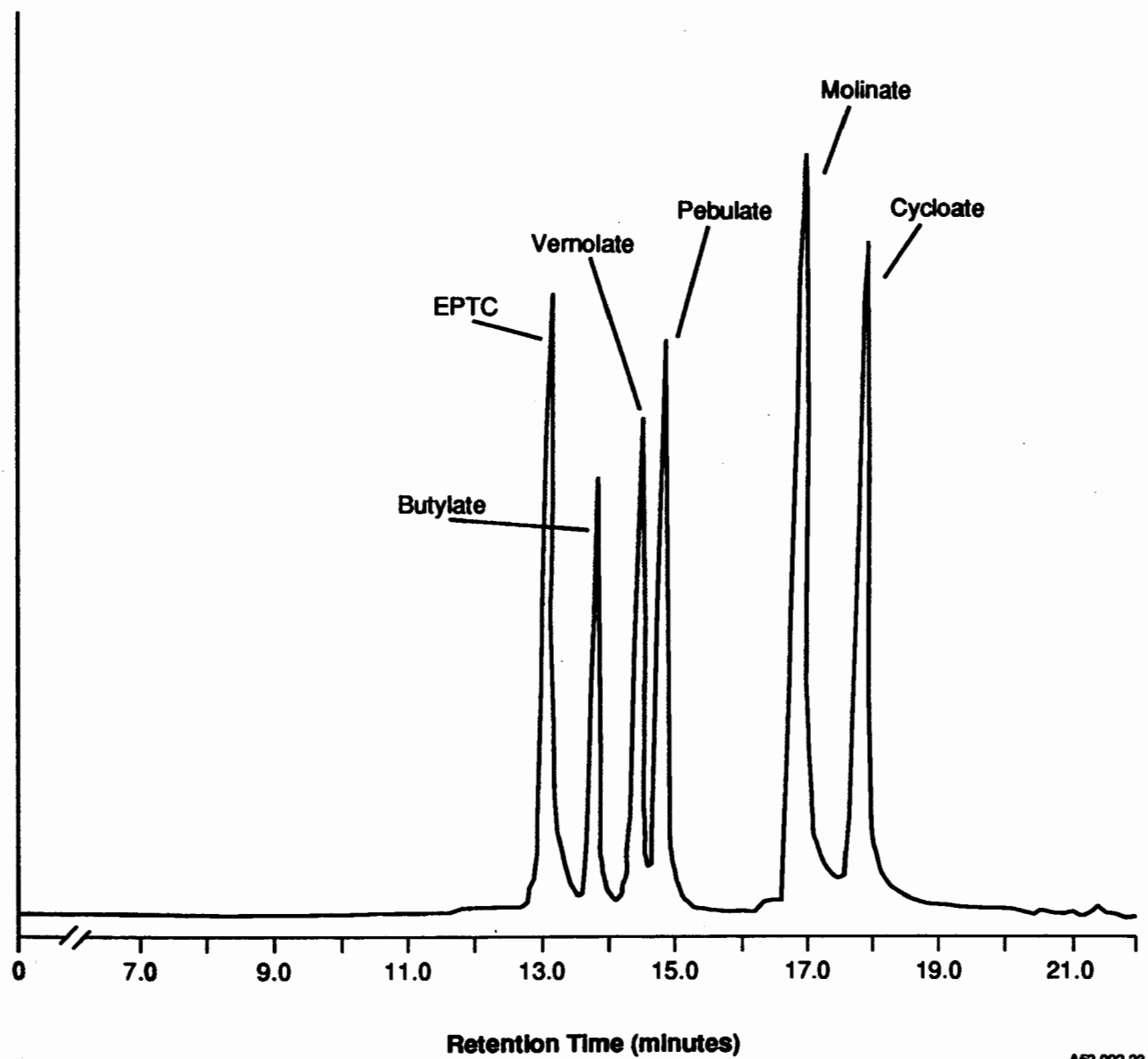
(b) 1 = secondary POTW effluent

Table 3. Elution Characteristics of the Thiocarbamates from 6% Deactivated Silica Gel

<i>Parameter</i>	<i>Appearance in Specified Fraction(a)</i>			
	<i>F1</i>	<i>F2</i>	<i>F3</i>	<i>F4</i>
Butylate		x	x	
Cycloate			x	
EPTC			x	
Molinate				x
Pebulate			x	
Vernolate			x	

Eluant composition by fraction:

- (a) F1 = 25 mL petroleum ether
F2 = 50 mL 6% ethyl ether in petroleum ether
F3 = 50 mL 15% ethyl ether in petroleum ether
F4 = 50 mL 50% ethyl ether in petroleum ether



A52-002-23

Figure 1. GC-AFD Chromatogram of 200 ng of Each Thiocarbamate (Column 1).

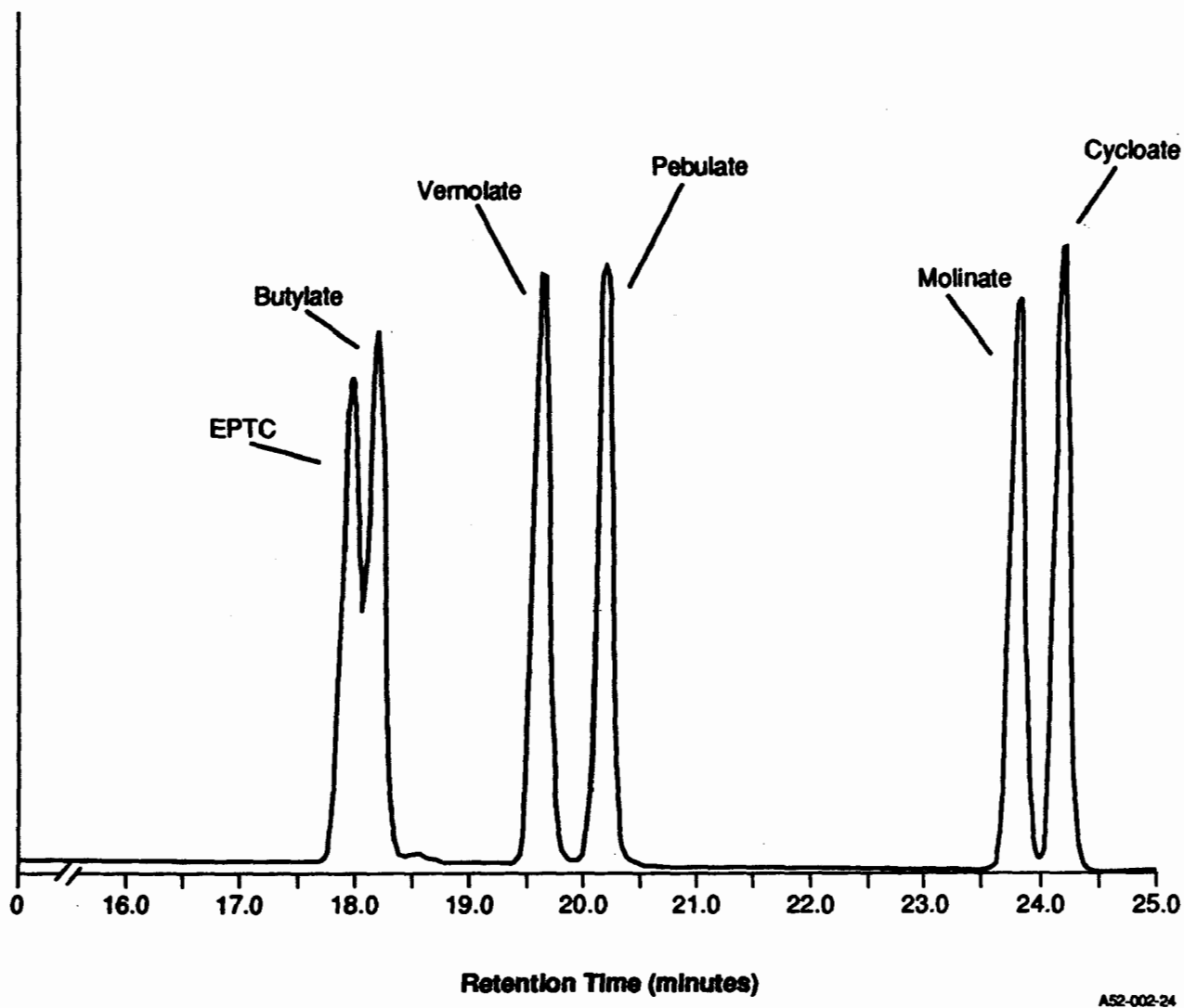


Figure 2. GC-AFD Chromatogram of 200 ng of Each Thiocarbamate (Column 2).

Method 635
The Determination of Rotenone
in Municipal and Industrial
Wastewaters

Method 635

The Determination of Rotenone in Municipal and Industrial Wastewaters

1. **SCOPE AND APPLICATION**

- 1.1 This method covers the determination of rotenone pesticide. The following parameter can be determined by this method:

<i>Parameter</i>	<i>CAS No.</i>
Rotenone	83-79-4

- 1.2 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compound listed above in municipal and industrial discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3 The method detection limit (MDL, defined in Section 14) for rotenone compound is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.5 When this method is used to analyze unfamiliar samples for the compound above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second liquid chromatographic column that can be used to confirm measurements made with the primary column.

2. **SUMMARY OF METHOD**

- 2.1 A measured volume of sample, approximately 1 L, is extracted with methylene chloride using a separatory funnel. Liquid chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by HPLC- UV.¹

3. **INTERFERENCES**

- 3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing hardware that lead to discrete artifacts or elevated baselines in liquid chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

- 3.1.1 Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot

water and detergent and thoroughly rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedure in Section 11 can be used to overcome these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified³⁻⁵ for the information of the analyst.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with PTFE. Aluminum foil may be substituted for PTFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel: 2000-mL, with PTFE stopcock.

5.2.2 Drying column: Chromatographic column 400 mm long by 10 mm ID with coarse frit.

- 5.2.3 Chromatographic column: 400 mm long by 19 mm ID with 250-mL reservoir at the top and PTFE stopcock (Kontes K-420290) or equivalent).
- 5.2.4 Concentrator tube, Kuderna-Danish: 25-mL, graduated (Kontes K-570050-2525 or equivalent). Calibration must be checked at the volumes employed in the test. A ground-glass stopper is used to prevent evaporation of extracts.
- 5.2.5 Evaporative flask, Kuderna-Danish: 250-mL (Kontes K-570001-0250 or equivalent). Attach to concentrator tube with springs.
- 5.2.6 Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).
- 5.2.7 Snyder column, Kuderna-Danish: Two-ball micro (Kontes K-569001-0219 or equivalent).
- 5.2.8 Vials: Amber glass, 5- to 10-mL capacity with PTFE-lined screw-cap.
- 5.2.9 Volumetric flask: 10-mL.
- 5.2.10 Erlenmeyer flask: 250-mL.
- 5.2.11 Graduated cylinder: 1000-mL.
- 5.3 Boiling chips: Approximately 10/40 mesh carborundum. Heat to 400°C for 4 hours or extract in a Soxhlet extractor with methylene chloride.
- 5.4 Water bath: Heated, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.
- 5.5 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6 Liquid chromatograph: Analytical system complete with liquid chromatograph and all required accessories including syringes, analytical columns, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.
 - 5.6.1 Pump: Isocratic pumping system, constant flow.
 - 5.6.2 Column 1: Normal-phase column, 5 μ Zorbax-CN, 250 mm long by 4.6 mm ID or equivalent.
 - 5.6.3 Column 2: Reversed-phase column, 5 μ Spherisorb-ODS, 250 mm long by 4.6 mm ID or equivalent.
 - 5.6.4 Detector: Ultraviolet absorbance detector, 254 nm.

6. REAGENTS

- 6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2 Methylene chloride, methanol, acetonitrile, acetone, hexane: Distilled-in-glass quality or equivalent.
- 6.3 Sodium sulfate: ACS, granular, anhydrous; heated in a muffle furnace at 400°C overnight.
- 6.4 1N sulfuric acid.
- 6.5 1N sodium hydroxide.
- 6.6 Silica gel, Davison grade 923, 100-200 mesh, dried for 12 hours at 150°C.

6.7 Stock standard solutions (1.00 µg/L): Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in distilled-in-glass quality methylene chloride for analyses performed using Column 1 and methanol for analyses performed using Column 2. Dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 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.

6.7.2 Transfer the stock standard solutions into PTFE-sealed screw-cap bottles. 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 calibration standards from them.

6.7.3 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

7.1 Establish liquid chromatographic operating parameters equivalent to those indicated in Table 1. The liquid chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 For each compound of interest, prepare calibration standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with 50/50 hexane/methylene chloride for Column 1 standards and acetonitrile for Column 2 standards. 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.

7.2.2 Using injections of 5 to 20 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each compound at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% 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.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each compound 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 50/50 hexane/methylene chloride for Column 1 standards and acetonitrile for Column 2 standards. 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.

7.3.2 Using injections of 5 to 20 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to pilot a calibration curve of response ratios, A_s/A_{is} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. QUALITY CONTROL

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

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

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methylene chloride, 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percentage recovery (R), and the standard deviation of the percentage recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 2, determine the recovery and single-operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁶ that are useful in observing trends in performance.

- 8.3.2** The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated with this method. This ability is established as described regularly.⁶
- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.
- 8.5** Before processing any samples, the analyst should demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.
- 8.6** 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 monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques, such as liquid chromatography with a dissimilar column, must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. SAMPLES COLLECTION, PRESERVATION, AND HANDLING

- 9.1** Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon and other potential sources of contamination.
- 9.2** The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3** Adjust the pH of the sample to 6 to 8 with 1N sodium hydroxide or 1N sulfuric acid immediately after sampling.

10. SAMPLE EXTRACTION

- 10.1** Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel. Check the pH of the sample with wide range pH paper and adjust to 7 with 1N sodium hydroxide or 1N H₂SO₄.
- 10.2** Add 60 mL of methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the

- funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.
- 10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, collecting the extract. Perform a third extraction in the same manner and collect the extract.
 - 10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 25-mL concentrator tube to a 250-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
 - 10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with an additional 30 to 40 mL of methylene chloride.
 - 10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
 - 10.7 Remove the macro-Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommended for this operation. Add one or two clean boiling chips and attach a two-ball micro-Snyder column to the concentrator tube. Prewet the micro-Snyder column with methylene chloride and concentrate the solvent extract as before. When an apparent volume of 0.5 mL is reached, or the solution stops boiling, remove the K-D apparatus and allow it to drain and cool for 10 minutes. If analysis is being performed using Column 1 or if sample cleanup is required, proceed with Section 10.9. If Column 2 is used and no sample cleanup is required, proceed with Section 10.8.
 - 10.8 Add 10 mL of acetonitrile to the concentrator tube along with one or two clean boiling chips. Attach a two-ball micro-Snyder column to the concentrator tube. Prewet the micro-Snyder column with acetonitrile and concentrate the solvent extract as before. When an apparent volume of 1 mL is reached, remove the K-D apparatus and allow it to drain and cool for 10 minutes. Transfer the liquid to a 10-mL volumetric flask and dilute to the mark with acetonitrile. Mix thoroughly prior to analysis. Proceed with Section 12 using Column 2.
 - 10.9 Remove the micro-Snyder column and adjust the volume of the extract to 1.0 mL with methylene chloride. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract is to be stored longer than two days, transfer the extract to a screw-capped vial with a PTFE-lined cap. If the sample extract requires no further cleanup,

proceed with the liquid chromatographic analysis in Section 12 using Column 1. If the sample requires cleanup, proceed to Section 11.

- 10.10** Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1** Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of additional cleanup, the analyst must demonstrate that the recovery of each compound of interest is no less than 85%.
- 11.2** Slurry 10 g of silica gel in 50 mL of acetone to which has been added 600 μ L of reagent water. Transfer the slurry to a chromatographic column (silica gel is retained with a plug of glass wool). Wash the column with 100 mL of methylene chloride. Use a column flow rate of 2 to 2.5 mL/min throughout the wash and elution profiles.
- 11.3** Add the extract from Section 10.9 to the head of the column. Allow the solvent to elute from the column until the silica gel is almost exposed to the air. Elute the column with 50 mL of methylene chloride. Discard this fraction.
- 11.4** Elute the column with 60 mL of 6% acetone in methylene chloride. Collect this fraction in a K-D apparatus. Concentrate the column fraction to 1 mL as described in Sections 10.6 and 10.7. If Column 1 is being used, proceed with Section 11.5. If Column 2 is being used, proceed with Section 11.7.
- 11.5** Add 5 mL of hexane to the concentrate along with one or two clean boiling chips. Attach a three-ball micro-Snyder column to the concentrator tube. Prewet the micro-Snyder column with hexane and concentrate the solvent extract to an apparent volume of 1 mL. Allow the K-D apparatus to drain and cool for 10 minutes.
- 11.6** Transfer the liquid to a 10-mL volumetric flask and dilute to the mark with hexane. Mix thoroughly prior to analysis. If the extracts will not be analyzed immediately, they should be transferred to PTFE-sealed screw-cap vials and refrigerated. Proceed with the liquid chromatographic analysis using Column 1.
- 11.7** Add 10 mL of acetonitrile to the concentrate along with one or two clean boiling chips. Attach a three-ball micro-Snyder column to the concentrator tube. Prewet the micro-Snyder column with acetonitrile and concentrate the solvent extract to an apparent volume of 1 mL. Allow the K-D apparatus to drain and cool for 10 minutes.
- 11.8** Transfer the liquid to a 10-mL volumetric flask and dilute to the mark with acetonitrile. Mix thoroughly prior to analysis. If the extracts will not be analyzed immediately, they should be transferred to PTFE-sealed screw-cap vials and refrigerated. Proceed with the liquid chromatographic analysis using Column 2.

12. LIQUID CHROMATOGRAPHY

- 12.1** Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. An example of the separations achieved by Column 1 and Column 2

are shown in Figures 1 and 2. Other columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.

- 12.2 Calibrate the liquid chromatographic system daily as described in Section 7.
- 12.3 If an internal standard approach is being used, the analyst must not add the internal standard to sample extracts until immediately before injection into the instrument. Mix thoroughly.
- 12.4 Inject 5 to 20 μL of the sample extract by completely filling the sample valve loop. Record the resulting peak sizes in area or peak height units.
- 12.5 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

- 13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_e)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_e = Volume of water extracted, in mL

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where

A_s = Response for parameter to be measured

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_o = Volume of water extracted, in L

- 13.2** Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 13.3** For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

14. METHOD PERFORMANCE

- 14.1** 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 about zero. The MDL concentrations listed in Table 1 were obtained using reagent water.¹ Similar results were achieved using representative wastewaters.
- 14.2** This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from $10 \times \text{MDL}$ to $1000 \times \text{MDL}$.
- 14.3** In a single laboratory, Battelle Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 2 were obtained. Seven replicates of each of two different wastewaters were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 2.¹

References

1. "Development of Methods for Pesticides in Wastewaters," Report for EPA Contract 68-03-2956 (In preparation).
2. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, Pennsylvania, p. 679, 1980.
3. "Carcinogens - Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August, 1977.
4. "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January, 1976).
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6. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U. S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory - Cincinnati, Ohio, March, 1979.
7. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, Pennsylvania, p. 76, 1980.
8. Glaser, J. A. et al, "Trace Analysis for Wastewaters," *Environmental Science and Technology*, 15, 1426 (1981).

Table 1. Chromatographic Conditions and Method Detection Limits

<i>Parameter</i>	<i>Retention Time (min.)</i>		<i>Method Detection Limit (µg/L)</i>
	<i>Column 1</i>	<i>Column 2</i>	
Rotenone	8.6	8.0	1.6

Rotenone

Column 1 conditions: Zorbax-CN, 5 micron, 250 by 4.6 mm; 1 mL/min flow; 30/70 methylene chloride/hexane.

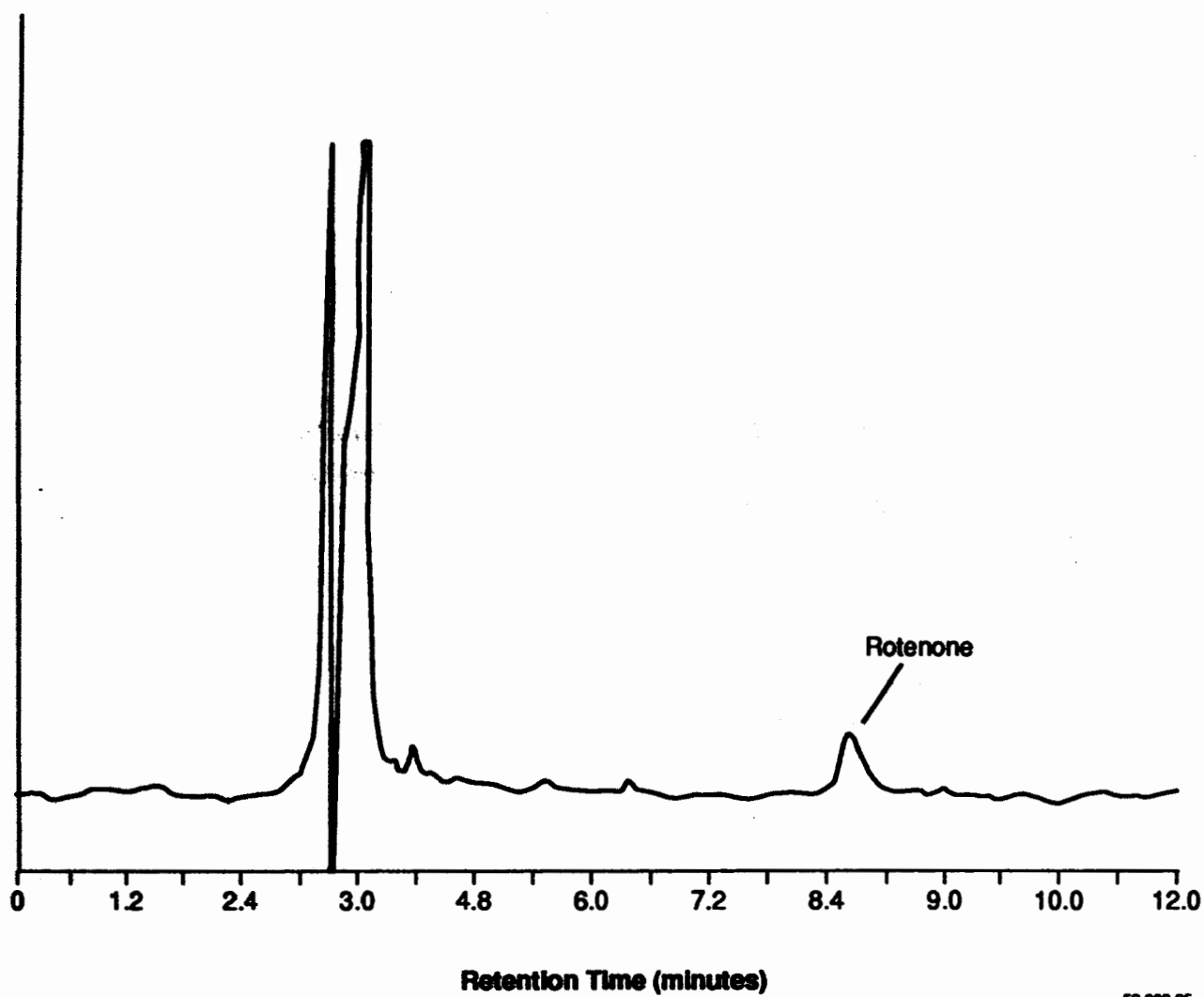
Column 2 conditions: Spherisorb-ODS, 5 micron, 250 by 4.6 mm; 1 mL/min flow; 60/40 acetonitrile/water.

Table 2. Single-Laboratory Accuracy and Precision (a)

<i>Parameter</i>	<i>Average Percent Recovery</i>	<i>Standard Deviation Percent</i>	<i>Spike Level (µg/L)</i>	<i>Number of Analyses</i>	<i>Matrix Type (b)</i>
Rotenone	85	8	5.5	7	1
	88	3	109	7	1

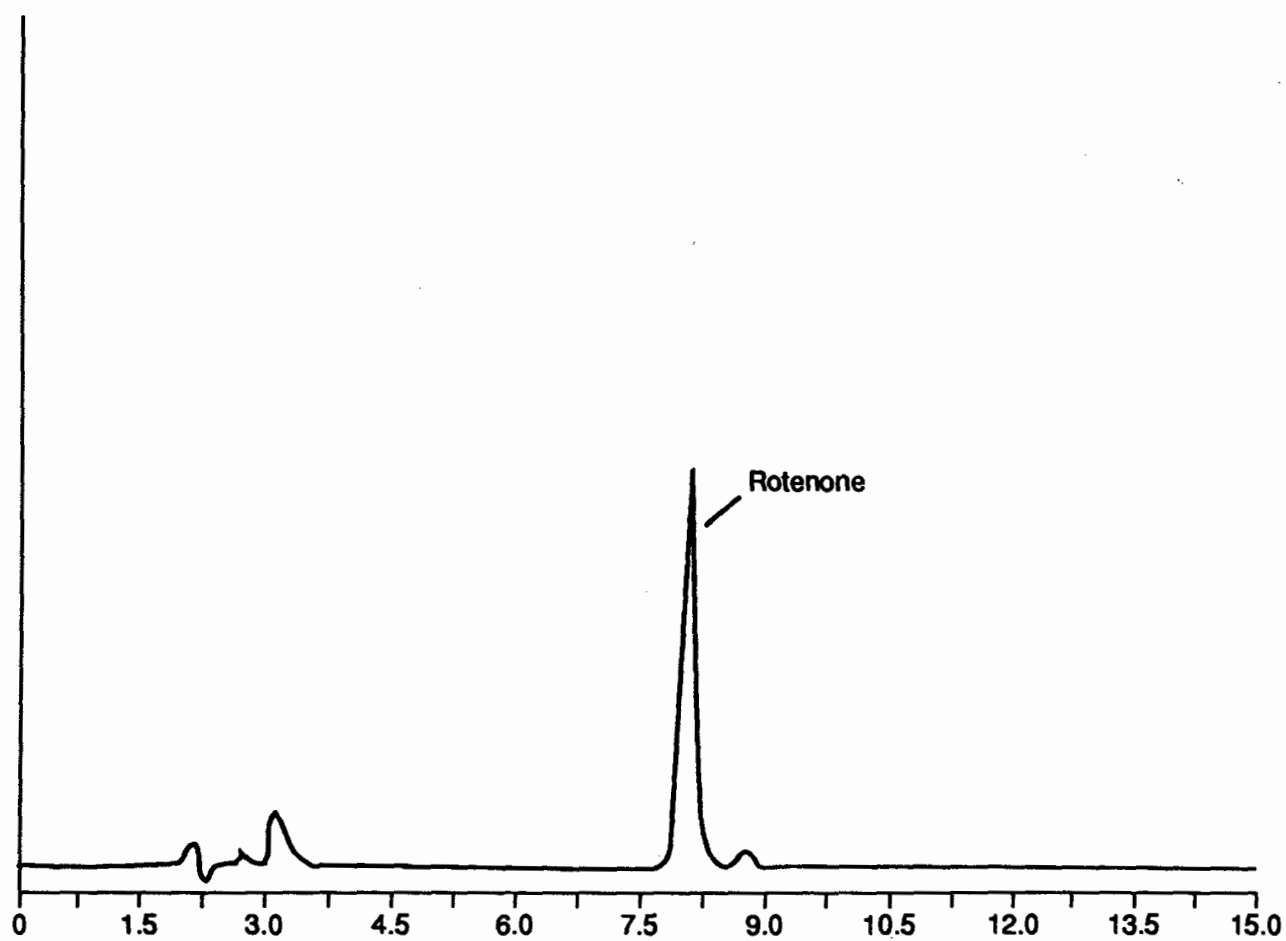
(a) Column 1 conditions were used.

(b) 1 = Pesticide manufacturing wastewater



62-002-25

Figure 1. HPLC-UV Chromatogram of Standard Solution Representing 5 µg/L of Rotenone in Water (Column 1).



A52-002-26

Figure 2. HPLC-UV Chromatogram of Standard Solution Representing 200 µg/L of Rotenone in Water (Column 2.)

Method 636
***The Determination of Bensulide
in Municipal and Industrial
Wastewater***

Method 636

The Determination of Bensulide in Municipal and Industrial Wastewater

1 SCOPE AND APPLICATION

- 1.1 This method covers the determination of bensulide pesticide. The following parameter can be determined by this method:

<i>Parameters</i>	<i>CAS No.</i>
Bensulide	741-58-2

- 1.2 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compound listed above in municipal and industrial discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3 The method detection limit (MDL, defined in Section 14) for bensulide compound is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.5 When this method is used to analyze unfamiliar samples for the compound above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second liquid chromatographic column that can be used to confirm measurements made with the primary column.

2. SUMMARY OF METHOD

- 2.1 A measured volume of sample, approximately 1 L, is extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to acetonitrile during concentration to a volume of 2 mL or less. Liquid chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by HPLC-UV.¹

3. INTERFERENCES

- 3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts or elevated baselines in liquid chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

- 3.1.1 Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.
- 3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedure in Section 11 can be used to overcome these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified^{3,5} for the information of the analyst.

5. APPARATUS AND MATERIALS

- 5.1 Sampling equipment, for discrete or composite sampling.
 - 5.1.1 Grab-sample bottle: Borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with PTFE. Aluminum foil may be substituted for PTFE if the sample is not corrosive. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.
 - 5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.
- 5.2 Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)
 - 5.2.1 Separatory funnel: 2000-mL, with PTFE stopcock.

- 5.2.2 Drying column: Chromatographic column 400 mm long by 10 mm ID with coarse frit.
- 5.2.3 Chromatographic column: 400 mm long by 19 mm ID with 250-mL reservoir at the top and PTFE stopcock (Kontes K-420290 or equivalent).
- 5.2.4 Concentrator tube, Kuderna-Danish: 25-mL, graduated (Kontes K-570050-2525 or equivalent). Calibration must be checked at the volumes employed in the test. A ground-glass stopper is used to prevent evaporation of extracts.
- 5.2.5 Evaporative flask, Kuderna-Danish: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.
- 5.2.6 Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).
- 5.2.7 Snyder column, Kuderna-Danish: Two-ball micro (Kontes K-569001-0219 or equivalent).
- 5.2.8 Vials: Amber glass, 5- to 10-mL capacity with PTFE-lined screw-cap.
- 5.3 Boiling chips: Approximately 10/40 mesh carborundum. Heat to 400°C for 4 hours or extract in a Soxhlet extractor with methylene chloride.
- 5.4 Water bath: Heated, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.
- 5.5 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6 Liquid chromatograph: Analytical system complete with liquid chromatograph and all required accessories including syringes, analytical columns, detector and strip-chart recorder. A data system is recommended for measuring peak areas.
 - 5.6.1 Pump: Isocratic pumping system, constant flow.
 - 5.6.2 Column 1: Reversed-phase column, 5 μ Spherisorb-ODS, 250 mm long by 4.6 mm ID or equivalent.
 - 5.6.3 Column 2: Reversed-phase column, 5 μ Lichrosorb RP-2, 250 mm long by 4.6 mm ID or equivalent.
 - 5.6.4 Detector: Ultraviolet absorbance detector, 270 nm.

6. REAGENTS

- 6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2 Methylene chloride, methanol, acetonitrile: Distilled-in-glass quality or equivalent.
- 6.3 Sodium sulfate: ACS, granular, anhydrous; heated in a muffle furnace at 400°C overnight.
- 6.4 Sodium phosphate, monobasic: ACS, crystal.
- 6.5 1N sulfuric acid: Slowly add 2.8 mL of concentrated H_2SO_4 (94%) to about 50 mL of distilled water. Dilute to 100 mL with distilled water.
- 6.6 1N sodium hydroxide: Dissolve 4.0 grams of NaOH in 100 mL of distilled water.
- 6.7 Florisil: PR grade (60/100 mesh). Purchase activated at 675 °C and store in brown glass bottle. To prepare for use, place 150 g in a wide-mouth jar and heat overnight at 160 to 170°C. Seal tightly with PTFE or aluminum foil-lined screw-cap and cool to room temperature.

- 6.8 Stock standard solutions (1.00 $\mu\text{g}/\mu\text{L}$):** Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.
- 6.8.1** Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in distilled-in-glass quality methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 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.
- 6.8.2** Transfer the stock standard solutions into PTFE-sealed screw-cap bottles. 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 calibration standards from them.
- 6.8.3** Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1** Establish liquid chromatographic operating parameters equivalent to those indicated in Table 1. The liquid chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2 External standard calibration procedure:**
- 7.2.1** For each compound of interest, prepare calibration standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with acetonitrile. 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.
- 7.2.2** Using injections of 2 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each compound at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% 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.
- 7.2.3** The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.
- 7.3 Internal standard calibration procedure:** To use this approach, the analyst must select one or more internal standards 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.

- 7.3.1** Prepare calibration standards at a minimum of three concentration levels for each compound 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 acetonitrile. 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.
- 7.3.2** Using injections of 2 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_i C_u)}{(A_u C_i)}$$

where

A_i = Response for the compound to be measured

A_u = Response for the internal standard

C_u = Concentration of the internal standard, in $\mu\text{g/L}$

C_i = Concentration of the compound to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, 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_i/A_u , against RF.

- 7.3.3** The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.
- 7.4** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

- 8.1.1** Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2** In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
- 8.1.3** The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2** To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
 - 8.2.1** Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol 1000 times more concentrated than the selected concentrations.
 - 8.2.2** Using a pipette, add 1.00 mL of the check sample of each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
 - 8.2.3** Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.
 - 8.2.4** Using the appropriate data from Table 2, determine the recovery and single-operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.
- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
 - 8.3.1** Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁶ that are useful in observing trends in performance.

- 8.3.2** The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated with this method. This ability is established as described regularly.⁶

- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.
- 8.5** Before processing any samples, the analyst should demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.
- 8.6** 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 monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as liquid chromatography with a dissimilar column, must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. SAMPLES COLLECTION, PRESERVATION, AND HANDLING

- 9.1** Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon and other potential sources of contamination.
- 9.2** The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3** Adjust the pH of the sample to 6 to 8 with 1N sodium hydroxide or 1N sulfuric acid immediately after sampling.

10. SAMPLE EXTRACTION

- 10.1** Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel. Check the pH of the sample with wide range pH paper and adjust to 7 with 1N sodium hydroxide or 1N H₂SO₄.
- 10.2** Add 60 mL of methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

- 10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, collecting the extract. Perform a third extraction in the same manner and collect the extract.
- 10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 25-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with 30 to 40 mL of methylene chloride.
- 10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball macro-Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least ten minutes. If the sample extract requires no further cleanup, proceed with solvent exchange to acetonitrile and chromatographic analysis as described in Sections 11.5 and 12 respectively. If the sample requires cleanup, proceed to Section 10.7.
- 10.7 Remove the macro-Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommended for this operation. Add one or two clean boiling chips and attach a two-ball micro-Snyder column to the concentrator tube. Prewet the micro-Snyder column with methylene chloride and concentrate the solvent extract as before. When an apparent volume of 0.5 mL is reached, or the solution stops boiling remove the K-D apparatus and allow it to drain and cool for 10 minutes.
- 10.8 Remove the micro-Snyder column and adjust the volume of the extract to 1.0 mL with methylene chloride. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract is to be stored longer than 2 days, transfer the extract to a screw-capped vial with a PTFE-lined cap.
- 10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of additional cleanup, the analyst must demonstrate that the recovery of each compound of interest is no less than 76%.
- 11.2 Slurry 10 g of Florisil in 100 mL of methylene chloride which has been saturated with reagent water. Transfer the slurry to a chromatographic column (Florisil may be retained with a plug of glass wool). Wash the column with 100 mL of methylene chloride. Use a column flow rate of 2 to 2.5 mL/min throughout the wash and elution profiles.

- 11.3 Add the extract from Section 10.8 to the head of the column. Allow the solvent to elute from the column until the Florisil is almost exposed to the air. Elute the column with 50 mL of methylene chloride. Discard this fraction.
- 11.4 Elute the column with 50 mL of 5% acetone in methylene chloride. Collect this fraction in a K-D apparatus. Concentrate the column fraction to 1 mL as described in Sections 10.6 and 10.7.
- 11.5 Add 15 mL of acetonitrile to the concentrate along with one or two clean boiling chips. Attach a three-ball micro-Snyder column to the concentrator tube. Prewet the micro-Snyder column with acetonitrile and concentrate the solvent extract to an apparent volume of 1 mL. Allow the K-D apparatus to drain and cool for 10 minutes.
- 11.6 Transfer the liquid to a 2-mL volumetric flask and dilute to the mark with acetonitrile. Mix thoroughly prior to analysis. If the extracts will not be analyzed immediately, they should be transferred to PTFE-sealed screw-cap vials and refrigerated. Proceed with the liquid chromatographic analysis.

12. LIQUID CHROMATOGRAPHY

- 12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. An example of the separations achieved by Column 1 and Column 2 are shown in Figure 1. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.
- 12.2 Calibrate the liquid chromatographic system daily as described in Section 7.
- 12.3 If an internal standard approach is being used, the analyst must not add the internal standard to the sample extracts until immediately before injections into the instrument. Mix thoroughly.
- 12.4 Inject 2 to 5 μ L of the sample extract into the sample valve loop. Record the resulting peak sizes in area or peak heights units.
- 12.5 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

- 13.1 Determine the concentration of individual compounds in the sample.
 - 13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_e)}$$

where

A = Amount of material injected, in nanograms

V_i = Volume of extract injected, in $\mu\text{g/L}$

V_t = Volume of total extract, in $\mu\text{g/L}$

V_e = Volume of water extracted, in mL

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_e)}$$

where

A_s = Response for parameter to be measured

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_e = Volume of water extracted, in liters

13.2 Report results in microgram per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

14. METHOD PERFORMANCE

14.1 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.⁸ The MDL concentrations listed in Table 1 were obtained using reagent water.⁸ Similar results were achieved using representative wastewaters.

14.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from $10 \times \text{MDL}$ to $1000 \times \text{MDL}$.

14.3 In a single laboratory, Battelle Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 2 were obtained. Seven replicates of each of two different wastewaters were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 2.¹

References

1. "Development of Methods for Pesticides in Wastewaters," EPA Contract Report 68-03-2956 (In preparation).
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7. ASTM Annual Book of Standards, Part 31, 03370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, Pennsylvania, p. 76, 1980.
8. Glaser, J. A. et al, "Trace Analysis for Wastewaters," *Environmental Science and Technology*, 15, 1426, (1981).

Table 1. Chromatographic Conditions and Method Detection Limits

<i>Parameter</i>	<i>Retention Time (min.)</i>		<i>Method Detection Limit (ug/L)</i>
	<i>Column 1</i>	<i>Column 2</i>	
Bensulide	14.1	7.2	1.6

Column 1 conditions: Spherisorb ODS, 5 μ , 250 mm long by 4.6 mm ID; 1 mL/min flow; 55/45 acetonitrile/water.

Column 2 conditions: Lichrosorb RP-2, 5 μ , 250 mm long by 4.6 mm ID; 1 mL/min flow; 60/40 acetonitrile/water.

Table 2. Single-Laboratory Accuracy and Precision^(a)

<i>Parameter</i>	<i>Average Percent Recovery</i>	<i>Standard Deviation, %</i>	<i>Spike Level (ug/L)</i>	<i>Number of Analyses</i>	<i>Matrix Type (b)</i>
Bensulide	86	18	25	7	1
	76	18	250	7	1

(a) Column 1 conditions were used.

(b) 1 = Relevant industrial wastewater

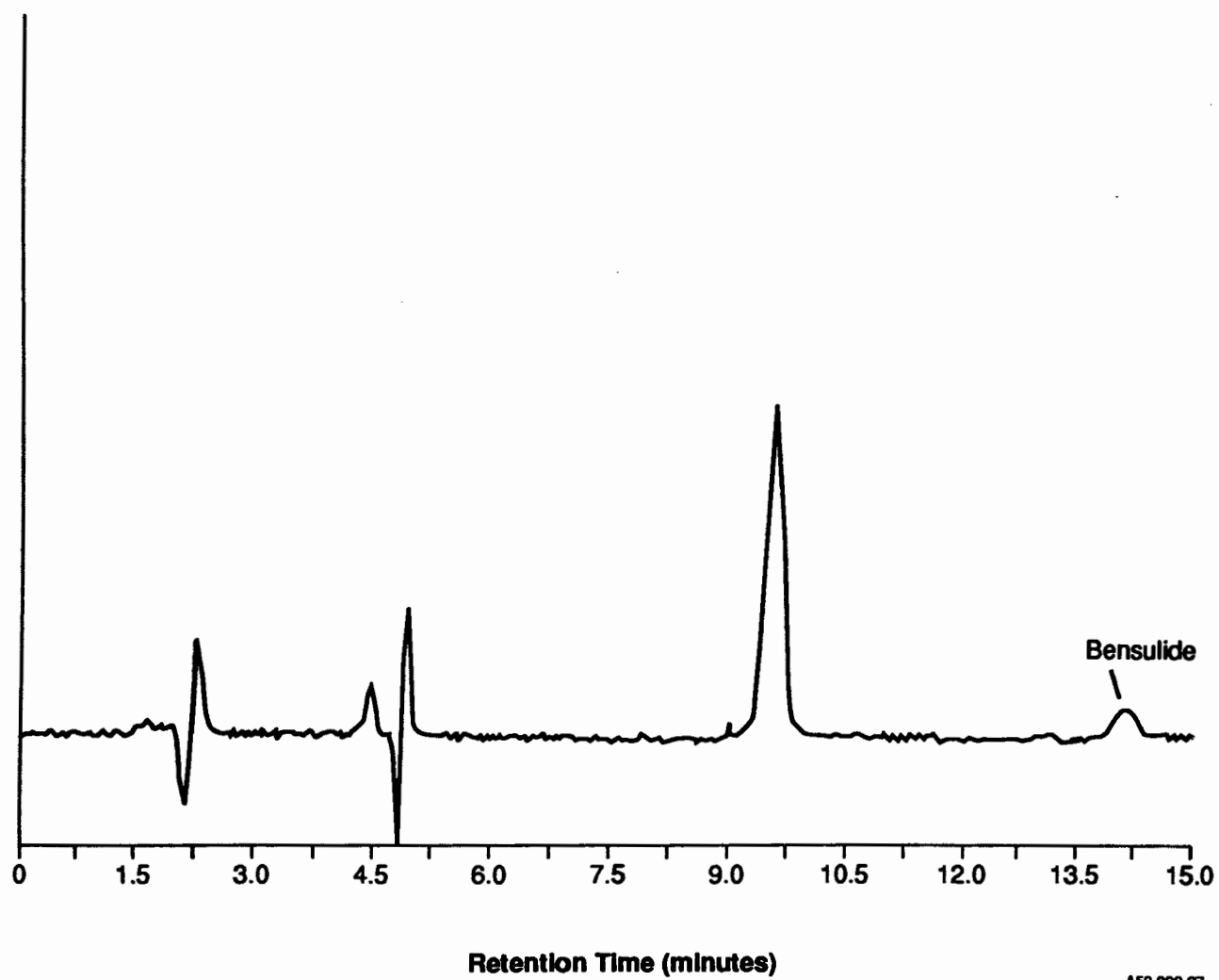


Figure 1. HPLC-UV Chromatogram of 60 ng of Bensulide (Column 1).

Method 637
***The Determination of MBTS
and TCMTB in Municipal and
Industrial Wastewaters***

Method 637

The Determination of MBTS and TCMTB in Municipal and Industrial Wastewaters

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of MBTS and TCMTB pesticides. The following parameters can be determined by this method.

<i>Parameter</i>	<i>CAS No.</i>
MBTS	120-78-5
TCMTB	21564-17-0

- 1.2 This is a liquid chromatographic (LC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3 The method detection limit (MDL, defined in Section 14) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4 The sample extraction and concentration steps in this method are essentially the same as in certain other 600-series methods. Thus, a single sample may be extracted to measure the compounds included in the scope of the methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures.
- 1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2
- 1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second liquid chromatographic column that can be used to confirm measurements made with the primary column.

2. SUMMARY OF METHOD

- 2.1 A measured volume of sample, approximately 1 L, is extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated to 5.0 mL. Liquid chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by high performance liquid chromatography with ultraviolet detection.¹
- 2.2 This method provides a silica gel column cleanup procedure to aid in the elimination of interferences which may be encountered.

3. INTERFERENCES

- 3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baselines in liquid chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.
- 3.1.1 Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.
- 3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified³⁻⁵ for the information of the analyst.

5. APPARATUS AND MATERIALS

- 5.1 Sampling equipment, for discrete or composite sampling.
- 5.1.1 Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with PTFE. Foil may be substituted for PTFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.
- 5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C

and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel: 2000-mL, with PTFE stopcock.

5.2.2 Drying column: Chromatographic column 400 mm long by 10 mm ID with coarse frit.

5.2.3 Chromatographic column: 400 mm long by 19 mm ID with 250-mL reservoir at the top and PTFE stopcock (Kontes K-420290 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. A ground-glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Vials: Amber glass, 10- to 15-mL capacity with PTFE-lined screw-cap.

5.2.8 Erlenmeyer flask: 250-mL.

5.2.9 Graduated cylinder 1000-mL.

5.2.10 Volumetric flask: 5-mL, 10-mL.

5.3 Boiling chips: Approximately 10/40 mesh carborundum. Heat at 400°C for 4 hours or perform a Soxhlet extraction with methylene chloride.

5.4 Water bath: Heated, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.

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

5.6 Liquid chromatograph: Analytical system complete with liquid chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column 1: 5 μ Dupont Zorbax-CN, 250 mm long by 4.6 mm ID or equivalent. This column was used to develop the method performance statements in Section 14. Alternative columns may be used in accordance with the provisions described in Section 12.1.

5.6.2 Column 2: 5 μ Dupont Zorbax Silica, 250 mm long by 4.6 mm ID or equivalent.

6. REAGENTS

6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

- 6.2 Methylene chloride, methanol, ethyl ether, and hexane: Distilled-in-glass quality of equivalent. Ethyl ether must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Catalog No. P1126-8 and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips.
- 6.3 Sodium sulfate: ACS, granular, anhydrous; heated in a muffle furnace at 40°C overnight.
- 6.4 Silica gel: Davison Grade 923, 100-120 mesh, dried for 12 hours at 150°C.
- 6.5 1N sodium hydroxide: Dissolve 4.0 g of NaOH (ACS) in 100 mL of distilled water.
- 6.6 1N sulfuric acid: Slowly add 2.8 mL of concentrated H₂SO₄ (94%) to about 50 mL of distilled water. Dilute to 100 mL with distilled water.
- 6.7 Sodium phosphate: Monobasic, ACS grade.
- 6.8 Sodium phosphate: Dibasic, ACS grade.
- 6.9 Stock standard solutions (1.00 µg/µL): Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.
 - 6.9.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in distilled-in-glass quality methylene chloride and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 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.
 - 6.9.2 Transfer the stock standard solutions into PTFE-sealed screw-cap bottles. Store at 4°C and protect from light. Frequently check standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
 - 6.9.3 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1 Establish liquid chromatographic operating parameters equivalent to those indicated in Table 1. The liquid chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2 External standard calibration procedure:
 - 7.2.1 For each compound of interest, prepare calibration standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with methylene chloride. One of the external standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
 - 7.2.2 Using injections of 5 to 20 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each compound at each

standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

- 7.2.3** The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.
- 7.3** Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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.
- 7.3.1** Prepare calibration standards at a minimum of three concentration levels for each compound 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 methylene chloride. One of the standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
- 7.3.2** Using injections of 5 to 20 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, 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} against RF.

- 7.3.3** The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

- 7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. QUALITY CONTROL

- 8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

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

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol, 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 3, determine the recovery and single-operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.

- 8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁶ that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁶

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 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 monitor the precision of the sampling technique. 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 perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. SAMPLES COLLECTION, PRESERVATION, AND HANDLING

9.1 Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 Adjust the pH of the sample to 6 to 8 with sodium hydroxide or sulfuric acid immediately after sampling.

10. SAMPLE EXTRACTION

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel. Check the pH of the sample with

- wide range pH paper and adjust to 6 to 8 with 1N sodium hydroxide or 1N sulfuric acid. Dissolve 5 g of monobasic sodium phosphate and 5 g of dibasic sodium phosphate in the sample.
- 10.2 Add 60 mL of methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.
 - 10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.
 - 10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
 - 10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with 30 to 40 mL of methylene chloride.
 - 10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. If the sample extract requires no cleanup, proceed with Section 10.7. If the sample extract requires cleanup, proceed to Section 11.
 - 10.7 Remove the macro-Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. Adjust the volume of the extract to 5.0 mL with methylene chloride. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract is to be stored longer than 2 days, transfer the extract to a screw-capped vial with a PTFE-lined cap. If the sample extract requires no further cleanup, proceed with the liquid chromatographic analysis in Section 12. If the sample requires cleanup, proceed to Section 11.
 - 10.8 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1** Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than the recovery values reported in Table 2.
- 11.2** The following silica gel column cleanup procedure has been demonstrated to be applicable to the pesticides listed in Table 1.
- 11.2.1** Add 10 g of silica gel to 100 mL of ethyl ether and 600 μ L of reagent water in a 250-mL Erlenmeyer flask. Shake vigorously for 15 minutes. Transfer the slurry to a chromatographic column (silica gel may be retained with a plug of glass wool). Allow the solvent to elute from the column until the silica gel is almost exposed to the air. Wash the column with 100 mL of 50% hexane in methylene chloride as before and discard. Use a column flow of 2 to 2.5 mL/min throughout the wash and elution profiles.
- 11.2.2** Quantitatively add the sample extract from Section 10.8 to the head of the column. Allow the solvent to elute from the column until the silica gel is almost exposed to the air. Elute the column with 50 mL of 50% hexane in methylene chloride. Discard this fraction.
- 11.2.3** Elute the column with 50 mL of methylene chloride (Fraction 1) and collect eluate in a K-D apparatus. Repeat process with 50 mL of 6% ethyl ether in methylene chloride (Fraction 2). The TCMTB elutes in Fraction 1 and the MBTS elutes in Fraction 2. Concentrate each fraction to 5.0 mL as described in Sections 10.6 and 10.7. Proceed with liquid chromatographic analysis.
- 11.2.4** The above-mentioned fractions can be combined before concentration at the discretion of the analyst.

12. LIQUID CHROMATOGRAPHY

- 12.1** Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Examples of the separations achieved by Column 1 and Column 2 are shown in Figures 1, 2, and 3. Other columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.
- 12.2** Calibrate the liquid chromatographic system daily as described in Section 7.
- 12.3** If the internal standard approach is being used, the analyst must not add the internal standard to the sample extracts until immediately before injection into the instrument. Mix thoroughly.
- 12.4** Inject 5 to 20 μ L of the sample extract by completely filling the sample value loop. Record the resulting peak sizes in areas of peak height units.
- 12.5** The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a

suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

- 12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

- 13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_e)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_e = Volume of water extracted, in mL

- 13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_e)}$$

where

A_s = Response for parameter to be measured

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_e = Volume of water extracted, in L

- 13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all (data obtained with the sample results).
- 13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

14. METHOD PERFORMANCE

- 14.1** 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.⁸ The MDL concentrations listed in Table 1 were obtained using reagent water.¹ Similar results were achieved using representative wastewaters.
- 14.2** This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from $10 \times \text{MDL}$ to $1000 \times \text{MDL}$.
- 14.3** In a single laboratory, Battelle Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 2 were obtained after silica gel cleanup. Seven replicates of each of two different wastewaters were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 2.¹

References

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8. Glaser, J. A., et al., "Trace Analysis for Wastewaters," *Environmental Science and Technology*, 15, 1426 (1981).

Table 1. Chromatographic Conditions and Estimated Detection Limits

<i>Parameter</i>	<i>Retention Time (Min.)</i>		<i>MDL (µg/L)</i>
	<i>Column 1</i>	<i>Column 2</i>	
MBTS	6.6	6.3	0.5
TCMTB	9.3	7.9	1.0

Column 1 conditions: Dupont Zorbax-CN, 5 µ, 250 by 4.6 mm; 1 mL/min flow; 15/85 methylene chloride/hexane.

Column 2 conditions: Dupont Zorbax silica, 5 µ, 250 by 4.6 mm; 1 mL/min flow; 90/9.5/0.5 hexane/methylene chloride/methanol.

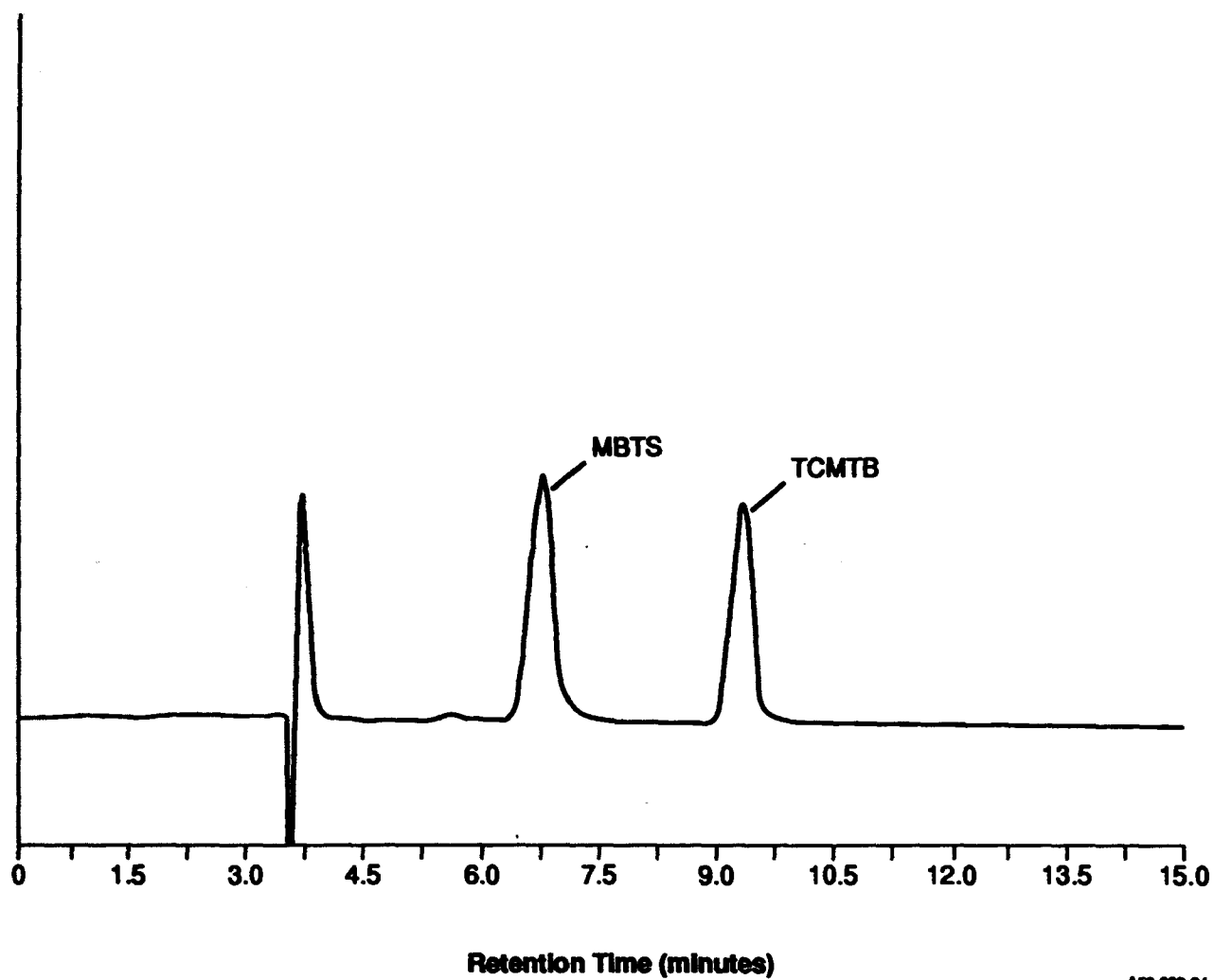
Table 2. Single Laboratory Accuracy and Precision(a)

<i>Parameter</i>	<i>Sample Type^b</i>	<i>Background µg/L</i>	<i>Spike µg/L</i>	<i>Mean Recovery, %</i>	<i>Standard Deviation</i>	<i>Number of Replicates</i>
MBTS	1	ND	5	35	23	7
	1	ND	100	69	6	7
TCMTB	1	ND	5	69	20	7
	1	ND	100	90	2	7

(a) Column 1 conditions were used.

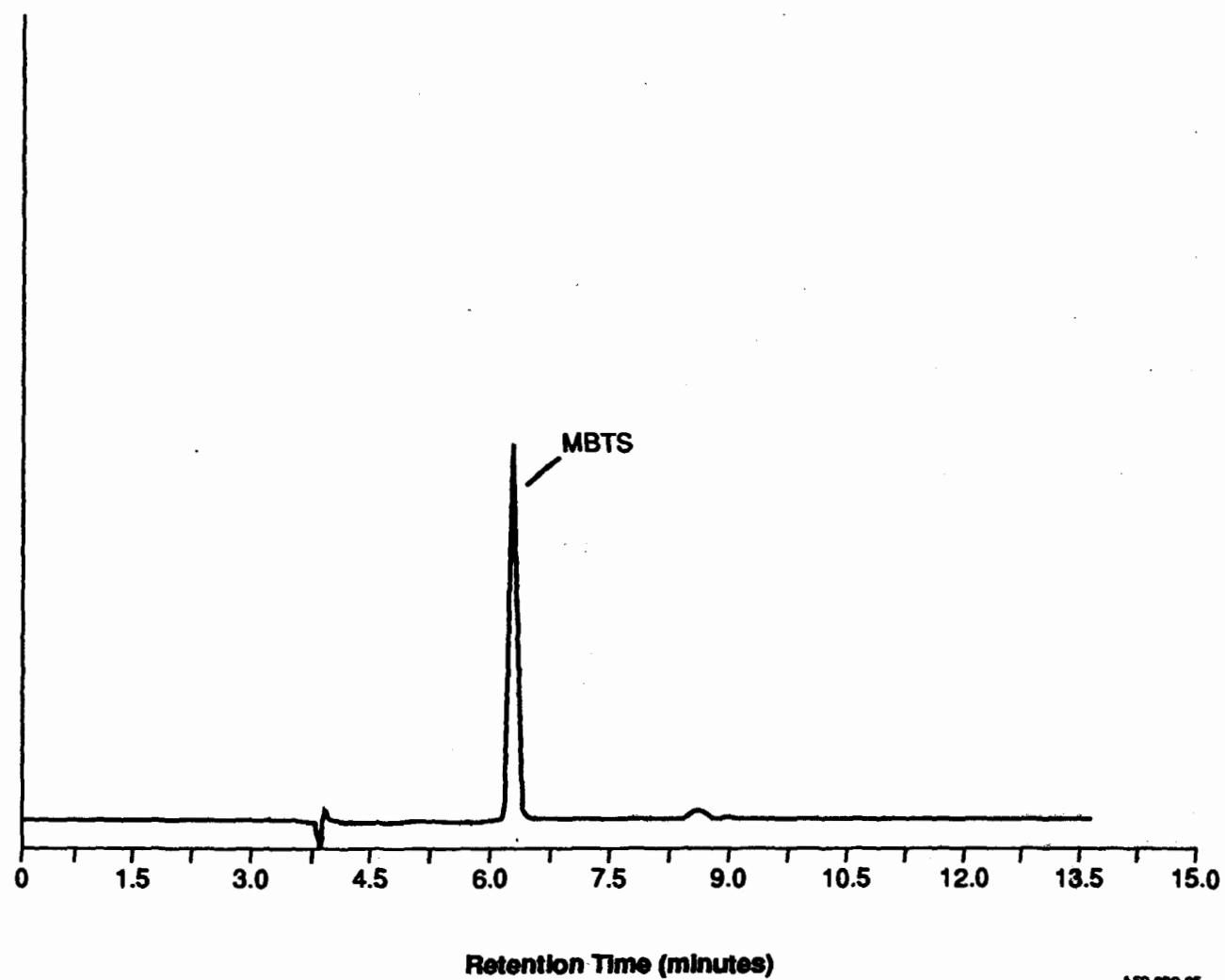
(b) 1 = Municipal sewage effluent

(c) ND = Not detected



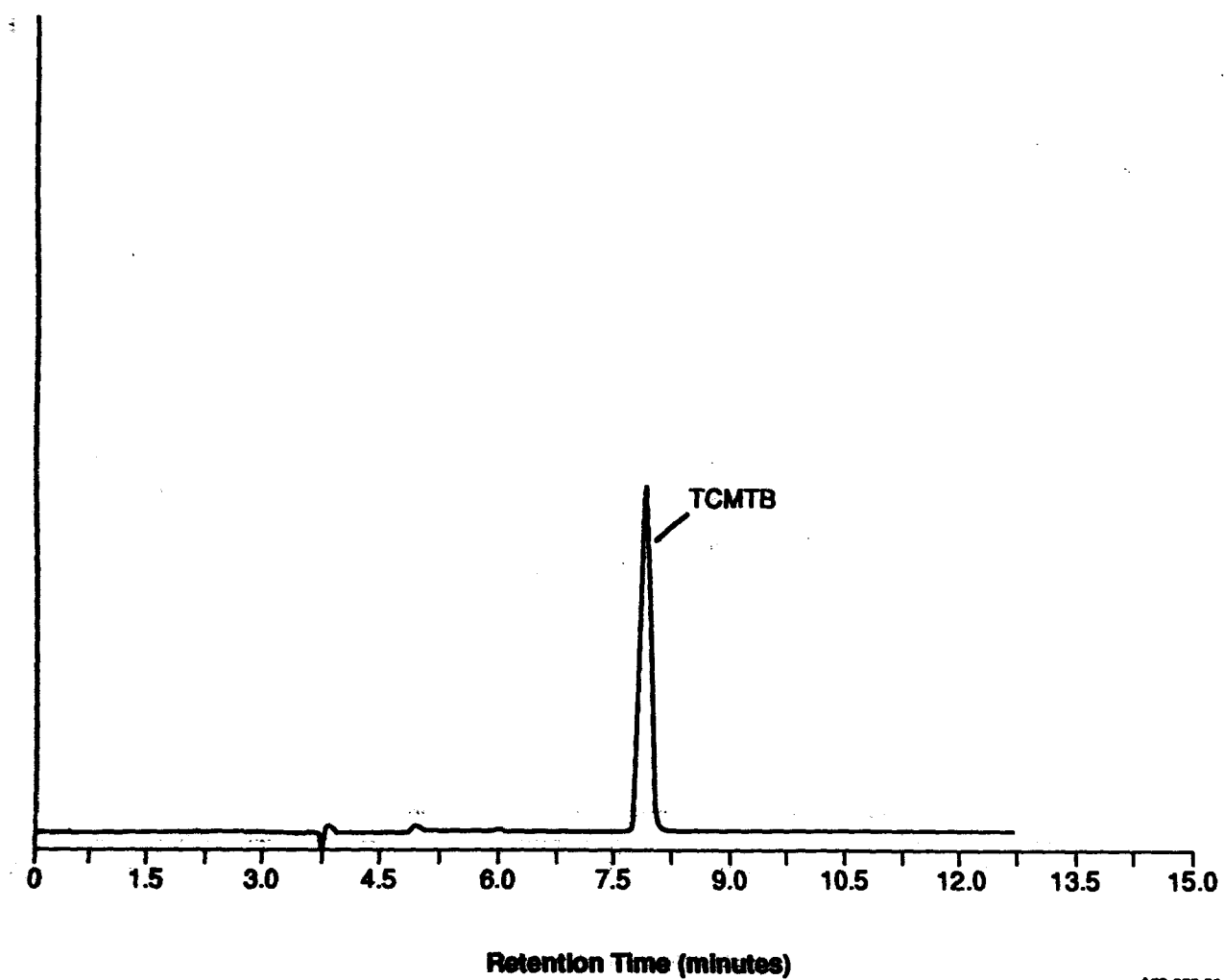
A62-002-34

Figure 1. HPLC-UV Chromatogram of 10 ng Each of MBTS and TCMTB (Column 1).



AS2-002-36

Figure 2. HPLC-UV Chromatogram of 100 ng of MBTS (Column 2).



A52-002-36

Figure 3. HPLC-UV Chromatogram of 100 ng of TCMTB (Column 2).

Method 638
The Determination of Oryzalin
in Municipal and Industrial
Wastewaters

Method 638

The Determination of Oryzalin in Municipal and Industrial Wastewaters

1. SCOPE AND APPLICATION

- 1.1** This method covers the determination of oryzalin pesticide. The following parameter can be determined by this method:

<i>Parameters</i>	<i>CAS No.</i>
Oryzalin	19044-88-3

- 1.2** This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compound listed above in municipal and industrial discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3** The estimated method detection limit (MDL, defined in Section 15) for oryzalin is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4** This method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.5** When this method is used to analyze unfamiliar samples for the compound above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second liquid chromatographic column that can be used to confirm measurements made with the primary column.

2. SUMMARY OF METHOD

- 2.1** A measured volume of sample, approximately 1 L, is extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to acetonitrile during concentration to a volume of 2 mL or less. Liquid chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by HPLC-UV.¹

3. INTERFERENCES

- 3.1** Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing hardware that lead to discrete artifacts or elevated baselines in liquid chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

- 3.1.1** Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.
 - 3.1.2** The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.2** Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedure in Section 11 can be used to overcome these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

- 4.1** The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified³⁻⁵ for the information of the analyst.

5. APPARATUS AND MATERIALS

- 5.1** Sampling equipment, for discrete or composite sampling.
 - 5.1.1** Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with PTFE. Aluminum foil may be substituted for PTFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.
 - 5.1.2** Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.
- 5.2** Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)

- 5.2.1 Separatory funnel: 2000-mL, with PTFE stopcock.
- 5.2.2 Drying column: Chromatographic column 400 mm long by 10 mm ID with coarse frit.
- 5.2.3 Chromatographic column: 400 mm long by 19 mm ID with 250-mL reservoir at the top and PTFE stopcock (Kontes K-420290 or equivalent).
- 5.2.4 Concentrator tube, Kuderna-Danish: 25-mL, graduated (Kontes K-570050-2525 or equivalent). Calibration must be checked at the volumes employed in the test. A ground-glass stopper is used to prevent evaporation of extracts.
- 5.2.5 Evaporative flask, Kuderna-Danish: 250-mL (Kontes K-570001-0250 or equivalent). Attach to concentrator tube with springs.
- 5.2.6 Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).
- 5.2.7 Snyder column, Kuderna-Danish: Two-ball micro (Kontes K-569001-0219 or equivalent).
- 5.2.8 Vials: Amber glass, 5- to 10-mL capacity with PTFE-lined screw-cap.
- 5.2.9 Volumetric flask: 2-mL with glass stopper.
- 5.3 Boiling chips: Approximately 10/40 mesh carborundum. Heat to 400°C for 4 hours or extract in a Soxhlet extractor with methylene chloride.
- 5.4 Water bath: Heated, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.
- 5.5 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6 Liquid chromatograph: Analytical system complete with liquid chromatograph and all required accessories including syringes, analytical columns, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.
 - 5.6.1 Pump: Isocratic pumping system, constant flow.
 - 5.6.2 Column 1: Reversed-phase column, 5 μ Spherisorb-ODS, 250 mm long by 4.6 mm ID or equivalent.
 - 5.6.3 Column 2: Reversed-phase column, 5 μ Lichrosorb RP-2, 250 mm long by 4.6 mm ID or equivalent.
 - 5.6.4 Detector: Ultraviolet absorbance detector, 254 nm.

6. REAGENTS

- 6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2 Methylene chloride, acetone, acetonitrile: Distilled-in-glass quality or equivalent.
- 6.3 Sodium sulfate: ACS, granular, anhydrous; heated in a muffle furnace at 400°C overnight.
- 6.4 Sodium hydroxide, 1N: Prepared by adding 4 g of sodium hydroxide in distilled water and diluting to 100 mL.
- 6.5 Sulfuric acid, 1N: Prepared by diluting 2.8 mL of concentrated sulfuric acid to distilled water and diluting to 100 mL.

- 6.6 Florisil: PR grade (60/100 mesh). Purchase activated at 675°C and store in brown glass bottle. To prepare for use, place 150 g in a wide-mouth jar and heat overnight at 160 to 170°C. Seal tightly with PTFE-or aluminum-foil-lined screw-cap and cool to room temperature.
- 6.7 Stock standard solutions (1.00 µg/µL): Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.
 - 6.7.1 Prepare stock standard solutions by accurately weighing about 0.010 g of pure material. Dissolve the material in distilled-in-glass quality acetonitrile and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 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.
 - 6.7.2 Transfer the stock standard solutions into PTFE-sealed screw-cap bottles. 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 calibration standards from them.
 - 6.7.3 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1 Establish liquid chromatographic operating parameters equivalent to those indicated in Table 1. The liquid chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2 External standard calibration procedure:
 - 7.2.1 For each compound of interest, prepare calibration standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with acetonitrile. 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.
 - 7.2.2 Using injections of 2 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each compound at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% 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.
 - 7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each compound 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 acetonitrile. 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.

7.3.2 Using injections of 2 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, 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} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.
- 8.1.1** Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2** In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
- 8.1.3** The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2** To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
- 8.2.1** Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol, 1000 times more concentrated than the selected concentrations.
- 8.2.2** Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
- 8.2.3** Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.
- 8.2.4** Using the appropriate data from Table 2, determine the recovery and single-operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.
- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
- 8.3.1** Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts that are useful in observing trends in performance.

- 8.3.2** The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated with this method. This ability is established as described regularly.⁶
- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.
- 8.5** Before processing any samples, the analyst should demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.
- 8.6** 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 monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as liquid chromatography with a dissimilar column, must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. SAMPLES COLLECTION, PRESERVATION, AND HANDLING

- 9.1** Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon and other potential sources of contamination.
- 9.2** The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3** Adjust the pH of the sample to 6 to 8 with 1N sodium hydroxide or 1N sulfuric acid immediately after sampling.
- 9.4** All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

10. SAMPLE EXTRACTION

- 10.1** Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel. Check the pH of the sample with wide range pH paper and adjust to 7 with 1N sodium hydroxide or 1N H₂SO₄.

- 10.2 Add 60 mL of methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.
- 10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, collecting the extract. Perform a third extraction in the same manner and combine the extracts.
- 10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 25-mL concentrator tube to a 250-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with 30 to 40 mL of methylene chloride.
- 10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.7 Remove the macro-Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommended for this operation. Add one or two clean boiling chips and attach a two-ball micro-Snyder column to the concentrator tube. Prewet the micro-Snyder column with methylene chloride and concentrate the solvent extract as before. When an apparent volume of 0.5 mL is reached, or the solution stops boiling, remove the K-D apparatus and allow it to drain and cool for 10 minutes.
- 10.8 Remove the micro-Snyder column and adjust the volume of the extract to 1.0 mL with methylene chloride. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract is to be stored longer than 20 days, transfer the extract to a screw-capped vial with a PTFE-lined cap. If the sample extract requires no further cleanup, proceed with solvent exchange to acetonitrile as described beginning with Section 11.5. If the sample requires cleanup, proceed to Section 11.
- 10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of additional cleanup, the analyst must demonstrate that the recovery of each compound of interest is no less than 85%.
- 11.2 Slurry 10 g of Florisil in 100 mL of methylene chloride which has been saturated with reagent water. Transfer the slurry to a chromatographic column (Florisil is retained with a plug of glass wool). Wash the column with 100 mL of methylene chloride. Use a column flow rate of 2 to 2.5 mL/min throughout the wash and elution profiles.
- 11.3 Add the extract from Section 10.8 to the head of the column. Allow the solvent to elute from the column until the Florisil is almost exposed to the air. Elute the column with 50 mL of methylene chloride. Discard this fraction.
- 11.4 Elute the column with 50 mL of 5% acetone in methylene chloride. Collect this fraction in a K-D apparatus. Concentrate the column fraction to 1 mL as described in Sections 10.6 and 10.7.
- 11.5 Add 15 mL of acetonitrile to the concentrate along with one or two clean boiling chips. Attach a three-ball micro-Snyder column to the concentrator tube. Prewet the micro-Snyder column with acetonitrile and concentrate the solvent extract to an apparent volume of 1 mL. Allow the K-D apparatus to drain and cool for 10 minutes.
- 11.6 Transfer the liquid to a 2-mL volumetric flask and dilute to the mark with acetonitrile. Mix thoroughly prior to analysis. If the extracts will not be analyzed immediately, they should be transferred to PTFE-sealed screw-cap vials and refrigerated. Proceed with the liquid chromatographic analysis.

12. LIQUID CHROMATOGRAPHY

- 12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. An example of the separations achieved by Column 1 and Column 2 are shown in Figures 1 and 2. Other columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.
- 12.2 Calibrate the liquid chromatographic system daily as described in Section 7.
- 12.3 If an internal standard approach is being used, the analyst must not add the internal standard to sample extracts until immediately before injection into the instrument.
- 12.4 Inject 2 to 5 μ L of the sample extract by completely filling the sample valve loop. Record the resulting peak sizes in area or peak height units.
- 12.5 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

- 12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

- 13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_i)(V_e)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_e = Volume of total extract, in μL

V_w = Volume of water extracted, in mL

- 13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_w)(RF)(V_w)}$$

where

A_s = Response for parameter to be measured

A_w = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_w = Volume of water extracted, in L

- 13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

14. METHOD PERFORMANCE

- 14.1 The method detection limit (MDL)⁸ 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 Table 1 were obtained using reagent water.¹ Similar results were achieved using representative wastewaters.

- 14.2** This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from $10 \times \text{MDL}$ to $1000 \times \text{MDL}$.
- 14.3** In a single laboratory, Battelle Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 2 were obtained. Seven replicates of each of two different wastewaters were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 2.¹

References

1. "Development of Methods for Pesticides in Wastewaters," EPA Contract Report 68-03-2956 (In preparation).
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5. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
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7. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, Pennsylvania, P. 76, 1980.
8. Glaser, J. A. et al , "Trace Analysis for Wastewaters," *Environmental Science and Technology*, 15, 1426 (1981).

Table 1. Chromatographic Conditions and Method Detection Limits

<i>Parameter</i>	<i>Retention Time (Min.)</i>		<i>MDL ($\mu\text{g/L}$)</i>
	<i>Column 1</i>	<i>Column 2</i>	
Oryzalin	6.2	10.7	0.5

Column 1 conditions: Spherisorb-ODS, 5 μ , 250 mm long by 4.6 mm ID; 1 mL/min flow; 40/60 acetonitrile/water. A UV detector was used with this column to determine the MDL.

Column 2 conditions: Lichrosorb RP-2, 5 μ , 250 mm long by 4.6 mm ID; 1 mL/min flow; 50/50 acetonitrile/water.

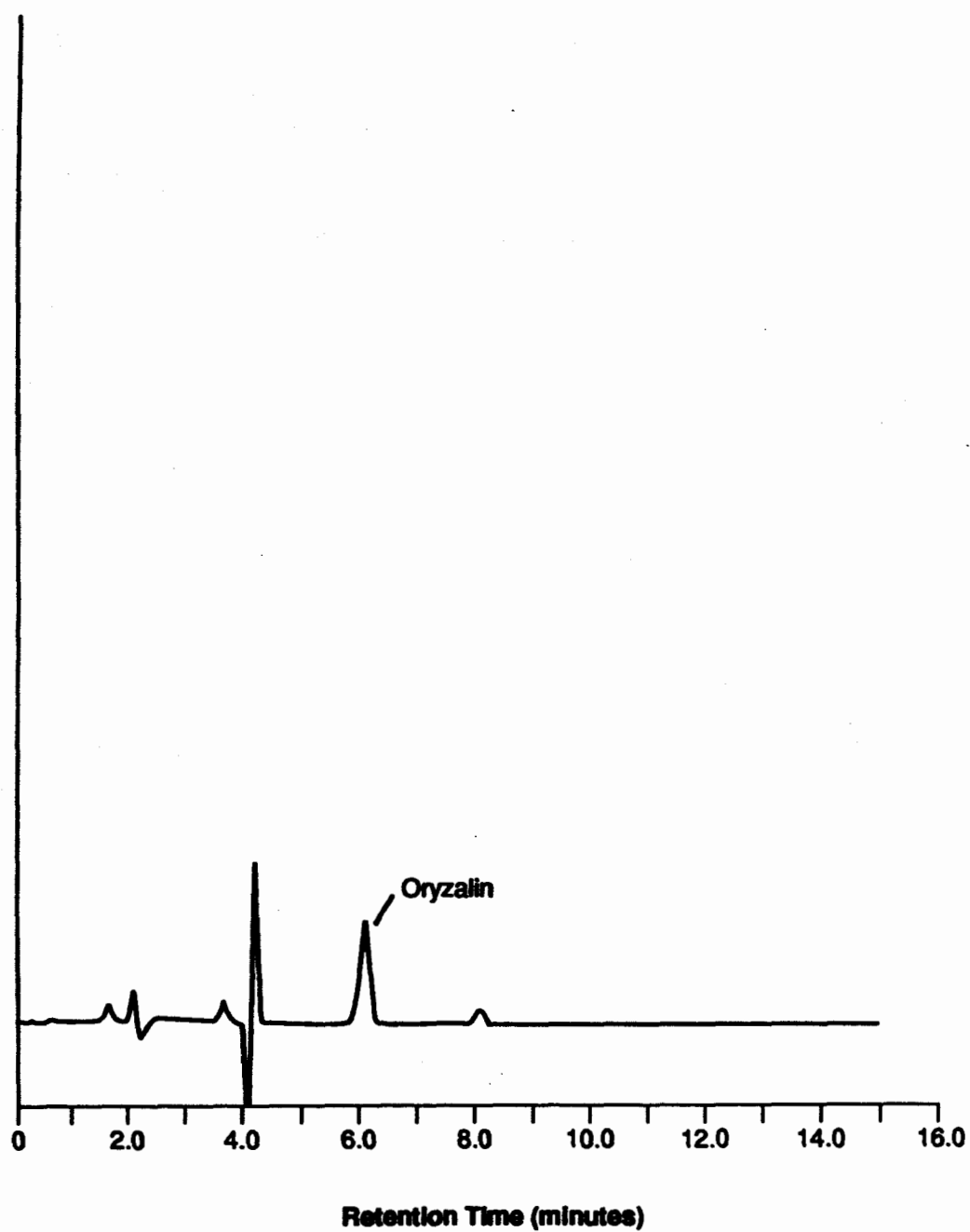
Table 2. Single-Laboratory Accuracy and Precision (a)

<i>Parameter</i>	<i>Sample Type (b)</i>	<i>Background $\mu\text{g/L}$</i>	<i>Spike Level $\mu\text{g/L}$</i>	<i>Mean Recovery (%)</i>	<i>Standard Deviation (%)</i>	<i>Number of Replicates</i>
Oryzalin	1	4	10	106	6	7
	2	40	200	100	10	7

(a) Column 1 conditions were used.

(b) 1 = Relevant industrial wastewater diluted 1000:1 with municipal sewage effluent

2 = Relevant industrial wastewater diluted 100:1 with municipal sewage effluent



52-002-28

Figure 1. HPLC-UV Chromatogram of 10 ng of Oryzalin (Column1).

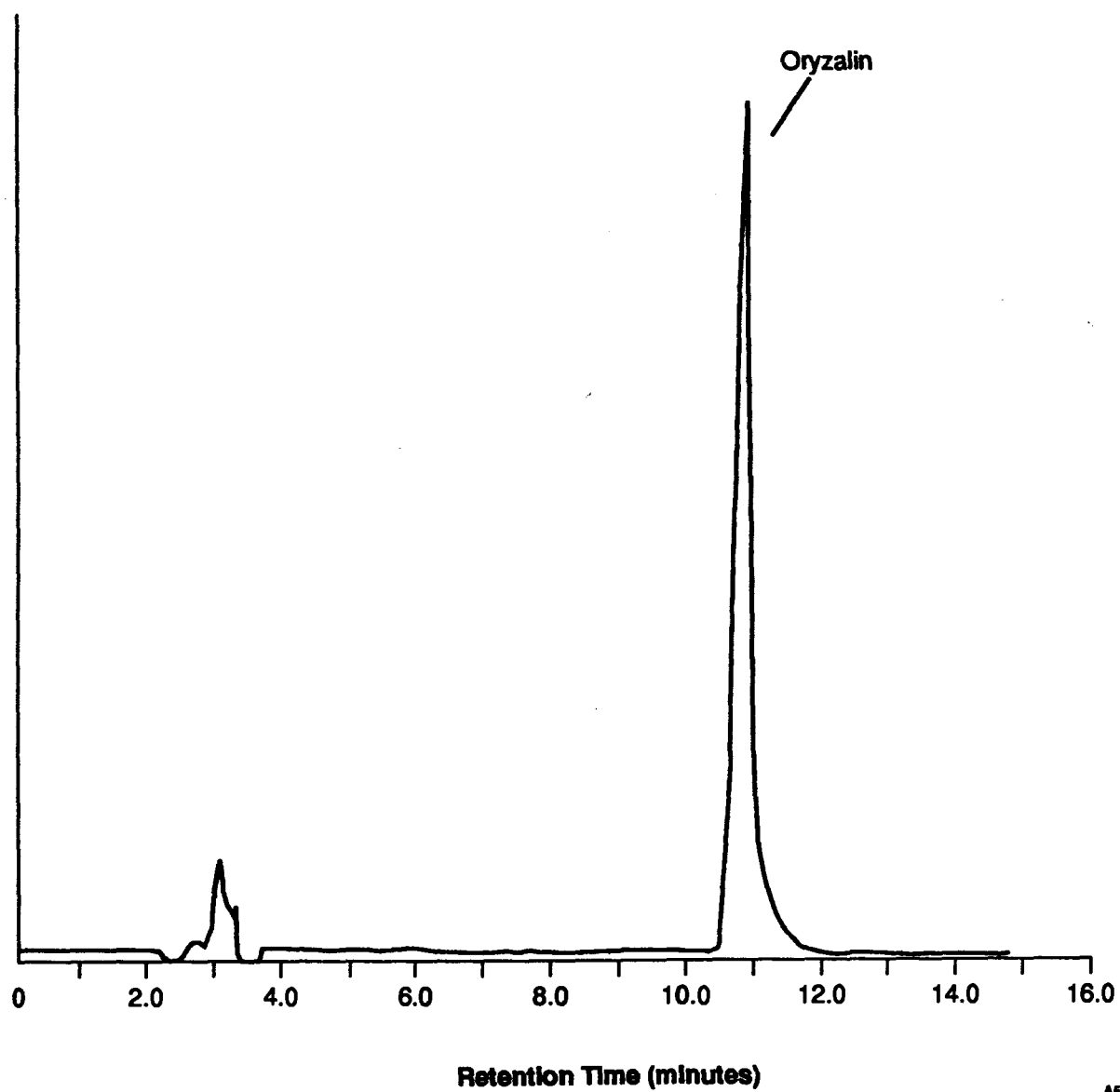


Figure 2. HPLC-UV Chromatogram of 250 ng of Oryzalin (Column 2).

Method 639
The Determination of
Bendiocarb in Municipal and
Industrial Wastewaters

Method 639

The Determination of Bendiocarb in Municipal and Industrial Wastewaters

1. SCOPE AND APPLICATION

- 1.1** This method covers the determination of bendiocarb pesticide. The following parameter can be determined by this method:

<i>Parameter</i>	<i>CAS No.</i>
Bendiocarb	22781-23-3

- 1.2** This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compound listed above in municipal and industrial discharges as provided under 40 *CFR* 136.1. When this method is used to analyze unfamiliar samples for the compound above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second liquid chromatographic column that can be used to confirm measurements made with the primary column.
- 1.3** The method detection limit (MDL, defined in Section 15) for bendiocarb is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4** Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.5** This method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

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- 2.1** A measured volume of sample, approximately 1 L, is extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to acetonitrile during concentration to a volume of 2 mL or less. Liquid chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by HPLC-UV.¹

3. INTERFERENCES

- 3.1** Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing hardware that lead to discrete artifacts or elevated baselines in liquid chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap and distilled water. It should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. Unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. SAFETY

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified³⁻⁵ for the information of the analyst.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab-sample bottle: Amber glass, 1-L or 1-quart volume, fitted with screw-caps lined with PTFE. Foil may be substituted for PTFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

- 5.2 Glassware.** (All specifications are suggested. Catalog numbers are included for illustration only.)
- 5.2.1** Separatory funnel: 2000-mL, with PTFE stopcock.
 - 5.2.2** Drying column: Chromatographic column 400 mm long by 10 mm ID with coarse frit.
 - 5.2.3** Chromatographic column: 400 mm long by 19 mm ID with 250-mL reservoir at the top and PTFE stopcock (Kontes K-420290 or equivalent).
 - 5.2.4** Concentrator tube, Kuderna-Danish: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. A ground-glass stopper is used to prevent evaporation of extracts.
 - 5.2.5** Evaporative flask, Kuderna-Danish: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.
 - 5.2.6** Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).
 - 5.2.7** Snyder column, Kuderna-Danish: Two-ball micro (Kontes K-569001-0219 or equivalent).
 - 5.2.8** Vials: Amber glass, 5- to 10-mL capacity with PTFE-lined screw-cap.
- 5.3** Boiling chips: Approximately 10/40 mesh carborundum. Heat to 400°C for 4 hours or extract in a Soxhlet extractor with methylene chloride.
- 5.4** Water bath: Heated, capable of temperature control ($\pm 2^{\circ}\text{C}$). The bath should be used in a hood.
- 5.5** Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6** Liquid chromatograph: Analytical system complete with liquid chromatograph and all required accessories including syringes, analytical columns, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.
- 5.6.1** Pump: Isocratic pumping system, constant flow.
 - 5.6.2** Column 1: Reversed-phase column, 5 μ Spherisorb-ODS, 250 mm long by 4.6 mm ID or equivalent.
 - 5.6.3** Column 2: Reversed-phase column, 5 μ Lichrosorb RP-2, 250 mm long by 4.6 mm ID or equivalent.
 - 5.6.4** Detector: Ultraviolet absorbance detector, 254 nm.

6. REAGENTS

- 6.1** Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2** Methylene chloride, methanol acetonitrile: Distilled-in-glass quality or equivalent.
- 6.3** Sodium sulfate: ACS, granular, anhydrous; heated in a muffle furnace at 400°C overnight.
- 6.4** Sodium hydroxide, 1N: Prepare by adding 4 g of sodium hydroxide to distilled water and diluting to 100 mL.
- 6.5** Sulfuric acid, 1N: Prepare by adding 2.8 mL of concentrated sulfuric acid to distilled water and diluting to 100 mL.

- 6.6** Florisil: PR grade (60/100 mesh). Purchase activated at 675°C and store in a brown glass bottle. To prepare for use, place 150 g in a wide-mouth jar and heat overnight at 160 to 170°C. Seal tightly with PTFE- or aluminum-foil-lined cap and cool to room temperature.
- 6.7** Stock standard solutions (1.00 µg/µL): Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.
- 6.7.1** Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in distilled-in-glass quality methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 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.
- 6.7.2** Transfer the stock standard solution into PTFE-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standard solution should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
- 6.7.3** Stock standard solution must be replaced after 6 months, or sooner if comparison with quality control check standards indicates a problem.

7. CALIBRATION

- 7.1** Establish liquid chromatographic operating parameters equivalent to those indicated in Table 1. The liquid chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2** External standard calibration procedure:
- 7.2.1** For the compound of interest, prepare calibration standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with acetonitrile. 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.
- 7.2.2** Using injections of 2 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for bendiocarb. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% 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.
- 7.2.3** The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for bendiocarb varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared.

- 7.3 Internal standard calibration procedure:** To use this approach, the analyst must select one or more internal standards similar in analytical behavior to bendiocarb. 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.
- 7.3.1** Prepare calibration standards at a minimum of three concentration levels for bendiocarb 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 acetonitrile. 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.
- 7.3.2** Using injections of 2 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for bendiocarb and internal standard. Calculate response factors (RF) as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, 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} against RF.

- 7.3.3** The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for bendiocarb varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared.
- 7.4** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. QUALITY CONTROL

- 8.1** Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

- 8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
- 8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
 - 8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol 1000 times more concentrated than the selected concentrations.
 - 8.2.2 Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
 - 8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.
 - 8.2.4 Using the appropriate data from Table 2, determine the recovery and single-operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.
- 8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
 - 8.3.1 Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

Where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁶ that are useful in observing trends in performance.

- 8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternatively, the analyst may use four wastewater data points gathered through the

requirement for continuing quality control in Section 8.4. The accuracy statements should be updated with this method. This ability is established as described regularly.⁶

- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.
- 8.5** Before processing any samples, the analyst should demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.
- 8.6** 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 monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as liquid chromatography with a dissimilar column must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. SAMPLES COLLECTION, PRESERVATION, AND HANDLING

- 9.1** Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon and other potential sources of contamination.
- 9.2** The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3** Adjust the pH of the sample to 6 to 8 with 1N sodium hydroxide or 1N sulfuric acid immediately after sampling.

10. SAMPLE EXTRACTION

- 10.1** Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel. Check the pH of the sample with wide range pH paper and adjust to 7 with 1N sodium hydroxide or 1N H₂SO₄.
- 10.2** Add 60 mL of methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample,

- but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.
- 10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, collecting the extract. Perform a third extraction in the same manner and collect the extract.
 - 10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 250-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
 - 10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with 30 to 40 mL of methylene chloride.
 - 10.6 Add one or two clean boiling chips to the evaporative flask and attach a macro-Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
 - 10.7 Remove the macro-Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommended for this operation. Add one or two clean boiling chips and attach a two-ball micro-Snyder column to the concentrator tube. Prewet the micro-Snyder column with methylene chloride and concentrate the solvent extract as before. When an apparent volume of 0.5 mL is reached, or the solution stops boiling, remove the K-D apparatus and allow it to drain and cool for 10 minutes.
 - 10.8 Remove the micro-Snyder column and adjust the volume of the extract to 1.0 mL with methylene chloride. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract is to be stored longer than 2 days, transfer the extract to a screw-capped vial with a PTFE-lined cap. If the sample extract requires no further cleanup, proceed with solvent exchange to acetonitrile as described beginning in Section 11.5. If the sample requires cleanup, proceed to Section 11.
 - 10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of additional cleanup, the analyst must demonstrate that the recovery of each compound of interest is no less than 65%.
- 11.2 Slurry 10 g of Florisil in 100 mL of methylene chloride which has been saturated with reagent water. Transfer the slurry to a chromatographic column. Wash the column with 100 mL of

methylene chloride. Use a column flow rate of 2 to 2.5 mL/min throughout the wash and elution profiles.

- 11.3 Add the extract from Section 10.8 to the head of the column. Allow the solvent to elute from the column until the Florisil is almost exposed to the air. Elute the column with 50 mL of methylene chloride. Discard this fraction.
- 11.4 Elute the column with 50 mL of 5% acetone in methylene chloride. Collect this fraction in a K-D apparatus. Concentrate the column fraction to 1 mL as described in Sections 10.6 and 10.7.
- 11.5 Add 10 mL of acetonitrile to the concentrate along with one or two clean boiling chips. Attach a three-ball micro-Snyder column to the concentrator tube. Prewet the micro-Snyder column with acetonitrile and concentrate the solvent extract to an apparent volume of 1 mL. Allow the K-D apparatus to drain and cool for 10 minutes.
- 11.6 Transfer the liquid to a 2-mL volumetric flask and dilute to the mark with acetonitrile. Mix thoroughly prior to analysis. If the extracts will not be analyzed immediately, they should be transferred to PTFE-sealed screw-cap vials and refrigerated. Proceed with the liquid chromatographic analysis.

12. LIQUID CHROMATOGRAPHY

- 12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. An example of the separations achieved by Column 1 and Column 2 are shown in Figures 1 and 2. Other columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.
- 12.2 Calibrate the liquid chromatographic system daily as described in Section 7.
- 12.3 If an internal standard approach is being used, the analyst must not add the internal standard to sample extracts until immediately before injection into the instrument. Mix thoroughly.
- 12.4 Inject 2 to 5 μ L of the sample extract by completely filling the sample valve loop. Record the resulting peak sizes in area or peak height units.
- 12.5 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of the retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

- 13.1 Determine the concentration of individual compounds in the sample.

- 13.1.1** If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_e)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_e = Volume of water extracted, in mL

- 13.1.2** If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_e)}$$

where

A_s = Response for parameter to be measured

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_e = Volume of water extracted, in L

- 13.2** Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 13.3** For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

14. METHOD PERFORMANCE

- 14.1** 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.⁸ The MDL concentrations listed in Table 1 were obtained using reagent water.¹ Similar results were achieved using representative wastewaters.
- 14.2** This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from $10 \times \text{MDL}$ to $1000 \times \text{MDL}$.

14.3 In a single laboratory, Battelle Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 2 were obtained. Seven replicates of each of two different wastewaters were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 2.¹

References

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7. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, Pennsylvania, p. 76, 1980.
8. Glaser, J. A. et al, "Trace Analysis for Wastewaters," *Environmental Science and Technology*, 15, 1426, (1981).

Table 1. Chromatographic Conditions and Method Detection Limits

<i>Parameter</i>	<i>Retention Time(Minutes)</i>		<i>Method Detection Limits (µg/L)</i>
	<i>Column 1</i>	<i>Column 2</i>	
Bendiocarb	9.3	6.0	1.8

Column 1 conditions: Spherisorb-ODS, 5 µ, 250 mm long by 4.6 mm ID; 1 mL/min flow; 40/60 acetonitrile/water.

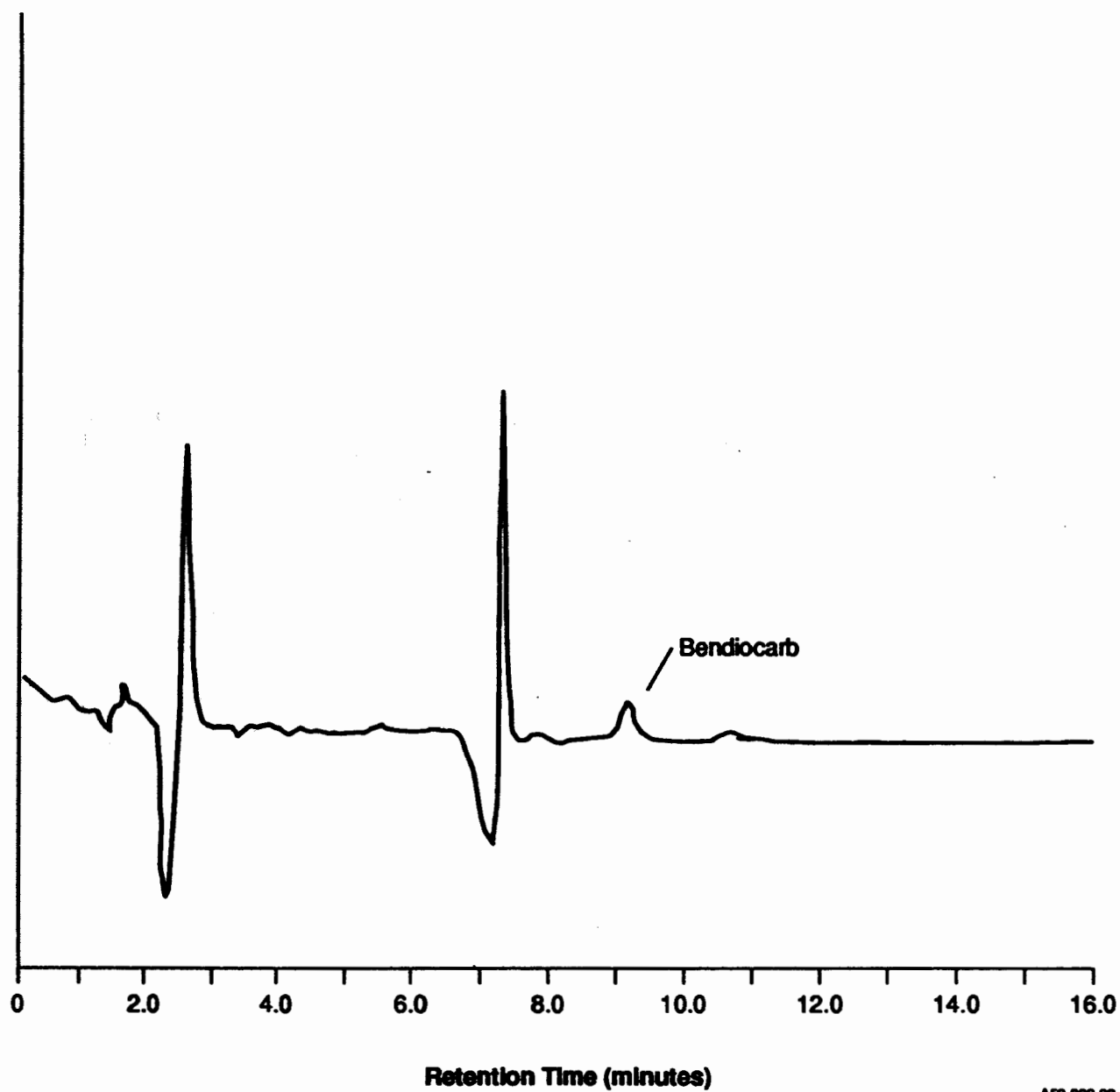
Column 2 conditions: Lichrosorb RP-2, 5 µ, 250 mm long by 4.6 mm ID; 1 mL/min flow; 50/50 acetonitrile/water.

Table 2. Single-Laboratory Accuracy and Precision (a)

<i>Parameter</i>	<i>Average Percent Relative Standard Spike Level</i>			<i>No. of Analyses</i>	<i>Matrix Type (b)</i>
	<i>Recovery</i>	<i>Deviation (%)</i>	<i>(µg/L)</i>		
Bendiocarb	65	35.6	8	7	1
	70	5.7	80	7	1

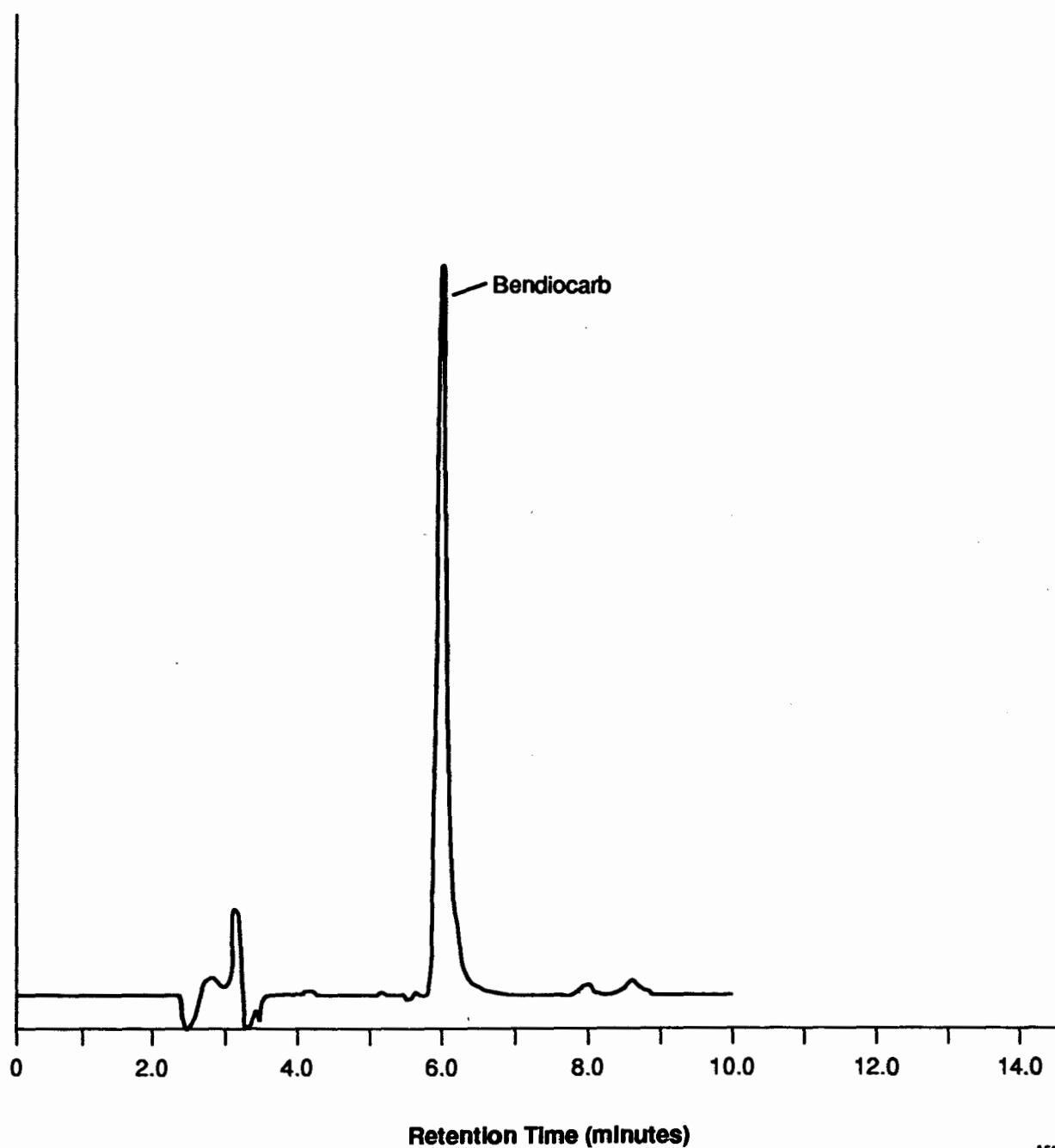
(a) Column 1 conditions were used.

(b) 1 = Relevant industrial wastewater



A52-002-22

Figure 1. HPLC-UV Chromatogram of 10 ng of Bendiocarb (Column 1).



A52-002-3

Figure 2. HPLC-UV Chromatogram of 600 ng of Bendiocarb (Column 2).

Method 640
The Determination of
Mercaptobenzothiazole in
Municipal and Industrial
Wastewaters

Method 640

The Determination of Mercaptobenzothiazole in Municipal and Industrial Wastewaters

1. SCOPE AND APPLICATION

- 1.1** This method covers the determination of mercaptobenzothiazole. The following parameter can be determined by this method:

<i>Parameter</i>	<i>CAS No.</i>
Mercaptobenzothiazole	149-30-4

- 1.2** This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compound listed above in municipal and industrial discharges as provided under 40 *CFR* 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3** The method detection limit (MDL, defined in Section 14) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4** The sample extraction and concentration steps in this method are essentially the same as in certain other 600-series methods. Thus, a single sample may be extracted to measure the compounds included in the scope of the methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures.
- 1.5** This method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.6** When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second liquid chromatographic column that can be used to confirm measurements made with the primary column.

2. SUMMARY OF METHOD

- 2.1** A measured volume of sample, approximately 1 L, is extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated to 1.0 mL. Liquid chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by high performance liquid chromatography with ultraviolet detection.
- 2.2** This method provides a silica gel column cleanup procedure to aid in the elimination of interferences which may be encountered.

3. INTERFERENCES

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing apparatus that lead to discrete artifacts or elevated baseline in liquid chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 minutes. Do not heat volumetric ware. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide-quality hexane may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 2.

4 SAFETY

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified³⁻⁵ for the information of the analyst.

5. APPARATUS AND MATERIALS

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab-sample bottle: Amber borosilicate or flint glass, 1-L or 1-quart volume, fitted with screw-caps lined with PTFE. Foil may be substituted for PTFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C

and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Glassware. (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel: 2000-mL, with PTFE stopcock.

5.2.2 Drying column: Chromatographic column 400 mm long by 10 mm ID with coarse frit.

5.2.3 Chromatographic column: 400 mm long by 19 mm ID with 250-mL reservoir at the top and PTFE stopcock (Kontes K-420290 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish: 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. A ground-glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Snyder column, Kuderna-Danish: Two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.8 Vials: Amber glass, 10- to 15-mL capacity with PTFE-lined screw-cap.

5.2.9 Erlenmeyer flask: 250-mL.

5.2.10 Graduated cylinder: 1000-mL.

5.2.11 Volumetric flask: 5-mL, 10-mL.

5.3 Boiling chips: Approximately 10/40 mesh carborundum. Heat at 400°C for 4 hours or perform a Soxhlet extraction with methylene chloride.

5.4 Water bath: Heated, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.

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

5.6 Liquid chromatograph: Analytical system complete with liquid chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, detectors, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column 1: Spherisorb-ODS, 5μ , 250 mm long by 4.6 mm ID or equivalent. This column was used to develop the method performance statements in Section 14. Alternative columns may be used in accordance with the provisions described in Section 12.1.

5.6.2 Column 2: Lichrosorb RP-2, 5μ , 250 mm long by 4.6 mm ID or equivalent.

6. REAGENTS

- 6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2 Methylene chloride, methanol, acetonitrile, ethyl ether, and acetone: Distilled-in-glass quality or equivalent. Ethyl ether must be free of peroxides as indicated by EM Quant test strips (available from Scientific Products Co., Catalog No. P1126-8 and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips.
- 6.3 Sodium sulfate: ACS, granular, anhydrous; heated in a muffle furnace at 400°C overnight.
- 6.4 Silica gel: Davison Grade 923, 100-200 mesh, dried for 12 hours at 150°C.
- 6.5 1N sodium hydroxide: Dissolve 4 g of sodium hydroxide in 100 mL of distilled water.
- 6.6 1N sulfuric acid: Slowly add 2.8 mL of concentrated H_2SO_4 (94%) to about 50 mL of distilled water. Dilute to 100 mL with distilled water.
- 6.7 Sodium phosphate: monobasic, ACS grade.
- 6.8 Sodium phosphate: dibasic, ACS grade.
- 6.9 Stock standard solutions (1.00 $\mu\text{g}/\mu\text{L}$): Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.
 - 6.9.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in distilled-in-glass quality methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 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.
 - 6.9.2 Transfer the stock standard solutions into PTFE-sealed screw-cap bottles. Store at 40°C and protect from light. Frequently check standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
 - 6.9.3 Stock standard solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. CALIBRATION

- 7.1 Establish liquid chromatographic operating parameters equivalent to those indicated in Table 1. The liquid chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2 External standard calibration procedure.
 - 7.2.1 For each compound of interest, prepare calibration standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with methanol. One of the external standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

- 7.2.2** Using injection of 5 to 20 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each compound at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.
- 7.2.3** The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.
- 7.3** Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards 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.
- 7.3.1** Prepare calibration standards at a minimum of three concentration levels for each compound 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 methanol. One of the standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
- 7.3.2** Using injections of 5 to 20 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, 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} , against RF.

- 7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.
- 7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from these agents.

8. QUALITY CONTROL

- 8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.
 - 8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
 - 8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
 - 8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
 - 8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol, 1000 times more concentrated than the selected concentrations.
 - 8.2.2 Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
 - 8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.
 - 8.2.4 Using the appropriate data from Table 2, determine the recovery and single-operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.

- 8.3** The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁶ that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s . Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁶

- 8.4** The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.
- 8.5** Before processing any samples, the analyst should demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.
- 8.6** 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 monitor the precision of the sampling technique. 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 perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. SAMPLES COLLECTION, PRESERVATION, AND HANDLING

- 9.1** Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2** The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

- 9.3 Adjust the pH of the sample to 6 to 8 with sodium hydroxide or sulfuric acid immediately after sampling.

10. SAMPLE EXTRACTION

- 10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel. Check the pH of the sample with wide range pH paper and adjust to 6 to 8 with 1N sodium hydroxide or 1N sulfuric acid. Dissolve 5 g of monobasic sodium phosphate and 5 g of dibasic sodium phosphate in the sample.
- 10.2 Add 60 mL of methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.
- 10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.
- 10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 25-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.
- 10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with 30 to 40 mL of methylene chloride.
- 10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.7 Remove the macro-Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. Add one or two clean boiling chips and attach a two-ball micro-Snyder column to the concentrator tube. Prewet the micro-Snyder column with methylene chloride and concentrate the solvent extract as before. When an apparent volume of 0.5 mL is reached, or the solution stops boiling, remove the K-D apparatus and allow it to drain and cool for 10 minutes.

- 10.8** Remove the micro-Snyder column and adjust the volume of the extract to 1.0 mL with methylene chloride. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract is to be stored longer than 2 days, transfer the extract to a screw-capped vial with a PTFE-lined cap. If the sample extract requires no further cleanup, proceed with Section 10.9. If the sample requires cleanup, proceed to Section 11.
- 10.9** Add one or two clean boiling chips to the concentrator tube along with 10-mL of methanol. Attach a two-ball micro-Snyder column and prewet the micro-Snyder column with about 1 mL of methanol. Concentrate the solvent extract as before to an apparent volume of 2 mL and allow it to drain and cool for 10 minutes. Transfer the solvent extract to a 5 mL volumetric flask and dilute to the mark with methanol. Proceed with the liquid chromatographic analysis in Section 12.
- 10.10** Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. CLEANUP AND SEPARATION

- 11.1** Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is not less than 85%.
- 11.2** The following silica gel column cleanup procedure has been demonstrated to be applicable to mercaptobenzothiazole.
- 11.2.1** Add 10 g of silica gel to 100 mL of ethyl ether and 600 μ L of reagent water in a 250-mL Erlenmeyer flask. Shake vigorously for 15 minutes. Transfer the slurry to a chromatographic column (silica gel may be retained with a plug of glass wool). Allow the solvent to elute from the column until the silica gel is almost exposed to the air. Wash the column with 100 mL of methylene chloride. Use a column flow of 2 to 2.5 mL/min throughout the wash and elution profiles.
- 11.2.2** Quantitatively add the sample extract from Section 10.8 to the head of the column. Allow the solvent to elute from the column until the silica gel is almost exposed to the air. Elute the column with 50 mL of methylene chloride. Discard this fraction.
- 11.2.3** Elute the column with 50 mL of 6% acetone in methylene chloride and collect eluate in a K-D apparatus. Concentrate this fraction to 1 mL as described in Sections 10.6 and 10.7. Exchange solvent with methanol as described in Section 10.9 and proceed with liquid chromatographic analysis.

12. LIQUID CHROMATOGRAPHY

- 12.1** Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Examples of the separations achieved by Column 1 and Column 2 are shown in Figures 1 and 2. Other columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.
- 12.2** Calibrate the liquid chromatographic system daily as described in Section 7.

- 12.3 If the internal standard approach is being used, the analyst must not add the internal standard to the sample extracts until immediately before injection into the instrument. Mix thoroughly.
- 12.4 Inject 5 to 20 μL of the sample extract. Record the resulting peak sizes in area or peak height units.
- 12.5 The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. CALCULATIONS

- 13.1 Determine the concentration of individual compounds in the sample.
 - 13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

Equation 2

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_w)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_w = Volume of water extracted, in mL

- 13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where

A_s = Response for parameter to be measured

A_{is} = Response for the internal standard

I_s = Amount of internal standard added to each extract, in μg

V_o = Volume of water extracted, in L

- 13.2** Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 13.3** For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

14. METHOD PERFORMANCE

- 14.1** 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.⁸ The MDL concentrations listed in Table 1 were obtained using reagent water.¹ Similar results were achieved using representative wastewaters.
- 14.2** This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from 5 to 1000 $\mu\text{g/L}$.
- 14.3** In a single laboratory, Battelle Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 2 were obtained after silica gel cleanup. Seven replicates of each of two different wastewaters were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 2.¹

References

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7. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, Pennsylvania, p. 76, 1980.
8. Glaser, J.A., et. al., "Trace Analysis for Wastewaters," *Environmental Science and Technology*, 15, 1426 (1981).

Table 1. Chromatographic Conditions and Method Detection Limits

<i>Parameter</i>	<i>Retention Time (min)</i>		<i>MDL ($\mu\text{g/L}$)</i>
	<i>Column 1</i>	<i>Column 2</i>	
Mercaptobenzothiazole	8.4	9.5	1.7

Column 1 conditions: Spherisorb-ODS, 5 μ , 250mm long by 4.6 mm ID; 1 mL/min flow; 50/50 acetonitrile/water.

Column 2 conditions: Lichrosorb RP-2, 5 μ , 250mm long by 4.6 mm ID; 1 mL/min flow; 10/90 acetonitrile/water.

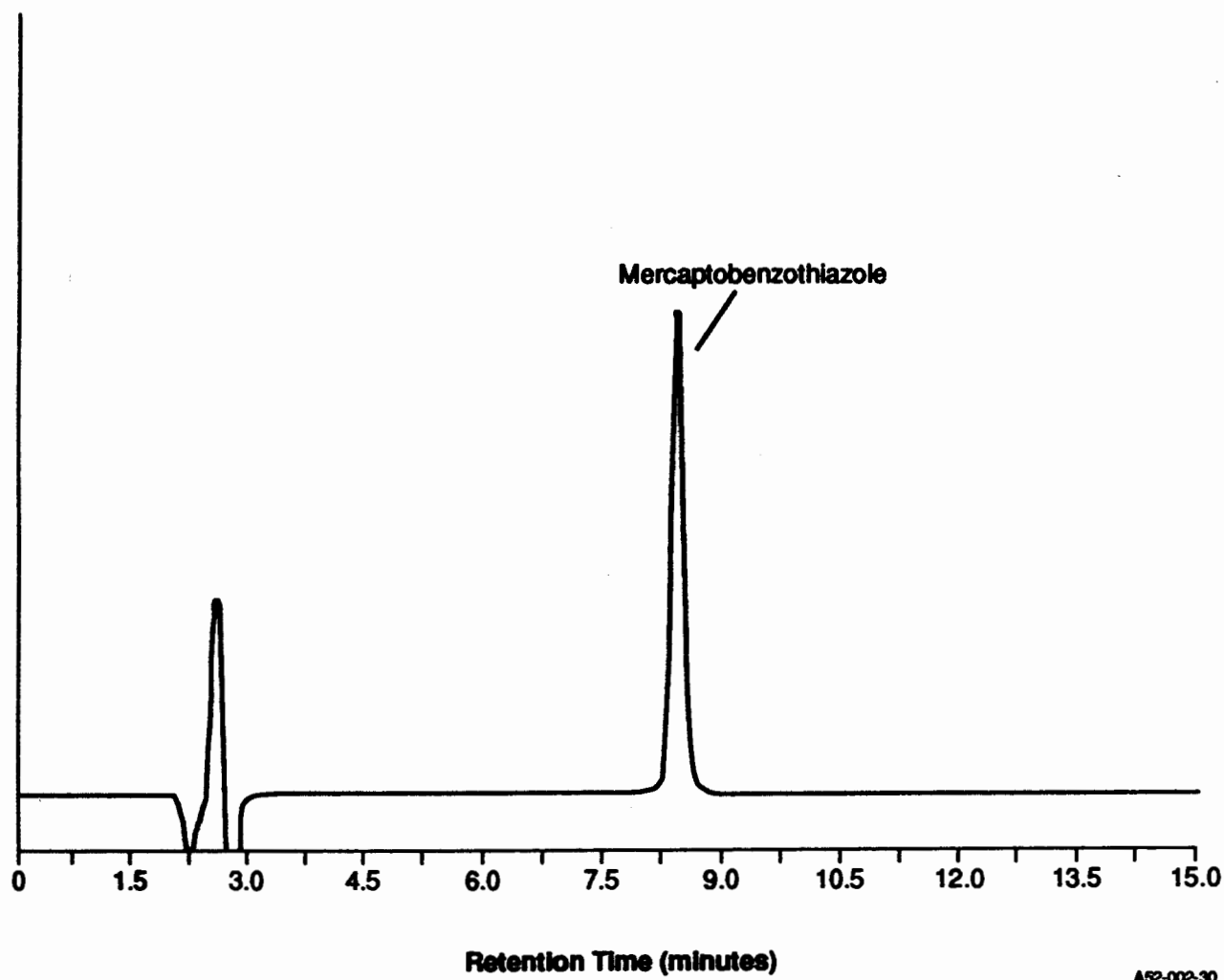
Table 2. Single-Laboratory Accuracy and Precision (a)

<i>Parameter</i>	<i>Sample Type (b)</i>	<i>Background ($\mu\text{g/L}$) (c)</i>	<i>Spike ($\mu\text{g/L}$)</i>	<i>Average Recovery (%)</i>	<i>Standard Deviation (%)</i>	<i>Number of Replicates</i>
Mercaptobenzo-thiazole	1	ND	5	79	5	7
	1	ND	10	87	4	7

(a) Column 1 conditions were used.

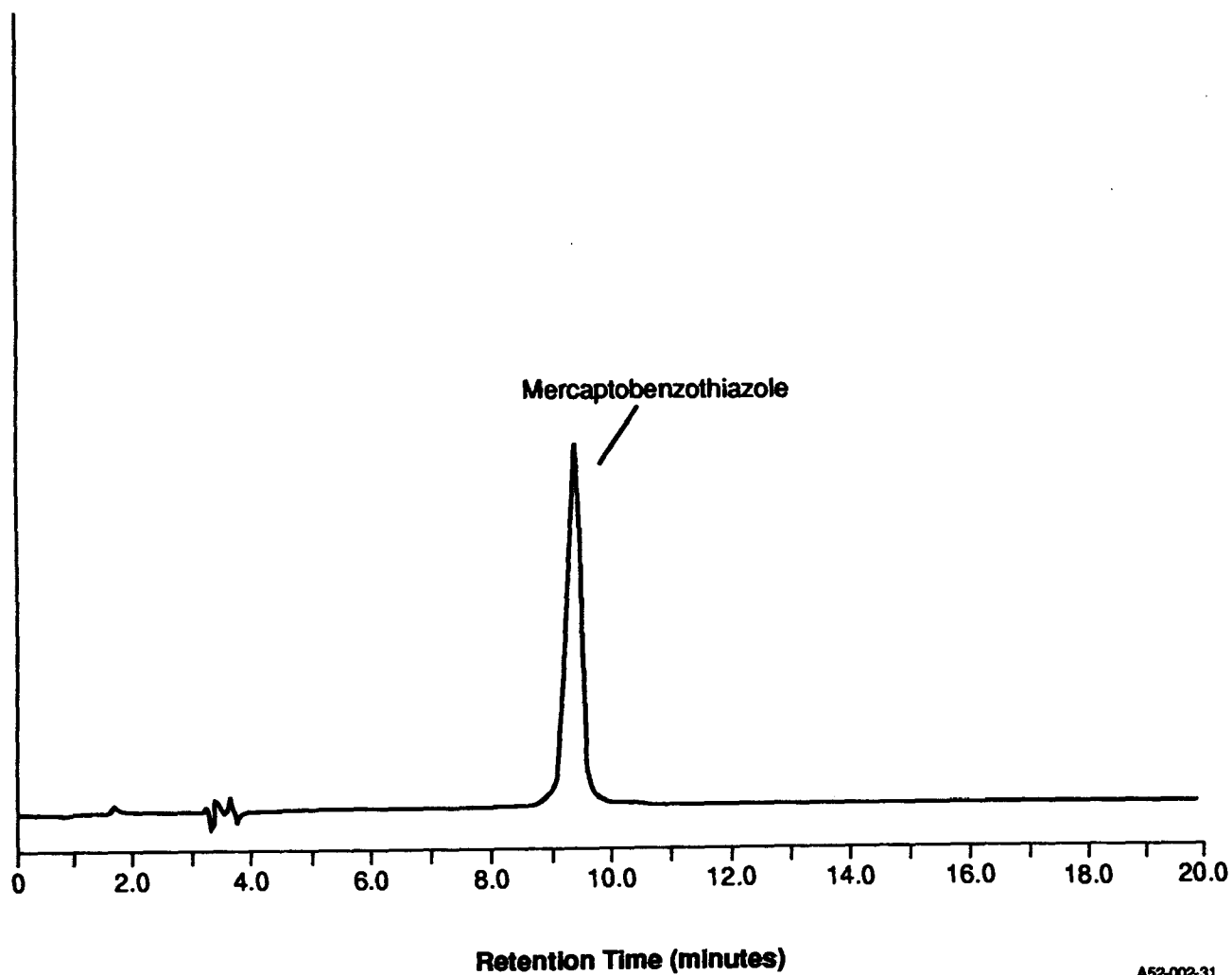
(b) 1 = Municipal sewage effluent

(c) ND = Not detected



A52-002-30

Figure 1. HPLC-UV Chromatogram of 10 ng of Mercaptobenzothiazole (Column 1).



A52-002-31

Figure 2. HPLC-UV Chromatogram of 10 ng of Mercaptobenzothiazole (Column 2).

Method 641
***The Determination of
Thiabendazole in Municipal
and Industrial Wastewater***

Method 641:

The Determination of Thiabendazole in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of thiabendazole in municipal and industrial wastewater.

<i>Parameter</i>	<i>CAS No.</i>
Thiabendazole	148-79-8

- 1.2 The estimated detection limit (EDL) for thiabendazole is listed in Table 1. The EDL was calculated from the minimum detectable response being equal to 5 times the background noise using a 100- μ L injection. The EDL for a specific wastewater may be different depending on the nature of interferences in the sample matrix.
- 1.3 This is a liquid chromatographic method applicable to the determination of thiabendazole in municipal and industrial discharges. When this method is used to analyze unfamiliar samples for thiabendazole, compound identification should be supported by at least one additional qualitative technique.
- 1.4 This method is restricted to use by or under the supervision of analysts experienced in the operation of liquid chromatographs and in the interpretation of chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Sections 9.2 and 9.3.

2. SUMMARY OF METHOD

- 2.1 Thiabendazole is analyzed in the sample matrix after solubilization with acid and filtration to remove particulate matter. Chromatographic conditions are described which permit the separation and accurate measurement of thiabendazole by direct aqueous injection and HPLC with fluorescence detection.

3. INTERFERENCES

- 3.1 Solvent, reagents, glassware, and other sample-processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of the chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 9.1.
- 3.1.1 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.1.2 Glassware must be scrupulously cleaned.¹ Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap water and reagent water. It should then be drained dry and heated in a muffle furnace at 400°C for 15 to 30 minutes. Solvent rinses

with acetone and pesticide-quality hexane may be substituted for the heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store the glassware inverted or capped with aluminum foil.

- 3.2 Matrix interferences may be caused by fluorescing contaminants that coelute with thiabendazole. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. Matrix interferences caused by the presence of particulate matter are removed by filtration. Unique samples may require additional cleanup approaches to achieve the detection limit listed in Table 1.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified²⁴ for the information of the analyst.

5. APPARATUS AND EQUIPMENT

- 5.1 Sampling equipment for discrete sampling.
- 5.1.1 Vial: 25-mL capacity or larger, equipped with a screw-cap with hole in center (Pierce 13074 or equivalent). Detergent wash, rinse with tap and distilled water, and dry at 105°C before use.
 - 5.1.2 Vial: 3.5-mL, equipped with a screw-cap with hole in center (Pierce 13019 or equivalent). Wash vial and cap as in Section 5.1.1.
 - 5.1.3 Septum: PTFE-faced silicone (Pierce 12722 or equivalent). Detergent wash and dry at 105°C for 1 hour before use.
 - 5.1.4 Septum: PTFE-faced silicone (Pierce 12712 or equivalent). Detergent wash and dry at 105° for 1 hour before use.
- 5.2 Syringe: Glass, 5-mL with Leur tip.
- 5.3 Syringe-filter holder: Stainless steel with Leur connection (Rainin 38 to 101 or equivalent).
- 5.4 Filters: 13 mm, Nylon 66, 0.45- μ pore (Rainin 38 to 112 or equivalent).
- 5.5 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6 High performance liquid chromatography (HPLC) apparatus: Analytical system complete with liquid chromatograph and all required accessories including syringes, analytical columns, and mobile phases. The system must be compatible with the specified detectors and strip-chart recorder. A data system is recommended for measuring peak areas.
- 5.6.1 Isocratic pumping system, constant flow.
 - 5.6.2 Injector valve (Rheodyne 7125 or equivalent) with 100- μ L loop.

- 5.6.3 Column: 250 mm long by 4.6 mm ID, stainless steel, packed with reverse-phase Ultrasphere ODS, 10 μ .
- 5.6.4 Fluorescence detector, for excitation at 300 nm and emission at 360 nm (Perkin Elmer 650 to 1S or equivalent). Fluorometer should have dispersive optics for excitation and utilize either filter or dispersive optics at the emission detector.
- 5.6.5 Strip-chart recorder compatible with detector, 250 mm. (A data system for measuring peak areas is recommended.)

6. REAGENTS AND CONSUMABLE MATERIALS

- 6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the EDL of each parameter of interest.
- 6.2 Sodium hydroxide solution (10N): Dissolve 40 grams of NaOH in reagent water and dilute to 100 mL.
- 6.3 Sodium thiosulfate: ACS, granular.
- 6.4 Sulfuric acid solution (1 + 1): Slowly add 50 mL of H₂SO₄ (specific gravity 1.84) to 50 mL of reagent water.
- 6.5 HPLC buffer (pH 8.2): Add 8 mL of triethanolamine (Eastman 1599) and 1 mL of glacial acetic acid (ACS) to 1 L of reagent water.
- 6.6 High-purity methanol: HPLC quality, distilled in glass.
- 6.7 Stock standard solution (1.0 μ g/ μ L): Stock standard solutions are prepared from pure standard material or purchased as a certified solution.
 - 6.7.1 Prepare the stock standard solution by accurately weighing about 0.0100g of pure material. Dissolve the material in pesticide-quality methanol, dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is certified at 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.
 - 6.7.2 Transfer the stock standard to a PTFE-sealed screw-cap bottle. Store at 4°C and protect from light. The stock standard should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards.
 - 6.7.3 The stock standard must be replaced after 6 months, or when comparison with quality control check samples indicates a problem.

7. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

- 7.1 Collect all samples in duplicate. Grab samples must be collected in glass containers. Conventional sampling practices³ should be followed, except that the bottle must not be prewashed with sample before collection.
- 7.2 The samples must be iced or refrigerated at 4°C from the time of collection until analysis. Chemical preservatives should not be used in the field unless more than 24 hours will elapse before delivery to the laboratory. If the samples will not be analyzed within 48 hours of

collection, the sample should be adjusted to a pH range of 1.0 to 3.0 with sodium hydroxide or sulfuric acid, and 35 mg of sodium thiosulfate per liter of sample for each part per million of free chlorine should be added.

- 7.3 All samples must be analyzed within 30 days of collection.⁶

8. CALIBRATION AND STANDARDIZATION

- 8.1 Establish liquid chromatographic operating parameters equivalent to those indicated in Table 1.
- 8.2 Prepare calibration standards at a minimum of three concentration levels of thiabendazole by adding volumes of the stock standard to a volumetric flask and diluting to volume with HPLC mobile phase. One of the standards should be at a concentration near, but greater than, the EDL, and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.
- 8.3 Using injections of 100 μ L of each calibration standard, tabulate peak height or area responses against the mass injected. The results are used to prepare a calibration curve for thiabendazole. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range ($<10\%$ relative standard deviation), linearity of the calibration curve can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.
- 8.4 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for thiabendazole varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or factor must be prepared.
- 8.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

9. QUALITY CONTROL

- 9.1 Monitoring for interferences.

9.1.1 Analyze a laboratory reagent blank as described in Section 10 each time a set of samples is extracted. A laboratory reagent blank is an aliquot of reagent water. If the reagent blank contains a reportable level of thiabendazole, immediately check the entire analytical system to locate and correct for possible interferences and repeat the test.

- 9.2 Assessing accuracy.

9.2.1 After every ten samples, and preferably in the middle of each day, analyze a laboratory control standard. Calibration standards may not be used for accuracy assessments and the laboratory control standard may not be used for calibration of the analytical system.

9.2.1.1 Laboratory control standard concentrate: From the stock standard prepared as described in Section 6.7, prepare a laboratory control standard concentrate that contains thiabendazole at a concentration of 2 μ g/mL in methanol or other suitable solvent.⁷

- 9.2.1.2** Laboratory control standard: using a pipette or microliter syringe, add 50.0 μL of the laboratory control standard concentrate to a 10-mL aliquot of reagent water contained in a 10-mL volumetric flask.
- 9.2.1.3** Analyze the laboratory control standard as described in Section 10. Calculate the percent recovery (P_i) with the equation:

Equation 1

$$P_i = \frac{100S_i}{T_i}$$

where

S_i = the analytical results from the laboratory control standard, in $\mu\text{g/L}$

T_i = the known concentration of the spike, in $\mu\text{g/L}$

-
- 9.2.2** At least annually, the laboratory should participate in formal performance evaluation studies, where solutions of unknown concentrations are analyzed and the performance of all participants is compared.

9.3 Assessing precision.

- 9.3.1** Precision assessments for this method are based upon the analysis of field duplicates (Section 7.1). Analyze both sample vials for at least 10% of all samples. To the extent practical, the samples for duplication should contain reportable levels of thiabendazole.
- 9.3.2** Calculate the relative range (RR_i) with the equation:

Equation 2

$$RR_i = \frac{100R_i}{X_i}$$

where

R_i = the absolute difference between the duplicate measurements X_1 and X_2 , in $\mu\text{g/L}$

X_i = the average concentration found $\left[\frac{X_1 + X_2}{2} \right]$ in $\mu\text{g/L}$

-
- 9.3.3** Individual relative range measurements are pooled to determine average relative range or to develop an expression of relative range as a function of concentration.

10. PROCEDURE

10.1 Sample preparation.

- 10.1.1** Adjust the pH of the sample to pH 1 to 3 with sulfuric acid solution.
- 10.1.2** Assemble the syringe-filtration assembly by attaching the filter holder (with filter) to a 5-mL glass syringe equipped with a Leur tip.
- 10.1.3** Remove the barrel from the syringe and pour a 4- to 5-mL aliquot of the acidified sample into the syringe, allowing room for reinsertion of the syringe barrel.

- 10.1.4** Filter a portion of the sample through 0.45- μ filter using a syringe-filter holder. The first few milliliters should be discarded. Collect the filtrate in a 4-mL vial equipped with a PTFE-sealed screw-cap.
- 10.1.5** The syringe and filter holder should be rinsed with acetone or methanol and then HPLC-grade water between samples.
- 10.2** Cleanup and separation.
- 10.2.1** Cleanup procedures may not be necessary for a relatively clean sample matrix. Use of fluorescent detectors, however, often obviates the necessity for cleanup of relatively clean sample matrices. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that recovery is no less than 85%.
- 10.3** Liquid chromatographic analysis.
- 10.3.1** Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are the estimated retention time and estimated detection limit that can be achieved by this method. An example chromatogram achieved by this column is shown in Figure 1. Figure 2 is a chromatogram of thiabendazole in a POTW wastewater sample. Other columns, chromatographic conditions, or detectors may be used if data quality comparable to Table 2 is achieved.
- 10.3.2** Calibrate the system daily as described in Section 8.
- 10.4** Inject 100 μ L of the filtered aqueous sample. Monitor the column eluent at excitation wavelength 300 nm (5-nm slit width) and emission wavelength 360 nm (10-nm slit width). Record the resulting peak size in area or peak height units.
- 10.5** The retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 10.6** If the response for the peak exceeds the working range of the system, dilute the sample with mobile phase and reanalyze.
- 10.7** If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

11. CALCULATIONS

- 11.1** Determine the concentration of thiabendazole in the sample.
- 11.1.1** Calculate the amount of thiabendazole injected from the peak response using the calibration curve or calibration factor in Section 8.2.2. The concentration in the sample can be calculated from the following equation:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(100)}{(V_i)}$$

where

A = Amount of the thiabendazole injected, in ng

V_i = Volume of sample injected, in μL

-
- 11.2** Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

12. METHOD PERFORMANCE

- 12.1** The EDL and associated chromatographic conditions for thiabendazole are listed in Table 1.⁵ The EDL is defined as the minimum response being equal to 5 times the background noise, using a 100- μL injection.
- 12.2** Single-operator accuracy and precision studies were conducted by Environmental Science and Engineering, Inc.,⁶ using a spiked POTW sample. The results of these studies are presented in Table 2.

References

1. ASTM Annual Book of Standards, Part 31, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, Pennsylvania, p. 679, 1980.
2. "Carcinogens - Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.
3. "OSHA Safety and Health Standards, General Industry" (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976).
4. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
5. ASTM Annual Book of Standards, Vol. 11.01, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, 1986.
6. Test procedures for Pesticides in Wastewaters, EPA Contract Report 68-03-2897, unpublished report available from U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.
7. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, March 1979.
8. "Evaluation of Ten Pesticides Methods," U.S. Environmental Protection Agency, Contract No. 68-03-1760, Task No. 11, U.S. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

Table 1. Liquid Chromatography of Thiabendazole*

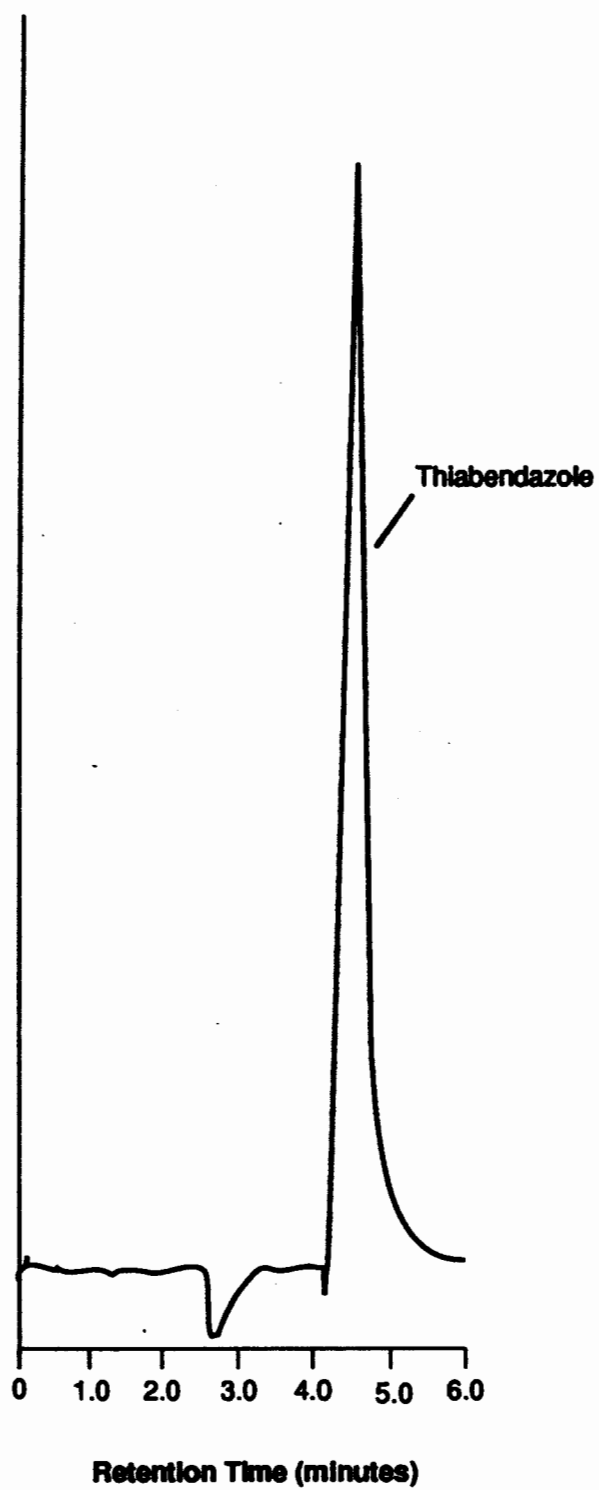
<i>Compound</i>	<i>Retention Time (min)</i>	<i>Estimated Detection Limit (µg/L)</i>
Thiabendazole	4.3	1.7

*HPLC conditions: 10 µ reverse-phase Ultrasphere ODS; Column: 250 mm long by 4.6 mm ID; isocratic 70% methanol/30% buffer; flow rate 1 mL/min.

Table 2. Single-Operator Accuracy and Precision*

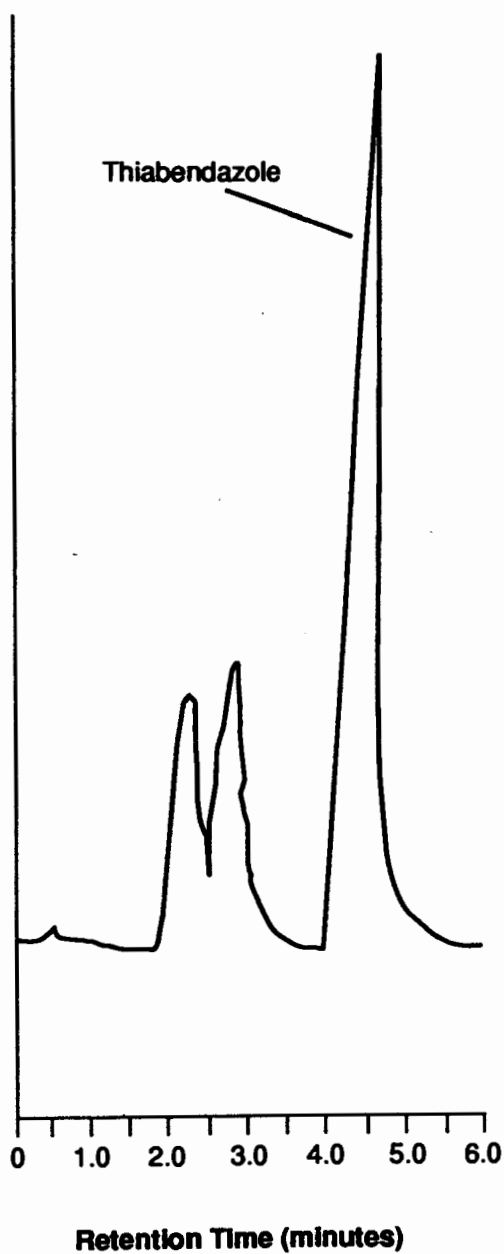
<i>Parameter</i>	<i>Spike Concentration (µg/L)</i>	<i>Number of Replicates</i>	<i>Average Percent Recovery</i>	<i>Standard Deviation (%)</i>
Thiabendazole	12.5	7	100	9.5
	625	7	92.8	4.5

*POTW effluent was used in this study.



A52-002-67

Figure 1. HPLC of Thiabendazole



A52-002-68

Figure 2. Chromatogram of Thiabendazole in Wastewater Sample.

Method 642
The Determination of
Biphenyl and Ortho-
Phenylphenol in Municipal
and Industrial Wastewater

Method 642

The Determination of Biphenyl and Ortho-Phenylphenol in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1** This method covers the determination of biphenyl and o-phenylphenol in municipal and industrial wastewater.

<i>Parameter</i>	<i>CAS No.</i>
Biphenyl	92-52-4
o-phenylphenol	132-27-4

- 1.2** The estimated detection limits (EDL) for the parameters above are listed in Table 1. The EDLs were calculated from the minimum detectable response being equal to 5 times the background noise using a 2-mL final extract volume of a 1-L sample and an injection volume of 50 μ L. The EDL for a specific wastewater may be different depending on the nature of interferences in the sample matrix.
- 1.3** This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compounds listed above in municipal and industrial discharges. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identification should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second HPLC column that can be used to confirm measurements made with the primary column.
- 1.4** This method is restricted to use by or under the supervision of analysts experienced in the operation of liquid chromatographs and in the interpretation of liquid chromatograms.

2. SUMMARY OF METHOD

- 2.1** The fungicides are removed from the sample matrix by extraction with methylene chloride. The extract is dried, exchanged to acetonitrile or methanol, and analyzed by liquid chromatography with ultraviolet (UV) detection.

3. INTERFERENCES

- 3.1** Solvent, reagents, glassware, and other sample-processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of liquid chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 9.1.
- 3.1.1** The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.1.2 Glassware must be scrupulously cleaned.¹ Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap water and reagent water. It should then be drained dry and heated in a muffle furnace at 400°C for 15 to 30 minutes. Solvent rinses with acetone and pesticide-quality hexane may be substituted for the heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store the glassware inverted or capped with aluminum foil.

3.2 Matrix interferences may be caused by UV-active contaminants that are coextracted from the samples. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. While general cleanup procedures are provided as part of this method, unique samples may require additional cleanup approaches to achieve the detection limits listed in Table 1.

4. SAFETY

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified²⁴ for the information of the analyst.

5. APPARATUS AND EQUIPMENT

5.1 Sample containers: Narrow-mouth glass bottles, 1-L or 1-quart volume, equipped with polytetrafluoroethylene (PTFE)-lined screw-caps. Wide-mouth glass bottles, 1-quart volume, equipped with PTFE-lined screw-caps may also be used. Prior to use, wash bottles and cap liners with detergent and rinse with tap and reagent water. Allow the bottles and cap liners to air dry, then muffle the bottles at 400°C for 1 hour. After cooling, rinse the bottles and cap liners with hexane, seal the bottles, and store in a dust-free environment.

5.1.1 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Kuderna-Danish (K-D) glassware.

5.2.1 Snyder column, Kuderna-Danish: Three-ball macro (Kontes K-503000-0121 or equivalent) and two-ball micro (Kontes K-569001-0219 or equivalent).

- 5.2.2 Concentrator tube: 10-mL, graduated (Kontes K-570050-1025 or equivalent) with ground-glass stopper.
- 5.2.3 Evaporative flask: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.
- 5.3 High performance liquid chromatography (HPLC) apparatus: Analytical system complete with liquid chromatograph and all required accessories including syringes, analytical columns, and mobile phases. The system must be compatible with the specified detectors and strip-chart recorder. A data system is recommended for measuring peak areas.
 - 5.3.1 Gradient pumping system, constant flow.
 - 5.3.2 Injector valve (Rheodyne 7125 or equivalent) with 50- μ L loop.
 - 5.3.3 Column 1: 250 mm long by 4.6 mm ID, stainless steel, packed with reverse-phase Perkin Elmer HC-ODS Sil-X 10 μ , or equivalent.
 - 5.3.4 Column 2: 250 mm long by 4.6 mm ID, packed with reverse-phase Dupont Zorbax ODS, 6 to 7 μ , or equivalent.
 - 5.3.5 Ultraviolet detector, capable of monitoring at 254 nm.
 - 5.3.6 Strip-chart recorder compatible with detector, 250 mm. (A data system for measuring peak areas is recommended.)
- 5.4 Chromatographic column: 300 mm long by 10 mm ID Chromaflex, equipped with coarse-fritted bottom plate and PTFE stopcock. (Kontes K-420540-0213 or equivalent).
- 5.5 Drying column: Approximately 400 mm long by 20 mm ID borosilicate glass, equipped with coarse-fritted bottom plate.
- 5.6 Miscellaneous.
 - 5.6.1 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
 - 5.6.2 Separatory funnels: 2-L, 500-mL, and 250-mL, equipped with PTFE stopcocks.
 - 5.6.3 Boiling chips: Approximately 10/40 mesh. Heat to 400°C for 30 minutes or perform a Soxhlet extraction with methylene chloride for 2 hours.
 - 5.6.4 Water bath: Heated with concentric ring cover, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.
 - 5.6.5 Standard solution storage containers: 15-mL bottles with PTFE-lined screw-caps.

6. REAGENTS AND CONSUMABLE MATERIALS

6.1 Reagents.

- 6.1.1 Acetone, acetonitrile, methanol, and methylene chloride: Demonstrated to be free of analytes and interferences.
- 6.1.2 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest. The water is held at 90°C. Store in clean, narrow-mouth bottles with PTFE-lined septa and screw-caps.
- 6.1.3 Sodium sulfate: Granular, anhydrous. Condition by heating at 400°C for 4 hours in a shallow tray.

- 6.1.4 HPLC Mobile Phase 1: Add 400 mL of acetonitrile to a 1-L volumetric flask and dilute to volume with reagent water.
- 6.1.5 HPLC Mobile Phase 2: Add 500 mL of methanol to a 1-L volumetric flask and dilute to volume with reagent water.
- 6.2 Standard stock solutions (1.00 $\mu\text{g}/\mu\text{L}$): These solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedures.
 - 6.2.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide-quality methanol or acetonitrile, dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is certified at 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.
 - 6.2.2 Transfer the stock standards to PTFE-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standards should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
 - 6.2.3 Stock standards must be replaced after 6 months, or when comparison with quality control check samples indicates a problem.

7. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

- 7.1 Collect all samples in duplicate. Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed, except that the bottle must not be prewashed with sample before collection.
- 7.2 The samples must be iced or refrigerated at 4°C from the time of collection until analysis.
- 7.3 All samples must be extracted and analyzed as soon as possible after sampling, since preservation studies⁶ have shown that these compounds undergo almost complete decomposition within 7 days.

8. CALIBRATION

- 8.1 Establish liquid chromatographic operating parameters equivalent to those indicated in Table 1.
- 8.2 Prepare calibration standards at a minimum of three concentration levels of the analytes by adding volumes of the stock standard to a volumetric flask and diluting to volume with HPLC mobile phase (40% acetonitrile in water or 50% methanol in water). One of the standards should be at a concentration near, but greater than, the EDL, and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.
- 8.3 Using injections of 50 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results are used to prepare a calibration curve for the analytes. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (<10% relative standard deviation), linearity of the calibration curve can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

- 8.4 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any analyte varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or factor must be prepared.
- 8.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

9. QUALITY CONTROL

- 9.1 Monitoring for interferences: Analyze a laboratory reagent blank each time a set of samples is extracted. A laboratory reagent blank is an aliquot of reagent water. If the reagent blank contains a reportable level of the analytes, immediately check the entire analytical system to locate and correct for possible interferences and repeat the test.
- 9.2 Assessing accuracy.
- 9.2.1 After every ten samples, and preferably in the middle of each day, analyze a laboratory control standard. Calibration standards may not be used for accuracy assessments and the laboratory control standard may not be used for calibration of the analytical system.
- 9.2.1.1 Laboratory control standard concentrate: From stock standards prepared as described in Section 6.2, prepare a laboratory control standard concentrate that contains the analytes at a concentration of 2 $\mu\text{g/mL}$ in methanol or acetonitrile.
- 9.2.1.2 Laboratory control standard: Using a pipette, add 1.0 mL of the laboratory control standard concentrate to a 1-L aliquot of reagent water contained in a 1000-mL volumetric flask.
- 9.2.1.3 Analyze the laboratory control standard as described in Section 10. Calculate the percent recovery (P_i) with the equation:

Equation 1

$$P_i = \frac{100S_i}{T_i}$$

where

S_i = The analytical results from the laboratory control standard, in $\mu\text{g/L}$

T_i = The known concentration of the spike, in $\mu\text{g/L}$

- 9.2.2 At least annually, the laboratory should participate in formal performance evaluation studies, where solutions of unknown concentrations are analyzed and the performance of all participants is compared.⁷
- 9.3 Assessing precision.
- 9.3.1 Precision assessments for this method are based upon the analysis of field duplicates (Section. 7.1). Analyze both sample bottles for at least 10% of all samples. To the

extent practical, the samples for duplication should contain reportable levels of the analytes.

9.3.2 Calculate the relative range' (RR_i) with the equation:

Equation 2

$$RR_i = \frac{100R_i}{X_i}$$

where

R_i = The absolute difference between the duplicate measurements X_1 and X_2 , in $\mu\text{g/L}$

X_i = The average concentration found $\left[\frac{X_1 + X_2}{2} \right]$, in $\mu\text{g/L}$

9.3.3 Individual relative range measurements are pooled to determine average relative range or to develop an expression of relative range as a function of concentration.

10. PROCEDURE

10.1 Sample extraction.

10.1.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel.

10.1.2 Add 60 mL of methylene chloride to the sample bottle and shake for 30 seconds to rinse the walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release vapor pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends on the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation. Collect the extract in a 250-mL Erlenmeyer flask.

10.1.3 Add an additional 60-mL volume of methylene chloride to the sample bottle and complete the extraction procedure a second time, combining the extracts in the Erlenmeyer flask.

10.1.4 Perform a third extraction in the same manner. Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, collecting the extract in a 500-mL K-D flask equipped with a 10-mL concentrator tube. Rinse the Erlenmeyer flask and column with about 30 mL of methylene chloride to complete the transfer.

10.1.5 Add one or two clean boiling chips to the flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (80 to 85°C) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed in steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of

distillation, the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. If the extract requires cleanup, proceed to Section 10.2. If the extract does not require cleanup, proceed with Sections 10.1.6 and 10.1.7.

10.1.6 Add 50 mL of methanol or acetonitrile and a clean boiling chip to the flask and repeat the concentration as described above. When the apparent volume of the liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methanol or acetonitrile. A 5-mL syringe is recommended for this operation.

10.1.7 Add a clean boiling chip to the concentrator tube. Attach a two-ball micro-Snyder column. Prewet the micro-Snyder column by adding about 0.5 mL of methanol or acetonitrile to the top. Place the micro K-D apparatus on a hot water bath (80 to 85°C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation, the balls will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a small volume of methanol or acetonitrile. Adjust the volume to 1.0 mL with methanol or acetonitrile. Add 1.0 mL of reagent water to the extract if methanol or 1.5 mL of reagent water to the extract if acetonitrile (Table 1).

NOTE: At high concentrations (approximately 1,000 mg/L or greater) of biphenyl in the extract, low recoveries may be obtained due to insolubility in the acetonitrile. Larger volumes of acetonitrile or acetone may be required to dissolve all the biphenyl and to prevent precipitation.

10.1.8 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1,000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

10.2 Cleanup and separation.

10.2.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. If particular circumstances demand the use of a cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.

10.2.2 Prior to HPLC analysis, the composition of the extracts must be as specified under chromatographic conditions in Table 1 and described in Sections 10.1.6 and 10.1.7.

10.2.3 Proceed with liquid chromatography as described in Section 10.3.

10.3 Liquid chromatography analysis.

10.3.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are the estimated retention times and estimated detection limits that

can be achieved by this method. An example of the separation achieved by Column 1 of the analytes in a POTW extract is shown in Figure 1. Other columns, chromatographic conditions,⁸ or detectors may be used if data quality comparable to Table 2 is achieved.

10.3.2 Calibrate the system daily as described in Section 8.

10.3.3 Inject 50 μL of the sample extract. Monitor the column eluent at 254 nm. Record the resulting peak size in area or peak height units.

10.3.4 The retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

10.3.5 If the response for the peak exceeds the working range of the system, dilute the sample with mobile phase and reanalyze.

10.3.6 If the measurement of the peak response is prevented by the presence of interferences, cleanup is required.

11. CALCULATIONS

11.1 Determine the concentration of analytes in the sample.

11.1.1 Calculate the amount of analytes injected from the peak response using the calibration curve or calibration factor in Section 8.2.2. The concentration in the sample can be calculated from the equation:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_s)}$$

where:

A = Amount of analytes injected, in nanograms.

V_i = Volume of extract injected in $\mu\text{g/L}$

V_t = Volume of total extract in $\mu\text{g/L}$

V_s = Volume of water extracted in mL

12. METHOD PERFORMANCE

12.1 The EDLs and associated chromatographic conditions for the analytes are listed in Table 1. The EDL is defined as the minimum response being equal to 5 times the background noise, assuming a 2-mL final extract volume of a 1-L sample and an HPLC injection volume of 50 μL .

12.2 Single-operator accuracy and precision studies were conducted by Environmental Science and Engineering, Inc.,⁶ in the designated matrices. The results of these studies are presented in Table 2.

References

1. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, Pennsylvania, p. 679, 1980.
2. "Carcinogens - Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.
3. "OSHA Safety and Health Standards, General Industry" (29 *CFR* 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976).
4. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
5. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.
6. Test procedures for Pesticides in Wastewaters, EPA Contract Report No. 68-03-2897. Unpublished report available from U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.
7. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory - Cincinnati, Ohio, March 1979.
8. Beernaert, H., Determination of Biphenyl and Ortho-Phenylphenol in Citrus Fruits by Gas Chromatography, *Journal of Chromatography*, 77: 331-8, 1973.
9. "Evaluation of Ten Pesticide Methods" U.S. Environmental Protection Agency, Contract No. 68-03-1760, Task No. 11, U.S. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

Table 1. Chromatographic Conditions and Estimated Detection Limits

<i>Parameter</i>	<i>Retention Time (min)</i>		<i>Estimated Detection Limit</i>
	<i>Column 1</i>	<i>Column 2</i>	<i>(µg/L)</i>
<i>o</i> -Phenylphenol	7.7	11.3	.01
Biphenyl	18.8	16.5	.04

Column 1: Reverse-phase column, 4.6 mm ID by 250 mm long; 10 µ, Perkin-Elmer HC-ODS Sil-X or equivalent; isocratic elution for 5 minutes using 40% acetonitrile in water, then linear gradient elution to 100% acetonitrile over 25 minutes; flow rate of 0.5 mL/min.

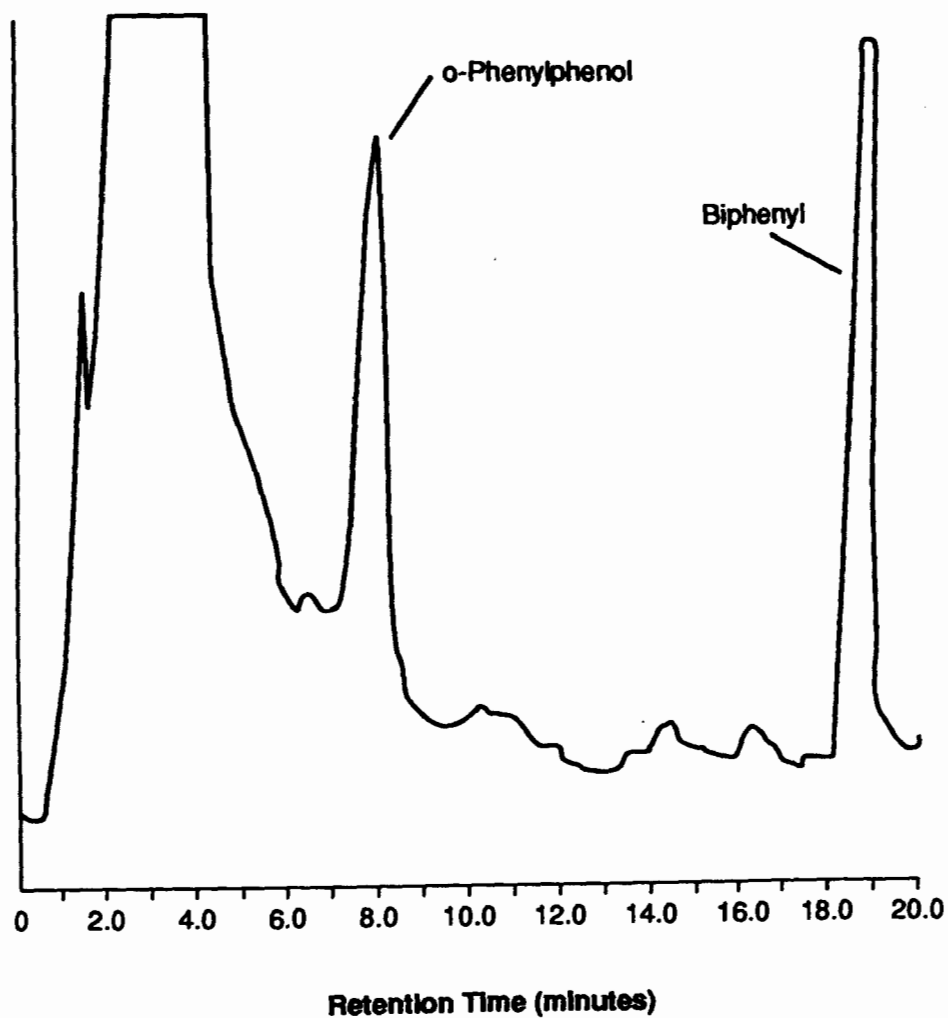
Column 2: Reverse-phase column, 4.6 mm ID by 250 mm long; 6 to 7 µ, Dupont Zorbax ODS or equivalent; isocratic elution for 3 minutes using 50% methanol in water, then linear gradient to 80-percent methanol over 10 minutes; flow rate 1.0 mL/min.

Table 2. Single-Operator Accuracy and Precision *

<i>Parameter</i>	<i>Spike Range (µg/L)</i>	<i>Number of Replicates</i>	<i>Average Percent Recovery**</i>	<i>Standard Deviation (%)</i>
<i>o</i> -Phenylphenol	2.5	7	102.3	36.3
	6,500	7	94.1	6.3
Biphenyl	2.4	7	86.3	16.2
	6,300	7	100.7	9.9

* POTW effluent was used in this study.

** No cleanup was employed in validation studies.



A52-002-69

Figure 1. Liquid Chromatogram of Wastewater Extract Fortified With o-Phenylphenol and Biphenyl (Column 1).

Method 643
The Determination of
Bentazon in Municipal and
Industrial Wastewater

Method 643

The Determination of Bentazon in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of bentazon in municipal and industrial wastewater.

Parameter	CAS No.
Bentazon (Basagran)	25057-89-0

- 1.2 The estimated detection limit (EDL) for bentazon is listed in Table 1. The EDL was calculated from the minimum detectable response being equal to 5 times the background noise using a 5-mL final extract volume of a 1-L sample and an injection volume of 100 μ L. The EDL for a specific wastewater may be different depending on the nature of interferences in the sample matrix.
- 1.3 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of bentazon in municipal and industrial discharges. When this method is used to analyze unfamiliar samples for bentazon, compound identification should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second HPLC column that can be used to confirm measurements made with the primary column.
- 1.4 This method is restricted to use by or under the supervision of analysts experienced in the operation of liquid chromatographs and in the interpretation of liquid chromatograms.

2. SUMMARY OF METHOD

- 2.1 Bentazon is removed from an acidified sample matrix by extraction with methylene chloride. The extract is discarded after back extraction with aqueous base. HPLC conditions are described which permit the separation and measurement of bentazon in the aqueous extract.

3. INTERFERENCES

- 3.1 Solvent, reagents, glassware, and other sample-processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of liquid chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 9.1
- 3.1.1 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.1.2 Glassware must be scrupulously cleaned.¹ Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap water and reagent water. It should then be drained dry and heated in a muffle furnace at 400°C for 15 to 30 minutes. Solvent rinses with acetone and pesticide-quality hexane may be substituted for the heating. Volumetric

ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store the glassware inverted or capped with aluminum foil.

- 3.2 Matrix interferences may be caused by UV-active contaminants that are coextracted from the samples. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. Unique samples may require additional cleanup approaches to achieve the detection limit listed in Table 1.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified²⁻⁴ for the information of the analyst.

5. APPARATUS AND EQUIPMENT

- 5.1 Sample containers: Narrow-mouth glass bottles, 1-L or 1-quart volume, equipped with polytetrafluoroethylene (PTFE)-lined screw-caps. Wide-mouth glass bottles, 1-quart volume, equipped with PTFE-lined screw-caps may also be used. Prior to use, wash bottles and cap liners with detergent and rinse with tap and reagent water. Allow the bottles and cap liners to air dry, then muffle the bottles at 400°C for 1 hour. After cooling, rinse the bottles and cap liners with hexane, seal the bottles, and store in a dust-free environment.
- 5.1.1 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.
- 5.2 High performance liquid chromatography (HPLC) apparatus: Analytical system complete with liquid chromatograph and all required accessories including syringes, analytical columns, and mobile phases. The system must be compatible with the specified detectors and strip-chart recorder. A data system is recommended for measuring peak areas.
- 5.2.1 Gradient pumping system, constant flow.
- 5.2.2 Injector valve (Rheodyne 7125 or equivalent) with 100-μL loop.
- 5.2.3 Column 1: 250 mm long by 4.6 mm ID, stainless steel, packed with reverse-phase Ultrasphere ODS, 10 μ, or equivalent.

- 5.2.4 Column 2: 300 mm long by 4.0 mm ID, packed with reverse phase μ Bondapak C18, 10 μ , (Waters Associates), or equivalent.
- 5.2.5 Ultraviolet detector, variable wavelength, capable of monitoring at 340 nm.
- 5.2.6 Strip-chart recorder compatible with detector, 250 mm. (A data system for measuring peak areas is recommended.)

5.3 Miscellaneous.

- 5.3.1 Balance: analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.3.2 Separatory funnels: 2-L, and 250-mL, equipped with PTFE stopcocks.
- 5.3.3 Standard solution storage containers: 15-mL bottles with PTFE-lined screw-caps.
- 5.3.4 Pasteur pipettes with bulbs.

6. REAGENTS AND CONSUMABLE MATERIALS

6.1 Reagents.

- 6.1.1 Acetone, hexane, methanol, and methylene chloride: Demonstrated to be free of analytes and interferences.
- 6.1.2 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.1.3 Sodium hydroxide solution (0.1N): Dissolve 0.4 g of NaOH in reagent water and dilute to 100 mL.
- 6.1.4 Sodium chloride: ACS, crystals.
- 6.1.5 Sodium thiosulfate: ACS, granular.
- 6.1.6 Sulfuric acid solution (1+1): Slowly add 50 mL of H₂SO₄ (specific gravity 1.84) to 50 mL of reagent water.
- 6.1.7 Sodium hydroxide solution (6N): Dissolve 24 g of NaOH in reagent water and dilute to 100 mL.
- 6.1.8 Acetate buffer solution: Dissolve 0.41 g of anhydrous sodium acetate (ACS) and 1.5 mL of glacial acetic acid (ACS) in 100 mL of reagent water.
- 6.1.9 Glacial acetic acid: ACS.
- 6.1.10 HPLC mobile phase buffer (pH 4.7, 0.062 M acetate): Dissolve 0.87 g of anhydrous sodium acetate (ACS) and 3.0 mL of glacial acetic acid (ACS) in 1 L of reagent water.

6.2 Standard stock solution (1.00 μ g/ μ L): This solution may be purchased as a certified solution or prepared from a pure standard material using the following procedures.

- 6.2.1 Prepare the stock standard solution by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide-quality methanol, dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is certified at 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.

- 6.2.2 Transfer the stock standards to a PTFE-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standards should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
- 6.2.3 Stock standards must be replaced after 6 months, or when comparison with quality control check samples indicates a problem.

7. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

- 7.1 Collect all samples in duplicate. Grab samples must be collected in glass containers. Conventional sampling practices⁵ should be followed, except that the bottle must not be prewashed with sample before collection.
- 7.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction. If the samples will not be extracted within 48 hours of collection, the sample should be adjusted to a pH range of 6.0 to 8.0 with sodium hydroxide or sulfuric acid and add 35 mg of sodium thiosulfate per liter of sample for each part per million of free chlorine.
- 7.3 All samples must be extracted within 7 days and completely analyzed within 30 days of extraction.⁶

8. CALIBRATION

- 8.1 Establish liquid chromatographic operating parameters equivalent to those indicated in Table 1.
- 8.2 Prepare calibration standards at a minimum of three concentration levels of bentazon by adding volumes of the stock standard to a volumetric flask and diluting to volume with HPLC mobile phase (35% methanol in HPLC mobile phase buffer or 40% methanol in HPLC mobile phase buffer). One of the standards should be at a concentration near, but greater than, the EDL, and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.
- 8.3 Using injections of 100 µL of each calibration standard, tabulate peak height or area response against the mass injected. The results are used to prepare a calibration curve for the analytes. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (<10% relative standard deviation, RSD), linearity of the calibration curve can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.
- 8.4 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for bentazon varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or factor must be prepared.
- 8.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

9. QUALITY CONTROL

9.1 Monitoring for interferences.

9.1.1 Analyze a laboratory reagent blank each time a set of samples is extracted. A laboratory reagent blank is an aliquot of reagent water. If the reagent blank contains a reportable level of bentazon, immediately check the entire analytical system to locate and correct for possible interferences and repeat the test.

9.2 Assessing accuracy.

9.2.1 After every ten samples, and preferably in the middle of each day, analyze a laboratory control standard. Calibration standards may not be used for accuracy assessments and the laboratory control standard may not be used for calibration of the analytical system.

9.2.1.1 Laboratory control standard concentrate: From stock standards prepared as described in Section 6.2, prepare a laboratory control standard concentrate that contains bentazon at a concentration of 2 µg/µL in methanol.

9.2.1.2 Laboratory control standard: Using a pipette or microliter syringe, add 50.0 µL of the laboratory control standard concentrate to a 1-L aliquot of reagent water.⁷

9.2.1.3 Analyze the laboratory control standard as described in Section 10. Calculate the percent recovery (P_i) with the equation:

Equation 1

$$P_i = \frac{100S_i}{T_i}$$

where

S_i = The analytical results from the laboratory control standard, in µg/L

T_i = The known concentration of the spike, in µg/L

9.2.2 At least annually, the laboratory should participate in formal performance evaluation studies, where solutions of unknown concentrations are analyzed and the performance of all participants is compared.⁷

9.3 Assessing precision.

9.3.1 Precision assessments for this method are based upon the analysis of field duplicates (Section 7.1). Analyze both samples for at least 10% of all samples. To the extent practical, the samples for duplication should contain reportable levels of bentazon.

9.3.2 Calculate the relative range⁷ (RR_i) with the equation:

Equation 2

$$RR_i = \frac{100 R_i}{X_i}$$

where

R_i = The absolute difference between the duplicate measurements X_1 and X_2 , in $\mu\text{g/L}$

X_i = The average concentration found $\left(\frac{X_1 + X_2}{2} \right)$, in $\mu\text{g/L}$

9.3.3 Individual relative range measurements are pooled to determine average relative range or to develop an expression of relative range as a function of concentration.

10. PROCEDURE

10.1 Sample extraction.

10.1.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel. Check the pH of the sample with wide-range pH paper and adjust to within the range of 2.5 to 3.5 with sulfuric acid. Add 200 g of sodium chloride and mix to dissolve.

10.1.2 Add 60 mL of methylene chloride to the sample bottle and shake for 30 seconds to rinse the walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release vapor pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends on the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation. Collect the extract in a 250-mL separatory funnel.

10.1.3 Add an additional 60-mL volume of methylene chloride to the sample bottle and complete the extraction procedure a second time, combining the extracts in the 250-mL separatory funnel.

10.1.4 Perform a third extraction in the same manner. Add 2 mL of 0.1M NaOH in reagent water to the 250-mL separatory funnel, and extract by shaking the funnel for 2 minutes with periodic venting to release vapor pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. Drain the methylene chloride into a 250-mL Erlenmeyer flask. Transfer the aqueous layer with a Pasteur pipette to a 5-mL volumetric flask.

10.1.5 Add the methylene chloride back to the 250-mL separatory funnel, and extract with an additional 2 mL of 0.1M NaOH. Combine the extracts in the 5-mL volumetric flask.

10.1.6 Add two drops of glacial acetic acid to the volumetric flask, and dilute to volume with acetate buffer solution (Section 6.1.7).

10.1.7 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1,000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

10.2 Cleanup and separation.

10.2.1 The cleanup procedure recommended in this method involves the back extraction of a methylene chloride extract with aqueous base, and has been used for the analysis of various clean waters and industrial effluents. If additional cleanup is required, a 1-L sample is adjusted to pH 12 with 6N NaOH and extracted with three 60-mL aliquots of methylene chloride in a 2-L separatory funnel. The methylene chloride extracts are discarded and the aqueous sample adjusted to pH range of 2.5 to 3.5 with 1:1 sulfuric acid solution for re-extraction as in Section 10.1.1. If additional cleanup is required, or if particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery for each compound of interest is no less than 85%.

10.3 Liquid chromatography analysis.

10.3.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are the estimated retention times and estimated detection limit that can be achieved by this method. An example of the separation achieved by Column 2 is shown in Figure 1. Other columns, chromatographic conditions, or detectors may be used if data quality comparable to Table 2 is achieved.

10.3.2 Calibrate the system daily as described in Section 8.

10.3.3 Inject 100 μ L of the sample extract. Monitor the column eluent at 340 nm. Record the resulting peak size in area or peak height units.

10.3.4 The retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

10.3.5 If the response for the peak exceeds the working range of the system, dilute the sample with mobile phase and reanalyze.

10.3.6 If the measurement of the peak response is prevented by the presence of interferences, additional cleanup is required.

11. CALCULATIONS

11.1 Determine the concentration of bentazon in the sample.

11.1.1 Calculate the amount of bentazon injected from the peak response using the calibration curve. The concentration in the sample can be calculated from the equation:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_s)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_s = Volume of water extracted, in mL

- 11.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

12. METHOD PERFORMANCE

- 12.1 The EDL and associated chromatographic conditions for bentazon are listed in Table 1.⁵ The EDL is defined as the minimum response being equal to 5 times the background noise, assuming a 5-mL final extract volume of a 1-L sample and an HPLC injection volume of 100 μL .
- 12.2 Single-operator accuracy and precision studies were conducted by Environmental Science and Engineering, Inc.,⁶ in the designated matrices. The results of these studies are presented in Table 2.

References

1. ASTM Annual Book of Standards, Vol. 11.02, D3694, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, Pennsylvania, 1986.
2. "Carcinogens - Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.
3. "OSHA Safety and Health Standards, General Industry" (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976).
4. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
5. ASTM Annual Book of Standards, Vol. 11.01, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, Pennsylvania, 1986.
6. Test procedures for Pesticides in Wastewaters, EPA Contract Report No. 68-03-2897 (In Preparation). Unpublished report available from U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.
7. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory - Cincinnati, Ohio 45268, March 1979.
8. "Evaluation of Ten Pesticide Methods," U.S. Environmental Protection Agency Contract No. 68-03-1760, Task No. 11, U.S. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

Table 1. Liquid Chromatography of Bentazon

<i>Parameter</i>	<i>Retention Time (min)</i>		<i>Estimated Detection Limit ($\mu\text{g/L}$)</i>
	<i>Column 1</i>	<i>Column 2</i>	
Bentazon	7.9	4.3	1.1

HPLC Column 1: Reverse-phase column, 250 mm long by 4.6 mm ID, stainless steel, packed with 10 μ Ultrasphere ODS or equivalent. Isocratic elution with 35% methanol/65% buffer; flow rate 2.0 mL/min.

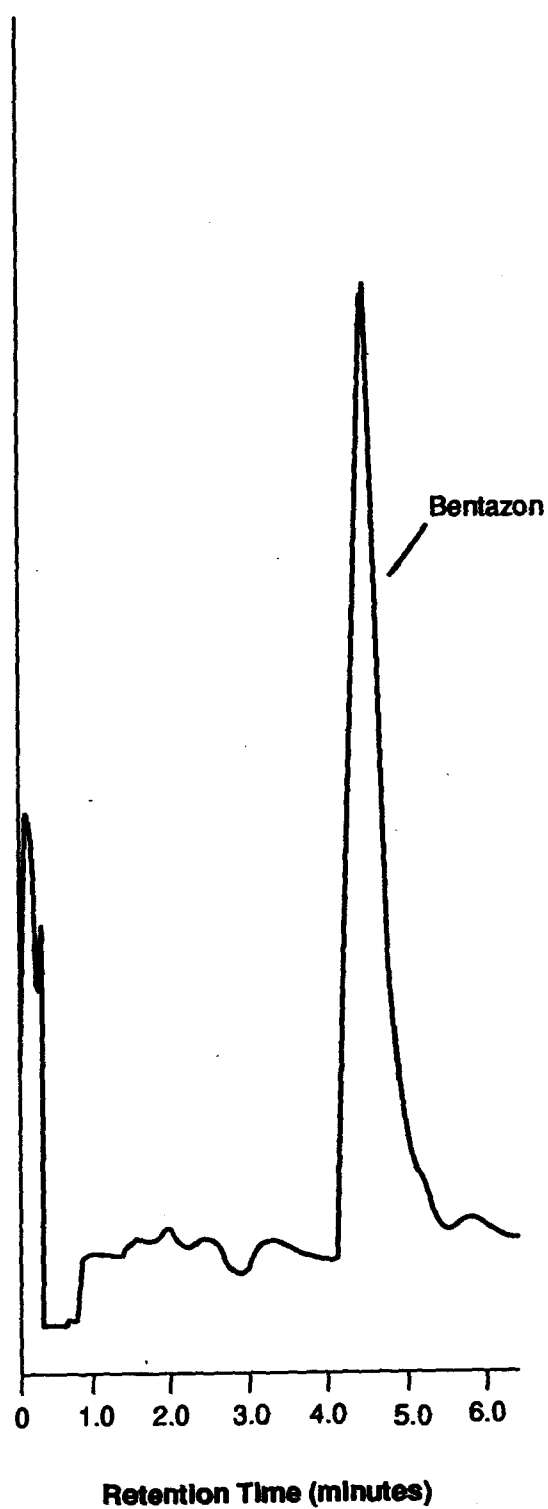
HPLC Column 2: Reverse-phase column, 300 mm long by 4 mm ID, stainless steel, packed with a μ Bondapak C18, 10 μ , Waters Associates or equivalent. Linear gradient elution of 40% methanol/60% buffer to 52% methanol/48% buffer over 9 minutes; flow rate 1 mL/min.

Table 2. Single-Laboratory Accuracy and Precision

<i>Parameter</i>	<i>Matrix Type*</i>	<i>Range $\mu\text{g/L}$</i>	<i>No. of Replicates</i>	<i>Average % Recovery</i>	<i>Standard Deviation (%)</i>
Bentazon	1	125	7	85.1	4.8
	2	20,400	7	88.4	8.4

*1 = 50% industrial effluent + 50% POTW effluent

2 = 100% industrial effluent



A52-002-70

Figure 1. HPLC of Bentazon (Column 2).

Method 644
The Determination of
Picloram in Municipal and
Industrial Wastewater

Method 644

The Determination of Picloram in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of picloram in municipal and industrial wastewater.

<i>Parameter</i>	<i>CAS No.</i>
Picloram	1918-02-1

- 1.2 The estimated detection limit (EDL) for picloram is listed in Table 1. The EDL was calculated from the minimum detectable response being equal to 5 times the background noise using a 100- μ L injection. The EDL for a specific wastewater may be different depending on the nature of interferences in the sample matrix.
- 1.3 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of picloram in municipal and industrial discharges. When this method is used to analyze unfamiliar samples for picloram, compound identification should be supported by at least one additional qualitative technique.
- 1.4 This method is restricted to use by or under the supervision of analysts experienced in the operation of liquid chromatographs and in the interpretation of liquid chromatograms.

2. SUMMARY OF METHOD

- 2.1 Picloram is removed from the acidified sample matrix by extraction with methylene chloride. The extract is dried, exchanged to HPLC mobile phase, and analyzed by HPLC with ultraviolet (UV) detection. An alkaline back-extraction is used as necessary to eliminate interferences which may be encountered.

3. INTERFERENCES

- 3.1 Solvent, reagents, glassware, and other sample-processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of liquid chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 9.1.
- 3.1.1 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.1.2 Glassware must be scrupulously cleaned.¹ Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap water and reagent water. It should then be drained dry and heated in a muffle furnace at 400°C for 15 to 30 minutes. Solvent rinses with acetone and pesticide-quality hexane may be substituted for the heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware

should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store the glassware inverted or capped with aluminum foil.

- 3.2 Matrix interferences may be caused by UV-active contaminants that coelute with picloram. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. While general cleanup procedures are provided as part of this method, unique samples may require additional cleanup approaches to achieve the detection limit listed in Table 1.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified^{2,4} for the information of the analyst.

5. APPARATUS AND EQUIPMENT

- 5.1 Sample containers: Narrow-mouth glass bottles, 1-L or 1-quart volume, equipped with polytetrafluoroethylene (PTFE)-lined screw-caps. Wide-mouth glass bottles, 1-quart volume, equipped with PTFE-lined screw-caps may also be used. Prior to use, wash bottles and cap liners with detergent and rinse with tap and reagent water. Allow the bottles and cap liners to air dry, then muffle at 400°C for 1 hour. After cooling, rinse the bottles and cap liners with hexane, seal the bottles, and store in a dust-free environment.
- 5.1.1 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.
- 5.2 Kuderna-Danish (K-D) glassware.
- 5.2.1 Synder column: Three-ball macro (Kontes K-503000-0121 or equivalent) and two-ball micro (Kontes K-569001-0219 or equivalent).
- 5.2.2 Concentrator tube: 10-mL, graduated (Kontes K-570050-1025 or equivalent) with ground-glass stopper.
- 5.2.3 Evaporative flask: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.
- 5.3 High performance liquid chromatography (HPLC) apparatus: Analytical system complete with liquid chromatograph and all required accessories including syringes, analytical columns, and

mobile phases. The system must be compatible with the specified detector and strip-chart recorder. A data system is recommended for measuring peak areas.

- 5.3.1 Isocratic pumping system, constant flow.
- 5.3.2 Injector valve (Rheodyne 7125 or equivalent) with 100- μ L loop.
- 5.3.3 Column: 250 mm long by 4.6 mm ID, stainless steel, packed with reverse-phase Ultrasphere ODS, 10 μ .
- 5.3.4 Ultraviolet detector, variable wavelength, capable of monitoring at 225 nm.
- 5.3.5 Strip-chart recorder compatible with detector, 250 mm. (A data system for measuring peak areas is recommended.)
- 5.4 Chromatographic column: 300 mm long by 10 mm ID Chromaflex, equipped with coarse-fritted bottom plate and PTFE stopcock. (Kontes K-420540-0213 or equivalent).
- 5.5 Drying column: Approximately 400 mm long by 20 mm ID borosilicate glass, equipped with coarse-fritted bottom plate.
- 5.6 Miscellaneous.
 - 5.6.1 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
 - 5.6.2 Separatory funnels: 2-L, 500-mL, and 250-mL, equipped with PTFE stopcocks.
 - 5.6.3 Boiling chips: Approximately 10/40 mesh. Heat to 400°C for 30 minutes or perform a Soxhlet extraction with methylene chloride for 2 hours.
 - 5.6.4 Water bath: Heated with concentric ring cover, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.
 - 5.6.5 Pasteur pipettes and bulbs.
 - 5.6.6 Standard solution storage containers: 15-mL bottles with PTFE-lined screw-caps.

6. REAGENTS AND CONSUMABLE MATERIALS

- 6.1 Reagents.
 - 6.1.1 Acetone, hexane, methanol, and methylene chloride: Demonstrated to be free of analytes and interferences.
 - 6.1.2 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
 - 6.1.3 Sodium hydroxide (NaOH) solution (0.3N): Dissolve 12 g NaOH in reagent water and dilute to 1000 mL.
 - 6.1.4 Sodium sulfate: Granular, anhydrous. Condition by heating at 400°C for 4 hours in a shallow tray.
 - 6.1.5 Sodium chloride: ACS, crystals.
 - 6.1.6 Sulfuric acid (H_2SO_4) solution (1+1): Add a measured volume of concentrated H_2SO_4 to an equal volume of reagent water.
 - 6.1.7 HPLC buffer (pH 2, 0.1M phosphate): Dissolve 5.83 g of KH_2PO_4 (ACS) and 3.9 mL of 85% phosphoric acid (ACS) in 1 L of reagent water.

6.1.8 HPLC mobile phase: Add 570 mL of HPLC buffer solution to a 1-L volumetric flask and dilute to volume with methanol.

6.2 Standard stock solutions (1.00 $\mu\text{g}/\mu\text{L}$): These solutions may be purchased as a certified solution or prepared from the pure standard material using the following procedures.

6.2.1 Prepare the stock standard solution by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide-quality methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is certified at 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.

6.2.2 Transfer the stock standard to a PTFE-sealed screw-cap bottle. Store at 4°C and protect from light. The stock standard should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from it.

6.2.3 The stock standard must be replaced after 6 months, or when comparison with a quality control check sample indicates a problem.

7. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

7.1 Collect all samples in duplicate. Grab samples must be collected in glass containers. Conventional sampling practices⁵ should be followed, except that the bottle must not be prewashed with sample before collection.

7.2 The samples must be iced or refrigerated at 4°C from the time of collection until analysis. Chemical preservatives should not be used in the field unless more than 24 hours will elapse before delivery to the laboratory. If the samples will not be analyzed within 48 hours of collection, the sample should be adjusted to a pH range of 1.0 to 3.0 with sodium hydroxide or sulfuric acid.

7.3 All samples must be extracted within 7 days of collection and analyzed within 30 days of extraction.⁶

8. CALIBRATION

8.1 Establish liquid chromatographic operating parameters equivalent to those indicated in Table 1.

8.2 Prepare calibration standards at a minimum of three concentration levels of picloram by adding volumes of the stock standard to a volumetric flask and diluting to volume with HPLC mobile phase. One of the standards should be at a concentration near, but greater than, the EDL, and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

8.3 Using injections of 100 μL of each calibration standard, tabulate peak height or area response against the mass injected. The results are used to prepare a calibration curve for picloram. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (<10% relative standard deviation), linearity of the calibration curve can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

- 8.4** The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for picloram varies from the predicted response by $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or factor must be prepared.

9. QUALITY CONTROL

9.1 Monitoring for interferences.

- 9.1.1** Analyze a laboratory reagent blank each time a set of samples is extracted. A laboratory reagent blank is an aliquot of reagent water. If the reagent blank contains a reportable level of picloram, immediately check the entire analytical system to locate and correct for possible interferences and repeat the test.

9.2 Assessing accuracy.

- 9.2.1** After every ten samples, and preferably in the middle of each day, analyze a laboratory control standard. Calibration standards may not be used for accuracy assessments and the laboratory control standard may not be used for calibration of the analytical system.
- 9.2.1.1** Laboratory control standard concentrate: From the stock standard prepared as described in Section 6.3, prepare a laboratory control standard concentrate that contains picloram at a concentration of $2 \mu\text{g/mL}$ in methanol or other suitable solvent.⁷
- 9.2.1.2** Laboratory control standard: Using a pipette add 1.0 mL of the laboratory control standard concentrate to a 1-L aliquot of reagent water.
- 9.2.1.3** Analyze the laboratory control standard as described in Section 10. Calculate the percent recovery (P_i) with the equation:

Equation 1

$$P_i = \frac{100S_i}{T_i}$$

where

S_i = The analytical results from the laboratory control standard, in $\mu\text{g/L}$

T_i = The known concentration of the spike, in $\mu\text{g/L}$

- 9.2.2** At least annually, the laboratory should participate in formal performance evaluation studies, where solutions of unknown concentrations are analyzed and the performance of all participants is compared.⁷

9.3 Assessing precision.

- 9.3.1** Precision assessments for this method are based upon the analysis of field duplicates (Section 7.1). Analyze both sample bottles for at least 10% of all samples. To the extent practical, the samples for duplication should contain reportable levels of picloram.
- 9.3.2** Calculate the relative range⁷ (RR_i) with the equation:

Equation 2

$$RR_i = \frac{100R_i}{X_i}$$

where

R_i = the absolute difference between the duplicate measurements X_1 and X_2 , in $\mu\text{g/L}$

X_i = the average concentration found $\left(\frac{X_1 + X_2}{2} \right)$, in $\mu\text{g/L}$

9.3.3 Individual relative range measurements are pooled to determine average relative range or to develop an expression of relative range as a function of concentration.

10. PROCEDURE

10.1 Sample extraction.

- 10.1.1** Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel. Check the pH of the sample with wide-range pH paper and adjust to within the range of 1.5 to 2.5 with sulfuric acid. Add 200 g of sodium chloride and mix to dissolve.
- 10.1.2** Add 60 mL of methylene chloride to the sample bottle and shake for 30 seconds to rinse the walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release vapor pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends on the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation. Collect the extract in a 250-mL Erlenmeyer flask.
- 10.1.3** Add an additional 60-mL volume of methylene chloride to the sample bottle and complete the extraction procedure a second time, combining the extracts in the Erlenmeyer flask.
- 10.1.4** Perform a third extraction in the same manner. Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate. If the extract requires cleanup, collect the extract in a 500-mL separatory funnel and proceed to Section 10.2 (cleanup and separation). If the extract does not require cleanup, collect the extract in a 500-mL K-D flask equipped with a 10-mL concentrator tube and proceed with Sections 10.1.5 and 10.1.6.
- 10.1.5** Add one or two clean boiling chips to the flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (80 to 85°C) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed in steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 5 mL, remove the K-D apparatus and allow

it to drain and cool for at least 10 minutes. Add 50 mL of methanol and a clean boiling chip to the flask and repeat the concentration as described above. When the apparent volume of the liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methanol. A 5-mL syringe is recommended for this operation.

10.1.6 Add a clean boiling chip to the concentrator tube. Attach a two-ball micro-Snyder column. Prewet the micro-Snyder column by adding about 0.5 mL of methanol to the top. Place the micro K-D apparatus on a hot water bath (80 to 85°C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation, the balls will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a small volume of methanol. Quantitatively transfer the extract to a 25-mL volumetric flask by means of a Pasteur pipette or other suitable device. Rinse the concentrator tube with about 0.5 mL of methanol and add to the volumetric flask. Adjust the final volume to 25 mL or to a volume suitable for liquid chromatography with HPLC mobile phase. Store refrigerated if further processing will not be performed immediately. Proceed with liquid chromatographic analysis.

10.1.7 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1,000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

10.2 Cleanup and separation.

10.2.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various clean waters and municipal effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than that recorded in Table 2.

10.2.2 Collect the dried extracts from Section 10.1.4 in a 500-mL separatory funnel. Add 10 mL of 0.3N NaOH and extract by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow a 10-minute separation time. Drain the methylene chloride and discard. Allow 2 minutes for the aqueous layer to drain from the walls, and collect it in a 25-mL volumetric flask.

10.2.3 Adjust the pH of the aqueous extract to 1.5 to 2.5 with sulfuric acid solution, and dilute to volume with HPLC mobile phase.

10.2.4 Proceed with liquid chromatography as described in Section 10.3.

10.3 Liquid chromatography analysis.

10.3.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are the estimated retention time and estimated detection limit that can be achieved by this method. An example of the separation achieved by this column

is shown in Figure 1. Figure 2 is a chromatogram of picloram in a POTW wastewater sample. Other columns, chromatographic conditions, or detectors may be used if data quality comparable to Table 2 is achieved.

10.3.2 Calibrate the system daily as described in Section 8.

10.3.3 Inject 100 μL of the sample extract. Monitor the column eluent at 225 nm. Record the resulting peak size in area or peak height units.

10.3.4 The retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

10.3.5 If the response for the peak exceeds the working range of the system, dilute the sample with mobile phase and reanalyze.

10.3.6 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

11. CALCULATIONS

11.1 Determine the concentration of picloram in the sample.

11.1.1 Calculate the amount of picloram injected from the peak response using the calibration curve or calibration factor in Section 8.2.2. The concentration in the sample can be calculated from the equation:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_e)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_e = Volume of water extracted, in mL

11.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

12. METHOD PERFORMANCE

12.1 The EDL and associated chromatographic conditions for picloram are listed in Table 1.⁸ The EDL is defined as the minimum response being equal to 5 times the background noise, using a 100- μL injection.

12.2 Single-operator accuracy and precision studies were conducted by Environmental Science and Engineering, Inc.,⁶ in the designated matrix. The results of these studies are presented in Table 2.

References

1. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, Pennsylvania, p. 679., 1980.
2. "Carcinogens - Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.
3. "OSHA Safety and Health Standards, General Industry" (29 *CFR* 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976).
4. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
5. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, Pennsylvania, p. 76 1980.
6. Test procedures for Pesticides in Wastewaters, EPA Contract Report 68-03-2897 Unpublished report, available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.
7. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory - Cincinnati, Ohio, March 1979.
8. "Evaluation of Ten Pesticide Methods," U.S. Environmental Protection Agency, Contract No. 68-03-1760, Task No. 11, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

Table 1. Liquid Chromatography of Picloram*

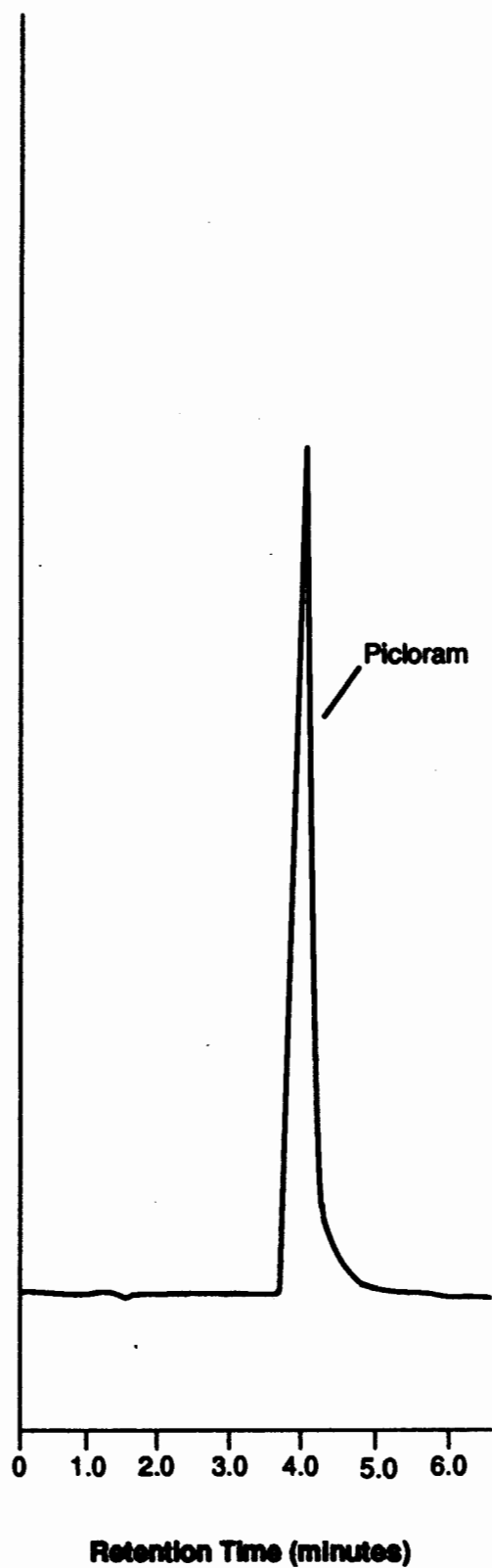
<i>Compound</i>	<i>Retention Time (min)</i>	<i>Estimated Detection Limit (µg/L)</i>
Picloram	4.0	0.3

*HPLC conditions: Reverse-phase 10 µm Ultrasphere ODS, 4.6 mm ID by 250 mm long column; isocratic elution; flow rate 1 mL/min. Mobile Phase: 57% HPLC Buffer solution in methanol (V/V).

Table 2. Single-Operator Accuracy and Precision

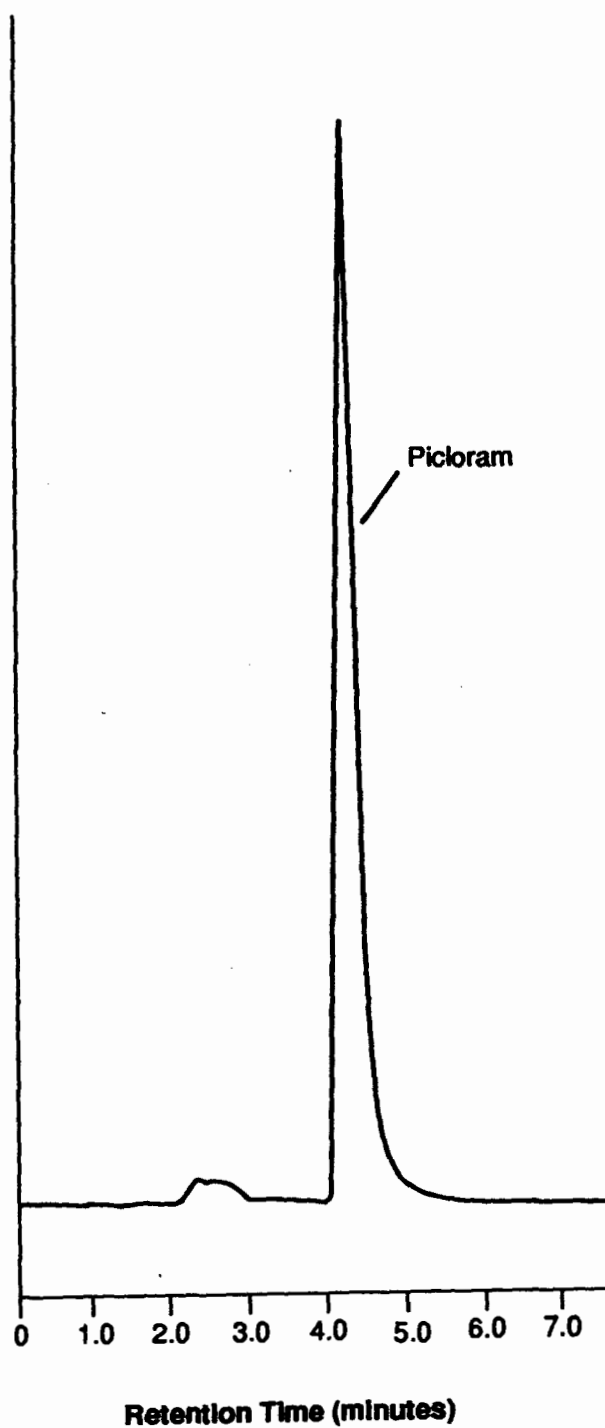
<i>Parameter</i>	<i>Matrix Type*</i>	<i>Spike Range (µg/L)</i>	<i>Number of Replicates</i>	<i>Average Percent Recovery</i>	<i>Standard Deviation (%)</i>
Picloram	1	25	7	93.9	9.1
	1	778	7	79.0	7.7

*1 = Municipal effluent



A52-002-71

Figure 1. HPLC of Picloram



A52-002-72

Figure 2. HPLC of Picloram in Wastewater Extract

Method 645
***The Determination of Certain
Amine Pesticides and Lethane
in Municipal and Industrial
Wastewater***

Method 645

The Determination of Certain Amine Pesticides and Lethane in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1** This method covers the determination of certain amine pesticides and lethane in municipal and industrial wastewater. The following parameters may be determined by this method.

<i>Parameter</i>	<i>CAS No.</i>
Alachlor	15972-60-8
Butachlor	23184-66-9
Diphenamid	957-51-7
Fluridone	59756-60-4
Lethane	112-56-1
Norflurazon	27314-13-2

- 1.2** The estimated detection limit (EDL) for each parameter is listed in Tables 1 and 2. The EDL was calculated from the minimum detectable response of the nitrogen/phosphorous detector equal to 5 times the gas chromatographic (GC) background noise assuming a 10 mL final extract volume of a 1-L reagent water sample and a GC injection of 5 μ L. The EDL for a specific wastewater may be different depending on the nature of interferences in the sample matrix.
- 1.3** This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges. When this method is used to analyze unfamiliar samples for any or all of the compounds listed above, compound identifications should be supported by at least one additional qualitative technique. Section 13 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative confirmation of compound identifications.
- 1.4** This method is restricted to use by or under the supervision of analysts experienced in the operation of gas chromatographs and in the interpretation of chromatograms.

2. SUMMARY OF METHOD

- 2.1** The amine pesticides and lethane are removed from the sample matrix by extraction with methylene chloride. The extract is dried, exchanged into hexane, and analyzed by gas chromatography. Column chromatography is used as necessary to eliminate interferences which may be encountered. Measurement of the pesticides is accomplished with a nitrogen/phosphorous specific detector.

- 2.2 Confirmatory analysis by gas chromatography/mass spectrometry is recommended (Section 13) when a new or undefined sample type is being analyzed if the concentration is adequate for such determination.

3. INTERFERENCES

- 3.1 Solvent, reagents, glassware, and other sample-processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 9.1

3.1.1 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.1.2 Glassware must be scrupulously cleaned.¹ Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap water and reagent water. It should then be drained dry and heated in a muffle furnace at 400°C for 15 to 30 minutes. Solvent rinses with acetone and pesticide-quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store the glassware inverted or capped with aluminum foil.

- 3.2 Interferences co-extracted from the samples will vary considerably from source to source, depending on the diversity of the industrial complex or municipality being sampled. While general cleanup procedures are provided as part of this method, unique samples may require additional cleanup approaches to achieve the detection limits listed in Tables 1 and 2.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified^{2,4} for the information of the analyst.

5. APPARATUS AND EQUIPMENT

- 5.1 Sample containers: Narrow-mouth glass bottles, 1-L or 1-quart volume, equipped with polytetrafluoroethylene (PTFE)-lined screw-caps. Wide-mouth glass bottles, 1-quart volume, equipped with PTFE-lined screw-caps may also be used. Prior to use, wash bottles and cap liners with detergent and rinse with tap and distilled water. Allow the bottles and cap liners to air dry, then muffle at 400°C for 1 hour. After cooling, rinse the bottles and cap liners with hexane, seal the bottles, and store in a dust-free environment.

5.1.1 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 Kuderna-Danish (K-D) glassware.

5.2.1 Synder column: Three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.2 Concentrator tube: 10-mL, graduated (Kontes K-570050-1025 or equivalent) with ground-glass stopper.

5.2.3 Evaporative flask: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.3 Gas chromatography system.

5.3.1 The gas chromatograph must be equipped with a glass-lined injection port compatible with the detector to be used. A data system is recommended for measuring peak areas.

5.3.1.1 Column 1: 180 cm long by 2 mm ID, glass, packed with 10% OV-11 on Gas Chrom W-HP (100/120 mesh) or equivalent.

5.3.1.2 Column 2: 180 cm long by 2 mm ID, Pyrex[®] glass, packed with 3% Dexsil 300 on Chromasorb W-HP (80/100 mesh) or equivalent.

5.3.1.3 Column 3: 180 cm long by 2mm ID Glass, packed with 3% SP-2100 on Supelcoport (100/120 mesh) or equivalent.

5.3.1.4 Column 1 was used to develop the accuracy and precision statements in Section 12. Guidelines for the use of alternative column packings are provided in Section 10.3.1.

5.3.1.5 Detector: Nitrogen/phosphorous. This detector has proven effective in the analysis of wastewaters for the parameters listed in Section 1.1 and was used to develop the method performance statements in Section 12. Guidelines for the use of alternate detectors are provided in Section 10.3.

5.4 Chromatographic column: 300 mm long by 10 mm ID Chromaflex, equipped with coarse-fritted bottom plate and PTFE stopcock. (Kontes K-420540-0213 or equivalent).

5.5 Drying column: Approximately 400 mm long by 20 mm ID borosilicate glass, equipped with coarse-fritted bottom plate.

5.6 Miscellaneous.

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

5.6.2 Separatory funnel: 2-L, equipped with PTFE stopcock.

5.6.3 Water bath: Heated with concentric ring cover, capable of temperature control ($\pm 2^\circ\text{C}$). The bath should be used in a hood.

5.6.4 Standard solution storage containers: 15-mL bottles with PTFE-lined screw-caps.

- 5.6.5 Boiling chips: Approximately 10/40 mesh. Heat to 400°C for 30 minutes or perform a Soxhlet extraction with methylene chloride.

6. REAGENTS AND CONSUMABLE MATERIALS

6.1 Reagents.

- 6.1.1 Acetone, hexane, and methylene chloride: demonstrated to be free of analytes.
- 6.1.2 Florisil: PR grade (60/100 mesh). Purchase activated at 675°C and store in glass containers with glass stoppers or foil-lined screw-caps. Before use, activate each batch overnight at 200°C in foil-covered glass containers. To prepare for use, place the amount necessary for the number of columns to be run in a 500-mL reagent bottle and add 2% by weight of reagent water. Seal and mix thoroughly by shaking or rolling for 10 minutes. Allow to stand for at least 2 hours prior to use. The mixture must be homogeneous. Keep the bottle tightly sealed to ensure proper activity.
- 6.1.3 Reagent water: reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.1.4 Sodium hydroxide (NaOH) solution (10N): Dissolve 40 g NaOH in reagent water and dilute to 100 mL.
- 6.1.5 Sodium sulfate: Granular, anhydrous. Condition by heating at 400°C for 4 hours in a shallow tray.
- 6.1.6 Sulfuric acid (H₂SO₄) solution (1+1): Add a measured volume of concentrated H₂SO₄ to an equal volume of reagent water.
- 6.1.7 Sodium thiosulfate: ACS, granular.

6.2 Standard stock solutions (1.00 µg/µL): These solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedures.

- 6.2.1 Prepare standard stock solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in hexane or other suitable solvent and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the standard stock.
- 6.2.2 Store standard stock solutions at 4°C in 15-mL bottles equipped with PTFE-lined screw-caps. Standard stock solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
- 6.2.3 Standard stock solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

- 7.1 Collect all samples in duplicate. Grab samples must be collected in glass containers. Conventional sampling practices⁵ should be followed, except that the bottle must not be prewashed with sample before collection.
- 7.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

- 7.3 Chemical preservatives should not be used in the field unless more than 24 hours will elapse before delivery to the laboratory. If the samples will not be extracted within 48 hours of collection, the sample should be adjusted to a pH range of 6.0 to 8.0 with sodium hydroxide or sulfuric acid.
- 7.4 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction⁶.

8. CALIBRATION AND STANDARDIZATION

8.1 Calibration.

- 8.1.1 A set of at least three calibration solutions containing the method analytes is needed. One calibration solution should contain each analyte at a concentration approaching but greater than the estimated detection limit (Tables 1 and 2) for that compound; the other two solutions should contain analytes at concentrations that bracket the range expected in samples. For example, if the detection limit for a particular analyte is 0.2 µg/L, and a sample expected to contain approximately 5 µg/L is analyzed, solutions of standards should be prepared at concentrations representing 0.3 µg/L, 5 µg/L, and 10 µg/L for the particular analyte.
- 8.1.2 To prepare a calibration solution, add an appropriate volume of a standard stock solution to a volumetric flask and dilute to volume with hexane.
- 8.1.3 Starting with the standard of lowest concentration, analyze each calibration standard according to Section 10.3 and tabulate peak height or area response versus the mass of analyte injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to concentration (calibration factor) is a constant over the working range (< 10% relative standard deviation), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.
- 8.1.4 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any analyte varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a new calibration curve.

9. QUALITY CONTROL

9.1 Monitoring for interferences.

- 9.1.1 Analyze a laboratory reagent blank each time a set of samples is extracted. A laboratory reagent blank is a 1-L aliquot of reagent water. If the reagent blank contains a reportable level of any analyte, immediately check the entire analytical system to locate and correct for possible interferences and repeat the test.

9.2 Assessing accuracy.

- 9.2.1 After every ten samples, and preferably in the middle of each day, analyze a laboratory control standard. Calibration standards may not be used for accuracy assessments and the laboratory control standard may not be used for calibration of the analytical system.

- 9.2.1.1** Laboratory control standard concentrate: From stock standards prepared as described in Section 6.3, prepare a laboratory control standard concentrate that contains each analyte of interest at a concentration of 2 µg/mL in acetone or other suitable solvent.
- 9.2.1.2** Laboratory control standard: Using a pipette, add 1.00 mL of the laboratory control standard concentrate to a 1-L aliquot of reagent water.
- 9.2.1.3** Analyze the laboratory control standard as described in Section 10. For each analyte in the laboratory control standard, calculate the percent recovery (P_i) with the equation:

Equation 1

$$P_i = \frac{100S_i}{T_i}$$

where

S_i = The analytical results from the laboratory control standard, in µg/L

T_i = The known concentration of the spike, in µg/L

- 9.2.2** At least annually, the laboratory should participate in formal performance evaluation studies, where solutions of unknown concentrations are analyzed and the performance of all participants is compared.

9.3 Assessing precision.

- 9.3.1** Precision assessments for this method are based upon the analysis of field duplicates (Sect. 7.1). Analyze both sample bottles for at least 10% of all samples. To the extent practical, the samples for duplication should contain reportable levels of most of the analytes.
- 9.3.2** For each analyte in each duplicate pair, calculate the relative range⁷ (RR_i) with the equation:

Equation 2

$$RR_i = \frac{100R_i}{X_i}$$

where

R_i = The absolute difference between the duplicate measurements X_1 and X_2 , in µg/L

X_i = The average concentration found $\left[\frac{X_1 + X_2}{2} \right]$, in µg/L

- 9.3.3** Individual relative range measurements are pooled to determine average relative range or to develop an expression of relative range as a function of concentration.

10. PROCEDURE

10.1 Sample extraction.

- 10.1.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-liter separatory funnel. Check the pH of the sample with wide-range pH paper and adjust to within the range of 5 to 9 with sodium hydroxide or sulfuric acid.
- 10.1.2 Add 60 mL of methylene chloride to the sample bottle and shake for 30 seconds to rinse the walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release vapor pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends on the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation. Collect the extract in a 250-mL Erlenmeyer flask.
- 10.1.3 Add an additional 60-mL volume of methylene chloride to the sample bottle and complete the extraction procedure a second time, combining the extracts in the Erlenmeyer flask.
- 10.1.4 Perform a third extraction in the same manner. Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect it in a 500-mL K-D flask equipped with a 10 mL concentrator tube. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.
- 10.1.5 Add one or two clean boiling chips to the flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (80 to 85°C) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed in steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride.
- 10.1.6 For Florisil[®] column cleanup or gas chromatography, the extract must be in hexane solution. To exchange the solvent to hexane, add one or two fresh boiling chips to the flask and ampule containing the extract, add 50 mL of hexane, and reattach the Snyder column. Pour about 1 mL of hexane into the top of the Snyder column, and concentrate the extract at 85 to 95°C in the hot water bath as above. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.
- 10.1.7 Remove the Snyder column, rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane. A 5-mL syringe is recommended for this operation. Dilute to 10 mL with hexane for analysis by gas chromatography (Section 10.3) if cleanup is not required. If the extract requires cleanup, proceed to Section 10.2. If the extracts will

be stored longer than 2 days, they should be transferred to PTFE-sealed screw-cap bottles. Proceed with gas chromatographic analysis.

- 10.1.8 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1,000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

10.2 Cleanup and separation.

- 10.2.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of alachlor, butachlor, diphenamid, and lethane in various clean waters and municipal effluents. The use of Florisil[®] as the cleanup material for fluridone and norflurazon has been demonstrated to yield recoveries of less than 50%, and is not recommended as a cleanup material for these compounds. Use of specific detectors may obviate the necessity for cleanup of relatively clean sample matrices. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.

- 10.2.2 Place the necessary amount of deactivated Florisil[®] into a 20 mm ID chromatographic column and tap the column to settle the Florisil[®]. Add 1 to 2 cm of anhydrous sodium sulfate to the top of the Florisil[®].

- 10.2.3 Pre-elute the column with 50 to 60 mL of hexane. Discard the eluate and, just prior to exposure of the sodium sulfate layer to the air, transfer the sample extract onto the column by decantation. Complete the transfer by rinsing with two additional 2-mL volumes of hexane. Alternatively, a measured aliquot of the extract may be taken for cleanup.

- 10.2.4 Just prior to exposure of the sodium sulfate layer to the air, elute the column with 100 mL hexane. Discard the eluate and repeat the elution with 200 mL of 6% acetone in hexane (V/V). Collect the eluate in a 500-mL K-D flask equipped with a 10-mL concentrator tube (Fraction 1). All elutions should be carried out using a flow rate of about 5 mL/min.

- 10.2.5 Perform a second elution with 200 mL of 15% acetone in hexane (Fraction 2). Collect each fraction in a separate K-D apparatus. The elution pattern for these compounds is shown in Table 3.

- 10.2.6 Determine, from Table 3, the fractions of interest and concentrate by standard K-D technique, as indicated in Section 10.1.5, using hexane in place of methylene chloride, to a volume of 10 mL.

- 10.2.7 Analyze the fractions by gas chromatography.

10.3 Gas chromatography analysis.

- 10.3.1 Recommended columns and detectors and operating conditions for the gas chromatography system are described in Section 5.3. Tables 1 and 2 summarize the recommended operating conditions for the gas chromatograph. Included in these tables are retention times and estimated detection limits that can be achieved by this method. Examples of the separations achieved are shown in Figures 1 through 3. Other packed columns, chromatographic conditions, or detectors may be used if data quality

comparable to Table 4 is achieved. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and data quality comparable to Table 4 is achieved.

- 10.3.2** Inject 2 to 5 μL of the sample extract using the solvent-flush technique.⁸ Record the volume injected to the nearest 0.05 μL , the total extract volume, and the resulting peak size in area or peak height units.
- 10.3.3** The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of the day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 10.3.4** If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 10.3.5** If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

11. CALCULATIONS

- 11.1** Determine the concentration (C) of individual compounds in the sample in micrograms per liter with the equation:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_s)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_s = Volume of water extracted, in mL

- 11.2** Report the results for the unknown samples in micrograms per liter. Round off the results to the nearest 0.1 $\mu\text{g/L}$ or two significant figures.

12. METHOD PERFORMANCE

- 12.1** Estimated detection limits (EDL) and associated chromatographic conditions are listed in Table 1.⁹ The detection limits were calculated from the minimum detectable response of the EC detector equal to 5 times the GC background noise, assuming a 10-mL final extract volume of a 1-L sample and a GC injection of 5 μL .
- 12.2** Single-laboratory accuracy and precision studies were conducted by Environmental Science and Engineering,⁶ using spiked samples. The results of these studies are presented in Table 2.

13. GC/MS CONFIRMATION

- 13.1** It is recommended that GC/MS techniques be judiciously employed to support qualitative identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compounds of interest. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak, but not to exceed 7 scans per peak utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.
- 13.2** Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved. The calculation of tailing factors is illustrated in Method 625.¹⁰
- 13.3** At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all DFTPP performance criteria are achieved.¹¹
- 13.4** To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
- 13.4.1** The molecular ion and other ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to $\pm 10\%$. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20 to 40%.
- 13.4.2** The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.
- 13.4.3** Compounds that have similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.
- 13.5** Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.
- 13.6** Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternative packed or capillary GC columns or additional cleanup.

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11. Eichelberger, J.W., Harris, L.E., and Budde, W.L., *Anal. Chem.*, 46, 1912 (1975).

Table 1. Gas Chromatography and Detection Limits of Certain Amines and Lethane

<i>Parameter</i>	<i>Retention Time (min)</i>			<i>Estimated Detection Limit</i>
	<i>Column 1</i>	<i>Column 2</i>	<i>Column 3</i>	<i>(µg/L)</i>
Alachlor	6.9	—	—	0.2
Butachlor	10.5	—	—	0.3
Diphenamide	10.8	—	—	0.2
Fluridone	2.2	2.45	2.1	0.5
Lethane	2.0	—	—	0.1
Norflurazon	18.4	—	—	0.02

Column 1: 180 cm long by 2 mm ID glass, packed with 10% OV-11 on Gas Chrom W-HP, 100/120 mesh; nitrogen carrier gas at a flow rate of 30 mL/min. Column temperature is held at 225°C for 4 minutes after injection and then programmed to 275°C at 4°/min and held for 8 minutes.

Column 2: 180 cm long by 2 mm ID glass, packed with 3% Dexsil 300 on Chromasorb W-HP, 80/100 mesh; nitrogen carrier gas at a flow rate of 30 mL/min. Column temperature at 300°C isothermal.

Column 3: 180 cm long by 2 mm ID glass, packed with 3% SP-2100 on Supelcoport, 100/120 mesh; nitrogen carrier gas at a flow rate of 40 mL/min. Column temperature at 275°C isothermal.

Table 2. Single-Laboratory Accuracy and Precision

<i>Parameter</i>	<i>Matrix Type*</i>	<i>Spike Range (µg/L)</i>	<i>No. of Replicates</i>	<i>Average Percent Recovery</i>	<i>Relative Standard Deviation (%)</i>
Alachlor	1	255	7	113	9.0
	1	996	7	104	13.3
Butachlor	1	286	7	93.1	8.2
	1	1,420	7	92.8	4.3
Diphenamid	2	9.3	7	100	14.2
	3	740	7	98.8	7.0
Fluridone**	1	20.8	7	92.0	11.5
	1	998	7	88.4	11.4
Lethane	1	167	7	93.3	19.9
	1	576	7	97.6	29.4
Norlurazone**	1	243	7	89.5	7.4
	3	1,048	7	102	6.1

* 1 = Manufacturing effluent wastewaters.

2 = Manufacturing effluent wastewater + POTW effluent at a ratio of 1:200.

3 = Manufacturing effluent wastewater + POTW effluent at a ratio of 1:1.

** Florisil cleanup not employed.

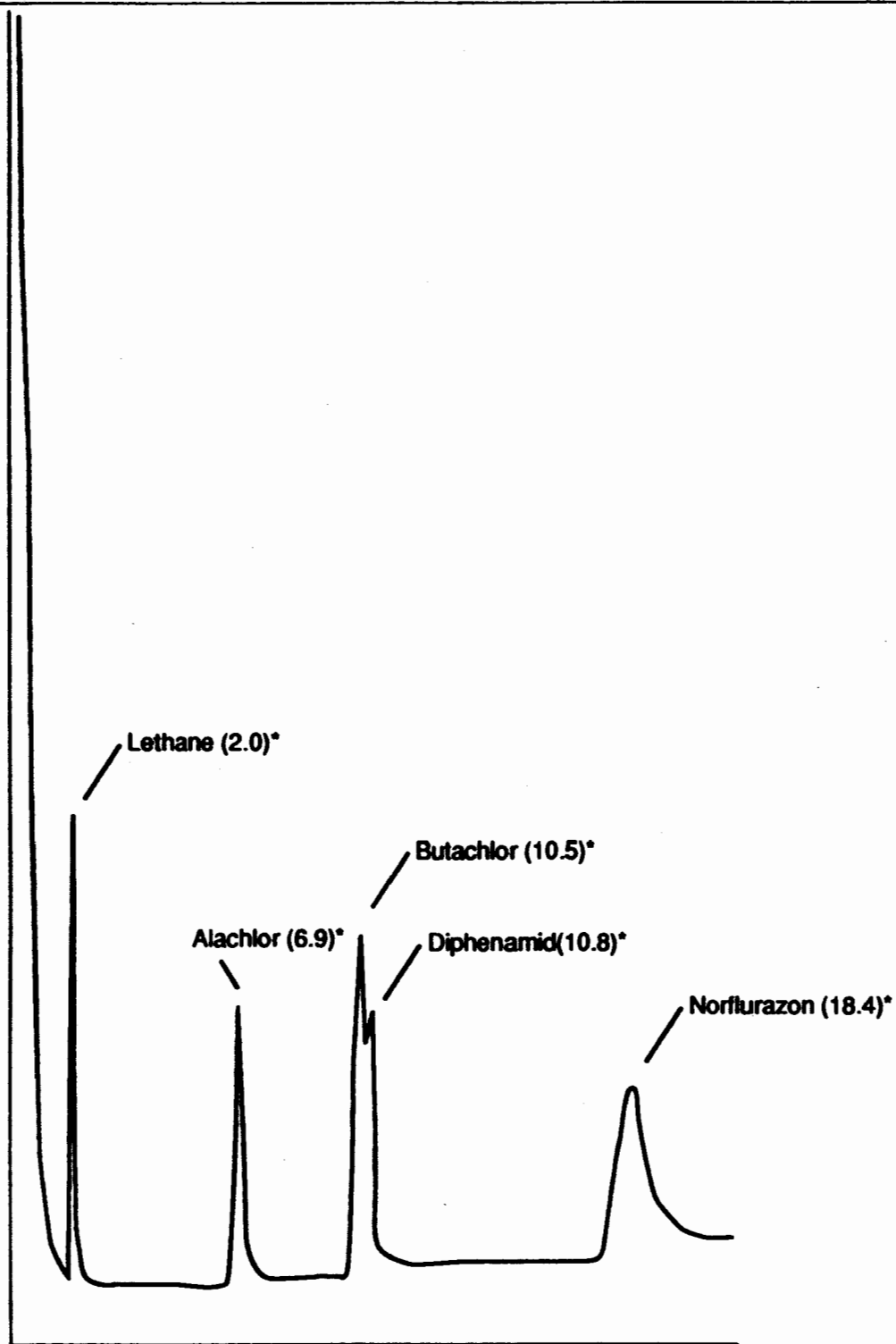
Table 3. Florisil® Cleanup Recoveries

<i>Solvent Fraction **</i>	<i>Alachlor</i>	<i>Average Percent Butachlor</i>	<i>Recoveries Diphenamid</i>	<i>Lethane</i>
1	103	95		106
2	ND	ND	96	ND

* 2% deactivated.

** 1 = 6% acetone/hexane

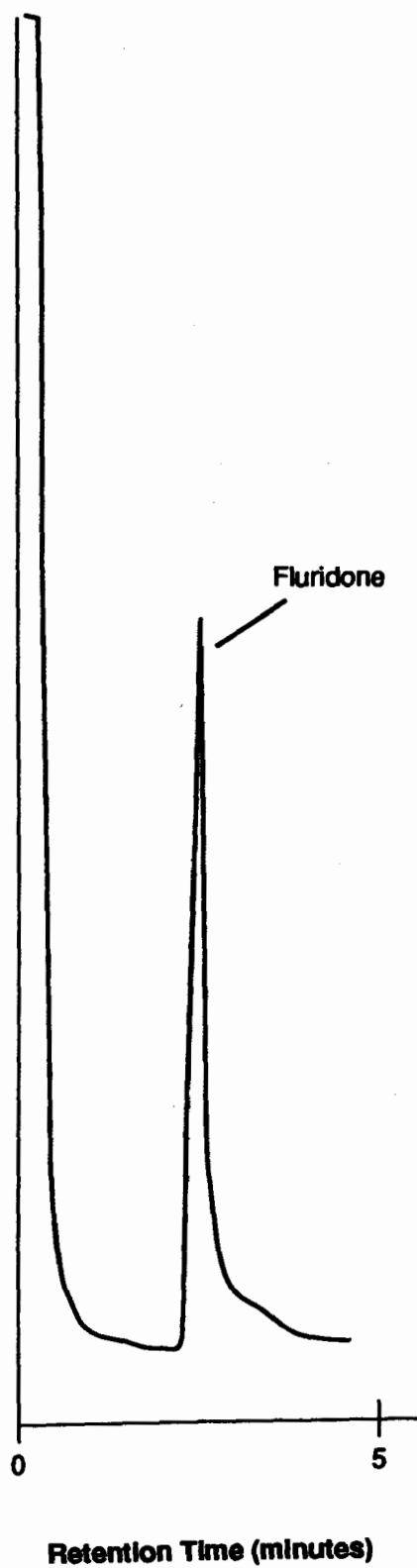
2 = 15% acetone/hexane



*Retention Time in parentheses

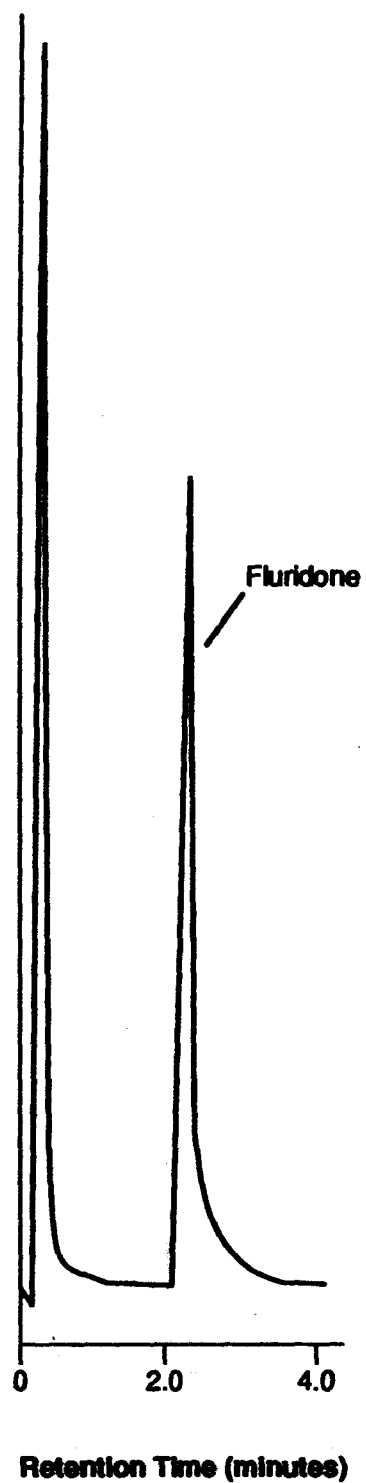
AS2-002-73

Figure 1. Gas Chromatogram of Amines/Lethane (Column 2).



A62-002-74

Figure 2. Gas Chromatogram of Fluridone (Column 2).



AS2-002-75

Figure 3. Gas Chromatogram of Fluridone (Column 3).

Method 646
The Determination of Dinitro
Aromatic Pesticides in
Municipal and Industrial
Wastewater

Method 646

The Determination of Dinitro Aromatic Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of certain dinitro aromatic pesticides in municipal and industrial wastewater. The following parameters may be determined by this method.

Parameter	CAS No.
Basalin (Fluchloralin)	33245-39-5
CDN	97-00-7
Dinocap	39300-45-3

- 1.2 The estimated detection limit (EDL) for each parameter is listed in Table 1. The EDL was calculated from the minimum detectable response of the electron capture detector (ECD) equal to 5 times the GC background noise assuming a 1.0 mL final extract volume of a 1-L reagent water sample and a GC injection of 5 μ L. The EDL for a specific wastewater may be different depending on the nature of interferences in the sample matrix.
- 1.3 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges. When this method is used to analyze unfamiliar samples for any or all of the compounds listed above, compound identifications should be supported by at least one additional qualitative technique. Section 13 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative confirmation of compound identifications.
- 1.4 This method is restricted to use by or under the supervision of analysts experienced in the operation of gas chromatographs and in the interpretation of chromatograms.

2. SUMMARY OF METHOD

- 2.1 Dinitroaromatic pesticides are removed from the sample matrix by extraction with 15% methylene chloride in hexane. The extract is dried, exchanged into hexane, and analyzed by gas chromatography (GC). Column chromatography is used as necessary to eliminate interferences which may be encountered. Measurement of the pesticides is accomplished with an electron capture detector.
- 2.2 Confirmatory analysis by gas chromatography/mass spectrometry (GC/MS) is recommended (Section 13) when a new or undefined sample type is being analyzed, if the concentration is adequate for such determination.

3. INTERFERENCES

- 3.1 Solvent, reagents, glassware, and other sample-processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 9.1.

- 3.1.1 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.
- 3.1.2 Glassware must be scrupulously cleaned.¹ Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap water and reagent water. It should then be drained dry and heated in a muffle furnace at 400°C for 15 to 30 minutes. Solvent rinses with acetone and pesticide-quality hexane may be substituted for the heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store the glassware inverted or capped with aluminum foil.
- 3.2 Interferences coextracted from the samples will vary considerably from source to source, depending on the diversity of the industrial complex or municipality being sampled. While general cleanup procedures are provided as part of this method, unique samples may require additional cleanup approaches to achieve the detection limits listed in Table 1.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified²⁻⁴ for the information of the analyst.

5. APPARATUS AND EQUIPMENT

- 5.1 Sample containers: Narrow-mouth glass bottles, 1-L or 1-quart volume, equipped with polytetrafluoroethylene (PTFE)-lined screw-caps. Wide-mouth glass bottles, 1-quart volume, equipped with PTFE-lined screw-caps may also be used. Prior to use, wash bottles and cap liners with detergent and rinse with tap and distilled water. Allow the bottles and cap liners to air dry, then muffle at 400°C for 1 hour. After cooling, rinse the cap liners with hexane, seal the bottles, and store in a dust-free environment.
 - 5.1.1 Automatic sampler (optional): Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.
- 5.2 Kuderna-Danish (K-D) glassware.
 - 5.2.1 Synder column: Three-ball macro (Kontes K-503000-0121 or equivalent) and two-ball micro (Kontes K-569001-0219 or equivalent).

- 5.2.2 Concentrator tube: 10-mL, graduated (Kontes K-570050-1025 or equivalent) with ground-glass stopper.
- 5.2.3 Evaporative flask: 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.
- 5.3 Gas chromatography system.
 - 5.3.1 The gas chromatograph must be equipped with a glass-lined injection port compatible with the detector to be used. A data system is recommended for measuring peak areas.
 - 5.3.1.1 Chromatography column: 180 cm long by 4 mm ID, glass, packed with 1.5% OV-17/1.95% OV-210 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 12. Guidelines for the use of alternative column packings are provided in Section 10.3.1.
 - 5.3.1.2 Detector: Electron capture. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 12. Guidelines for the use of alternative detectors are provided in Section 10.3.
- 5.4 Chromatographic column: 200 mm long by 10 mm ID Chromaflex, equipped with coarse-fritted bottom plate and PTFE stopcock. (Kontes K-420540-0213 or equivalent).
- 5.5 Drying column: Approximately 400 mm long by 20 mm ID borosilicate glass, equipped with coarse-fritted bottom plate.
- 5.6 Miscellaneous
 - 5.6.1 Balance: analytical, capable of accurately weighing to the nearest 0.0001 g.
 - 5.6.2 Separatory funnel: 2-L, equipped with PTFE stopcock.
 - 5.6.3 Water bath: heated, with concentric ring cover, capable of temperature control ($\pm 2^{\circ}\text{C}$). The bath should be used in a hood.
 - 5.6.4 Standard solution storage containers: 15-mL bottles with PTFE-lined screw-caps.

6. REAGENTS AND CONSUMABLE MATERIALS

6.1 Reagents.

- 6.1.1 Acetone, hexane, and methylene chloride: Demonstrated to be free of analytes.
- 6.1.2 Florisil: PR grade (60/100 mesh). Purchase activated at 675°C and store in dark in glass containers with glass stoppers or foil-lined screw-caps. Before use, activate each batch overnight at 200°C in foil-covered glass container.
- 6.1.3 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.1.4 Sodium hydroxide (NaOH) solution (10N): Dissolve 40 g NaOH in reagent water and dilute to 100 mL.
- 6.1.5 Sodium sulfate: Granular, anhydrous. Condition by heating at 400°C for 4 hours in a shallow tray.

- 6.1.6 Sulfuric acid (H_2SO_4) solution (1 + 1): Add measured volume of concentrated H_2SO_4 to equal volume of reagent water.
- 6.2 Standard stock solutions ($1.00 \mu\text{g}/\mu\text{L}$): These solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedures.
 - 6.2.1 Prepare standard stock solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in hexane and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the standard stock.
 - 6.2.2 Store standard stock solutions at 4°C in 15-mL bottles equipped with PTFE-lined screw-caps. Standard stock solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.
 - 6.2.3 Standard stock solutions must be replaced after 6 months, or sooner if comparison with check standards indicates a problem.

7. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

- 7.1 Collect all samples in duplicate. Grab samples must be collected in glass-containers. Conventional sampling practices⁵ should be followed, except that the bottle must not be prewashed with sample before collection.
- 7.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 7.3 All samples must be extracted within 30 days of collection.⁶

8. CALIBRATION AND STANDARDIZATION

- 8.1 Calibration.
 - 8.1.1 A set of at least three calibration solutions containing the method analytes is needed. One calibration solution should contain each analyte at a concentration approaching but greater than the estimated detection limit (Table 1) for that compound; the other two solutions should contain analytes at concentrations that bracket the range expected in samples. For example, if the detection limit for a particular analyte is $0.2 \mu\text{g}/\text{L}$, and a sample expected to contain approximately $5 \mu\text{g}/\text{L}$ is analyzed, standard solutions should be prepared at concentrations of $0.3 \mu\text{g}/\text{L}$, $5 \mu\text{g}/\text{L}$, and $10 \mu\text{g}/\text{L}$.
 - 8.1.2 To prepare a calibration solution, add an appropriate volume of a standard stock solution to a volumetric flask and dilute to volume with hexane.
 - 8.1.3 Starting with the standard of lowest concentration, analyze each calibration standard according to Section 10.3 and tabulate peak height or area responses versus the mass of analyte injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to concentration (calibration factor) is a constant over the working range ($< 10\%$ relative standard deviation), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

8.1.4 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any analyte varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a new calibration curve.

8.2 Florisil standardization.

8.2.1 Florisil from different batches or sources may vary in absorptive capacity. To standardize the amount of Florisil which may be used in the cleanup procedure (Section 10.2) use of the lauric acid value⁷ is suggested. The referenced procedure determines the adsorption from hexane solution of lauric acid, in milligrams per gram of Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.

9. QUALITY CONTROL

9.1 Monitoring for interferences: Analyze a laboratory reagent blank each time a set of samples is extracted. A laboratory reagent blank is a 1-L aliquot of reagent water. If the reagent blank contains a reportable level of any analyte, immediately check the entire analytical system to locate and correct for possible interferences and repeat the test.

9.2 Assessing accuracy.

9.2.1 After every ten samples, and preferably in the middle of each day, analyze a laboratory control standard. Calibration standards may not be used for accuracy assessments and the laboratory control standard may not be used for calibration of the analytical system.

9.2.1.1 Laboratory control standard concentrate: From stock standards prepared as described in Section 6.3, prepare a laboratory control standard concentrate that contains each analyte of interest at a concentration of 2 $\mu\text{g/mL}$ in acetone or other suitable solvent.⁸

9.2.1.2 Laboratory control standard: Using a pipette, add 1.00 mL of the laboratory control standard concentrate to a 1-L aliquot of reagent water.

9.2.1.3 Analyze the laboratory control standard as described in Section 10. For each analyte in the laboratory control standard, calculate the percent recovery (P_i) with the equation:

Equation 1

$$P_i = \frac{100S_i}{T_i}$$

where

S_i = The analytical results from the laboratory control standard, in $\mu\text{g/L}$

T_i = The known concentration of the spike, in $\mu\text{g/L}$

9.2.2 At least annually, the laboratory should participate in formal performance evaluation studies, where solutions of unknown concentrations are analyzed and the performance of all participants is compared.

9.3 Assessing precision.

9.3.1 Precision assessments for this method are based upon the analysis of field duplicates (Section 7.1). Analyze both sample bottles for at least 10% of all samples. To the extent practical, the samples for duplication should contain reportable levels of most of the analytes.

9.3.2 For each analyte in each duplicate pair, calculate the relative range (RR_i) with the equation:

Equation 2

$$RR_i = \frac{100R_i}{X_i}$$

where

R_i = The absolute difference between the duplicate measurements X_1 and X_2 , in $\mu\text{g/L}$

X_i = The average concentration found $\left[\frac{X_1 + X_2}{2} \right]$, in $\mu\text{g/L}$

9.3.3 Individual relative range measurements are pooled to determine average relative range or to develop an expression of relative range as a function of concentration.

10. PROCEDURE

10.1 Sample extraction.

10.1.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-L separatory funnel. Check the pH of the sample with wide-range pH paper and adjust to within the range of 5 to 9 with sodium hydroxide or sulfuric acid.

10.1.2 Add 60 mL of 15% methylene chloride/hexane to the sample bottle and shake for 30 seconds to rinse the walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release vapor pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends on the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation. Collect the extract in a 250-mL Erlenmeyer flask.

10.1.3 Add an additional 60-mL volume of 15% methylene chloride/hexane to the sample bottle and complete the extraction procedure a second time, combining the extracts in the Erlenmeyer flask.

- 10.1.4** Perform a third extraction in the same manner. Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect it in a 500-mL K-D flask equipped with a 10-mL concentrator tube.
- 10.1.5** Add one or two clean boiling chips to the flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL of hexane to the top. Place the K-D apparatus on a hot water bath (80 to 85°C) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed in steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane. A 5-mL syringe is recommended for this operation. If the extract requires cleanup proceed to Section 10.2 (cleanup and separation). If cleanup has been performed or if the extract does not require cleanup, proceed with Section 10.1.6.
- 10.1.6** Add a clean boiling chip to the concentrator tube. Attach a two-ball micro-Snyder column. Prewet the micro-Snyder column by adding about 0.5 mL of hexane to the top. Place this micro K-D apparatus on a steaming-water bath (80 to 85°C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation, the balls will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a small volume of hexane. Adjust the final volume to 1.0 mL, and stopper the concentrator tube; store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than 2 days, they should be transferred to PTFE-sealed screw-cap bottles. Proceed with gas chromatographic analysis.
- 10.1.7** Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

10.2 Cleanup and separation.

- 10.2.1** Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various clean waters and municipal effluents. The Florisil cleanup procedure allows for a select fractionation of the compounds and will eliminate non-polar materials. The single-operator precision and accuracy data in Table 2 were gathered using the recommended cleanup procedures. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than that recorded in Table 2.

- 10.2.2** Prepare a slurry of 10 g of Florisil in methylene chloride. Use it to pack a 10-mm ID chromatography column, gently tapping the column to settle the Florisil. Add a 1-cm layer of anhydrous sodium sulfate to the top of the Florisil.
- 10.2.3** Just prior to exposure of the sodium sulfate layer to the air, transfer the sample extract onto the column using an additional 2 mL of hexane to complete the transfer.
- 10.2.4** Just prior to exposure of the sodium sulfate layer to the air, add 30 mL of 50% methylene chloride/hexane and continue the elution of the column. Elution of the column should be at a rate of about 2 mL/min. Discard the eluate from this fraction.
- 10.2.5** Next, elute the column with 30 mL of methylene chloride, collecting the eluate in a 500-mL K-D flask equipped with a 10-mL concentrator tube. Add 50 mL of hexane to the flask and concentrate the collected fraction by the standard technique prescribed in Sections 10.1.5 and 10.1.6. This fraction should contain DCN and basalin.
- 10.2.6** Elute the column with 30 mL of 10% acetone/methylene chloride collecting the eluate in a 500-mL K-D flask equipped with a 10 mL concentrator tube. Add 50-mL of hexane to the flask and concentrate the collected fraction by the standard technique prescribed in Sections 10.1.5 and 10.1.6. This fraction should contain dinocap.
- 10.2.7** Analyze the fractions by gas chromatography.
- 10.3** Gas chromatography analysis.
- 10.3.1** Recommended columns and detectors for the gas chromatography system are described in Section 5.3. Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and detection limits that can be achieved by this method. Examples of the separations achieved are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if data quality comparable to Table 2 are achieved. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and data quality comparable to Table 2 are achieved.
- 10.3.2** Inject 2 to 5 μL of the sample extract using the solvent-flush technique.⁹ Record the volume injected to the nearest 0.05 μL , the total extract volume, and the resulting peak size in area or peak height units.
- 10.3.3** The width of the retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of the day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 10.3.4** If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.
- 10.3.5** If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

11. CALCULATIONS

- 11.1 Determine the concentration (C) of individual compounds in the sample in micrograms per liter with the equation:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_i)}{(V_t)(V_s)}$$

where

A = Amount of material injected, in ng

V_i = Volume of extract injected, in μL

V_t = Volume of total extract, in μL

V_s = Volume of water extracted, in mL

- 11.3 Report the results for the unknown samples in micrograms per liter. Round off the results of the nearest 0.1 $\mu\text{g/L}$ or two significant figures.

12. METHOD PERFORMANCE

- 12.1 Estimated detection limits and associated chromatographic conditions are listed in Table 1.¹⁰ The detection limits were calculated from the minimum detectable response of the ECD equal to 5 times the GC background noise, assuming a 1.0-mL final extract volume of a 1-L sample and a GC injection of 5 μL .
- 12.2 Single-laboratory accuracy and precision studies were conducted by Environmental Science and Engineering, Inc.,⁶ using spiked wastewater samples. The results of these studies are presented in Table 2.

13. GC/MS CONFIRMATION

- 13.1 It is recommended that GC/MS techniques be judiciously employed to support qualitative identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak, but not to exceed 7 seconds per scan utilizing a 70 V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all glass or glass-lined materials is recommended. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program.
- 13.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC/MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved. The calculation of tailing factors is illustrated in Method 625.¹¹
- 13.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all DFTPP performance criteria are achieved.¹²

- 13.4** To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 25 ng of material be injected into the GC/MS. The criteria below must be met for qualitative confirmation.
- 13.4.1** The molecular ion and other ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to $\pm 10\%$. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20 to 40%.
- 13.4.2** The retention time of the compound in the sample must be within seven seconds of the same compound in the standard solution.
- 13.4.3** Compounds that have similar mass spectra can be explicitly identified by GC/MS only on the basis of retention time data.
- 13.5** Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.
- 13.6** Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternative packed or capillary GC columns or additional cleanup.

References

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11. "Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater," U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.
12. Eichelberger, J.W., Harris, L.E., and Budde, W.L., *Anal. Chem.*, 46, 1912, 1975.

Table 1. Chromatographic Conditions and Estimated Detection Limits

<i>Parameter</i>	<i>Retention Time (min)</i>	<i>Estimated Detection Limit (µg/L)</i>
CDN	2.0	.0005
Basalin	6.4	.0005
Dinocap*	10-16	0.1

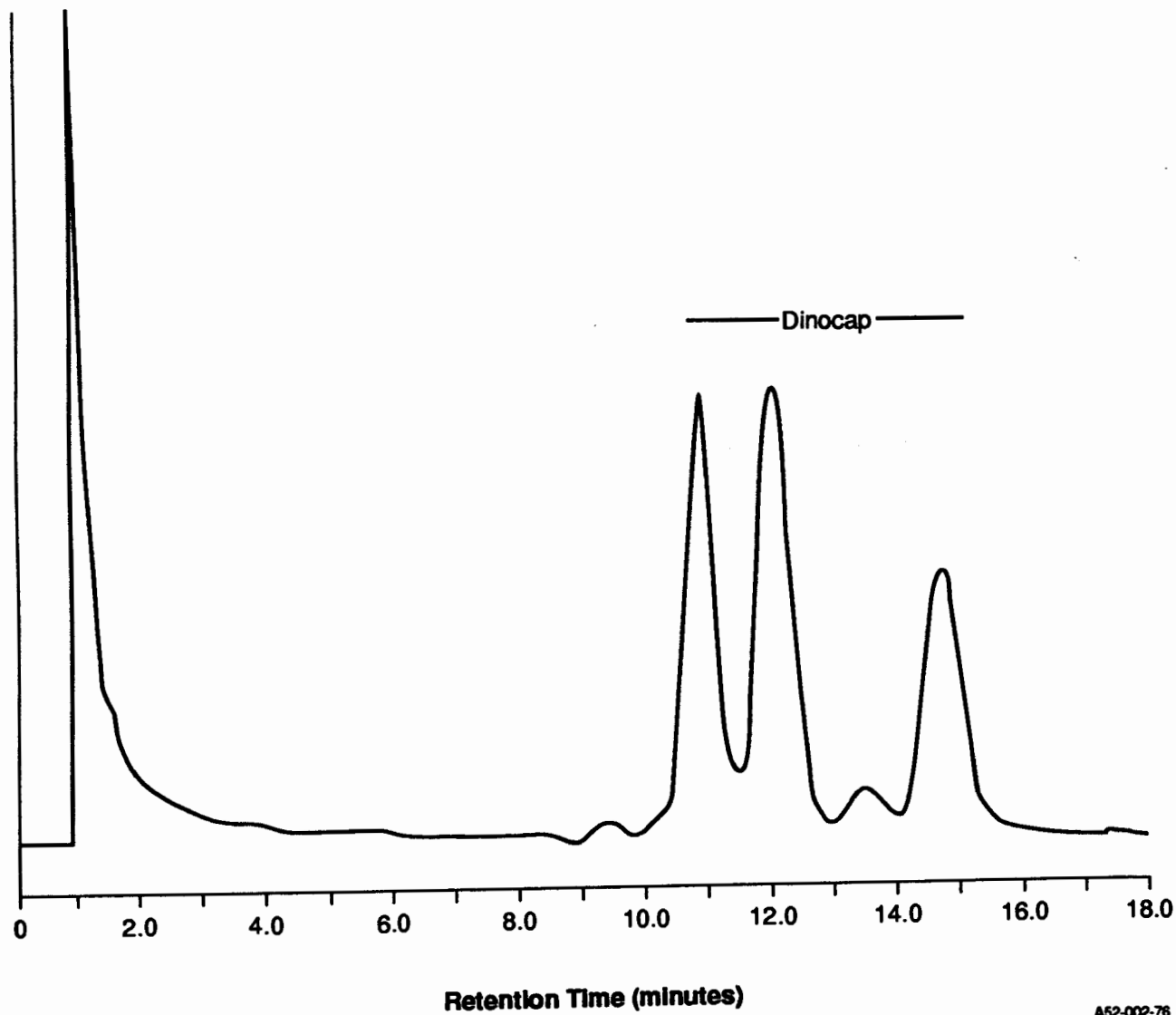
* Oven temperature 200°C isothermal.

Conditions: 180 cm long by 4 mm ID, glass column, packed with 1.5% OV-17/1.95% OV-210 on Supelcoport (100/120 mesh) or equivalent; 5% methane/95% argon carrier gas at 33 mL/min flow rate. Oven temperature 160°C isothermal.

Table 2. Single-Laboratory Accuracy and Precision

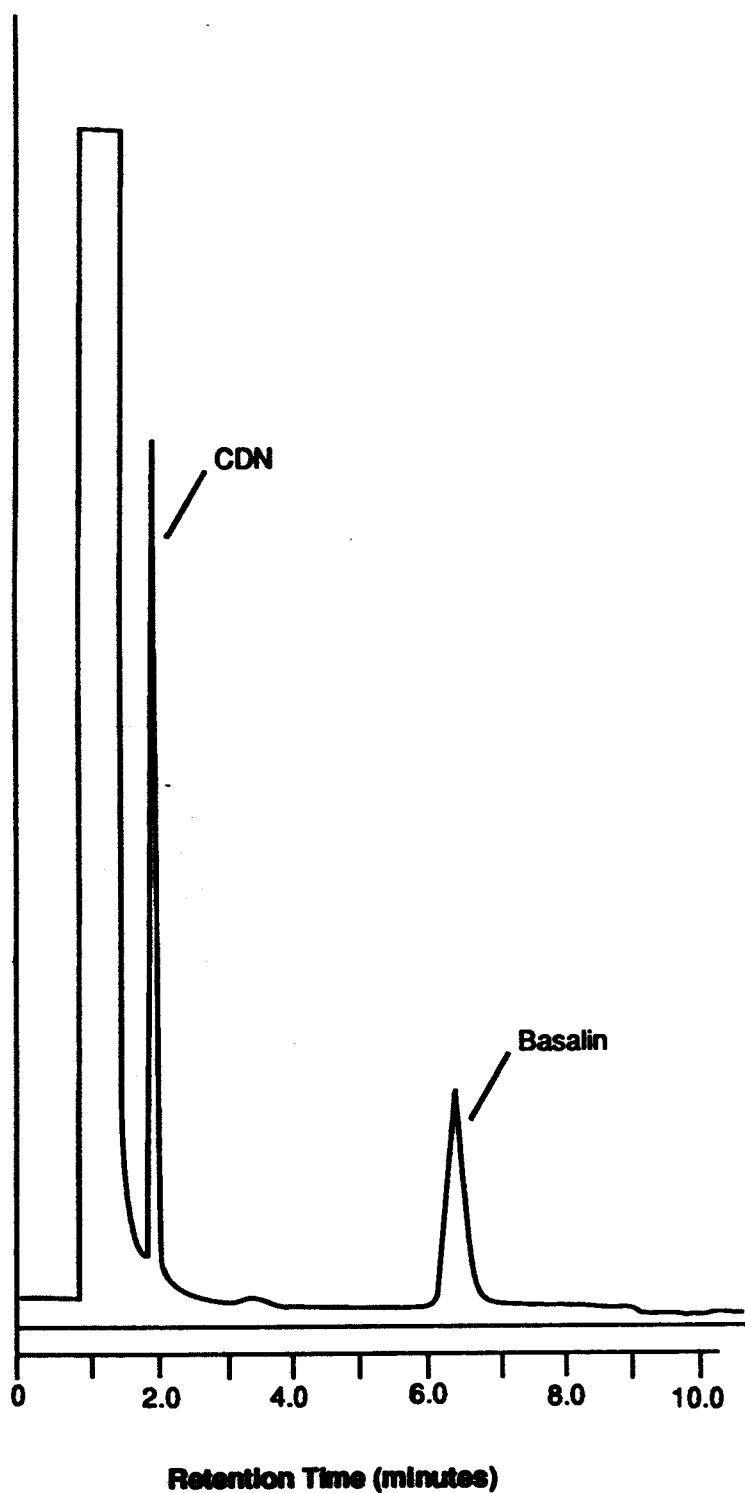
<i>Parameter</i>	<i>Matrix Type*</i>	<i>Spike Range (µg/L)</i>	<i>Number of Replicates</i>	<i>Average Percent Recovery</i>	<i>Standard Deviation (%)</i>
Basalin	1	10	7	79.0	7.0
	1	121	7	99.3	10.1
CDN	1	10	7	78.6	7.6
	1	99.2	7	99.5	6.1
Dinocap	1	10	7	108.5	4.5
	1	161	7	100.3	4.4

*1 = Publicly Owned Treatment Works (POTW) wastewater



A52-002-76

Figure 1. 4-Peak Gas Chromatogram of Dinocap.



A52-002-77

Figure 2. Gas Chromatogram of CDN and Basalin

Method 1656
The Determination of
Organo-Halide Pesticides in
Municipal and Industrial
Wastewater

Method 1656

The Determination of Organo-Halide Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method is designed to meet the survey requirements of the Environmental Protection Agency (EPA). It is used to determine (1) the organo-halide pesticides and polychlorinated biphenyls (PCBs) associated with the Clean Water Act, the Resource Conservation and Recovery Act, and the Comprehensive Environmental Response, Compensation and Liability Act; and (2) other compounds amenable to extraction and analysis by wide-bore capillary column gas chromatography (GC) with halogen-specific detectors.
- 1.2 The compounds listed in Table 1 may be determined in waters, soils, sediments, and sludges by this method. The method is a consolidation of several EPA wastewater methods. For waters, the sample extraction and concentration steps are essentially the same as in these methods. However, the extraction and concentration steps have been extended to other sample matrices. The method may be applicable to other pesticides as well. The quality control requirements in this method give the steps necessary to determine this applicability.
- 1.3 This method is applicable to a large number of compounds. Calibrating the GC systems for all compounds is time-consuming. If only a single compound or small number of compounds is to be tested for, it is necessary to calibrate the GC systems and meet the performance specifications in this method for these compounds only. In addition, the GC conditions can be optimized for these compounds provided that all performance specifications in this method are met.
- 1.4 When this method is applied to analysis of unfamiliar samples, compound identity must be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Gas chromatography/mass spectrometry (GC/MS) can be used to confirm compounds in extracts produced by this method when analyte levels are sufficient.
- 1.5 The detection limits of this method are usually dependent on the level of interferences rather than instrumental limitations. The limits in Table 2 typify the minimum quantities that can be detected with no interferences present.
- 1.6 This method is for use by or under the supervision of analysts experienced in the use of a gas chromatograph and in the interpretation of gas chromatographic data. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 8.2.

2. SUMMARY OF METHOD

2.1 Extraction.

2.1.1 The percent solids content of a sample is determined.

2.1.2 Samples containing low solids: If the solids content is 1% or less, a 1-L sample is extracted with methylene chloride using continuous extraction techniques.

2.1.3 Samples containing less than 1% solids.

2.1.3.1 Non-sludge samples: If the solids content is 1 to 30%, the sample is diluted to 1% solids with reagent water, homogenized ultrasonically, and extracted with methylene chloride using continuous extraction techniques. If the solids content is greater than 30%, the sample is extracted with methylene chloride:acetone using ultrasonic techniques.

2.1.3.2 Municipal sludge samples and other intractable sample types: If the solids content is less than 30%, the sample is diluted to 1% solids and extracted with methylene chloride using continuous extraction techniques. If the solids content is greater than 30%, the sample is extracted with acetonitrile and then methylene chloride using ultrasonic techniques. The extract is back-extracted with 2% (W/V) sodium sulfate in reagent water to remove water-soluble interferences and residual acetonitrile.

2.2 Concentration and cleanup: The extract is dried over sodium sulfate, concentrated using a Kuderna-Danish evaporator, cleaned up (if necessary) using gel permeation chromatography (GPC) and/or adsorption chromatography and/or solid-phase extraction, and then concentrated to 1 mL. Sulfur is removed from the extract, if required.

2.3 Gas chromatography: A 1- μ L aliquot of the extract is injected into the gas chromatograph (GC). The compounds are separated on a wide-bore, fused-silica capillary column. The analytes are detected by an electron capture, microcoulometric, or electrolytic conductivity detector.

2.4 Identification of a pollutant (qualitative analysis) is performed by comparing the GC retention times of the compound on two dissimilar columns with the respective retention times of an authentic standard. Compound identity is confirmed when the retention times agree within their respective windows.

2.5 Quantitative analysis is performed using an authentic standard to produce a calibration factor or calibration curve, and using the calibration data to determine the concentration of a pollutant in the extract. The concentration in the sample is calculated using the sample weight or volume and the extract volume.

2.6 Quality is assured through reproducible calibration and testing of the extraction and GC systems.

3. CONTAMINATION AND INTERFERENCES

3.1 Solvents, reagents, glassware, and other sample-processing hardware may yield artifacts and/or elevated baselines causing misinterpretation of chromatograms. All materials used in the analysis shall be demonstrated to be free from interferences under the conditions of analysis by running method blanks as described in Section 8.5.

3.2 Glassware and, where possible, reagents are cleaned by solvent rinse and baking at 450°C for 1 hour minimum in a muffle furnace or kiln. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment, and thorough rinsing with acetone and pesticide-quality hexane may be required.

3.3 Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

- 3.4 Interference by phthalate esters can pose a major problem in pesticide analysis when using the electron capture detector. Phthalates usually appear in the chromatogram as large, late-eluting peaks. Phthalates may be leached from common flexible plastic tubing and other plastic materials during the extraction and cleanup processes. Cross-contamination of clean glassware routinely occurs when plastics are handled during extraction, especially when solvent-wetted surfaces are handled. Interferences from phthalates can best be minimized by avoiding the use of plastics in the laboratory, or by using a microcoulometric or electrolytic conductivity detector.
- 3.5 Interferences coextracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled. The cleanup procedures given in this method can be used to overcome many of these interferences, but unique samples may require additional cleanup to achieve the minimum levels given in Table 2.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 1 through 3.
- 4.2 The following compounds covered by this method have been tentatively classified as known or suspected human or mammalian carcinogens: 4,4'-DDD, 4,4'-DDT, the BHCs and the PCBs. Primary standards of these compounds shall be prepared in a hood, and a NIOSH/MESA-approved toxic gas respirator should be worn when high concentrations are handled.
- 4.3 Mercury vapor is highly toxic. If mercury is used for sulfur removal, all operations involving mercury shall be performed in a hood.
- 4.4 Unknown samples may contain high concentrations of volatile toxic compounds. Sample containers should be opened in a hood and handled with gloves that will prevent exposure. The oven used for sample drying to determine percent moisture should be located in a hood so that vapors from samples do not create a health hazard in the laboratory.

5. APPARATUS AND MATERIALS

NOTE: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance meeting requirements of this method is the responsibility of the laboratory.

- 5.1 Sampling equipment for discrete or composite sampling.
- 5.1.1 Sample bottles and caps.
- 5.1.1.1 Liquid samples (waters, sludges, and similar materials that contain less than 5% solids): Sample bottle, amber glass, 1-L or 1-quart, with screw-cap.

5.1.1.2 Solid samples (soils, sediments, sludges, filter cake, compost, and similar materials that contain >5% solids): Sample bottle, wide mouth, amber glass, 500-mL minimum.

5.1.1.3 If amber bottles are not available, samples shall be protected from light.

5.1.1.4 Bottle caps: Threaded to fit sample bottles. Caps shall be lined with PTFE.

5.1.1.5 Cleaning.

5.1.1.5.1 Bottles are detergent-water washed, then rinsed with solvent or baked at 450°C for 1 hour minimum before use.

5.1.1.5.2 Liners are detergent-water washed, then rinsed with reagent water and solvent, and baked at approximately 200°C for 1 hour minimum prior to use.

5.1.2 Compositing equipment: Automatic or manual compositing system incorporating glass containers cleaned per bottle-cleaning procedure above. Sample containers are kept at 0 to 4°C during sampling. Glass or PTFE tubing only shall be used. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, the tubing shall be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.

5.2 Equipment for determining percent moisture.

5.2.1 Oven, capable of maintaining a temperature of 110°C ($\pm 5^\circ\text{C}$).

5.2.2 Desiccator.

5.2.3 Crucibles, porcelain.

5.2.4 Weighing pans, aluminum.

5.3 Extraction equipment.

5.3.1 Equipment for ultrasonic extraction.

5.3.1.1 Sonic disrupter: 375 watt with pulsing capability and ½- or ¾-inch disrupter horn (Ultrasonics, Inc, Model 375C, or equivalent).

5.3.1.2 Sonabox (or equivalent), for use with disrupter.

5.3.2 Equipment for liquid-liquid extraction.

5.3.2.1 Continuous liquid-liquid extractor: PTFE or glass connecting joints and stopcocks without lubrication, 1.5- to 2-L capacity (Hershberg-Wolf Extractor, Cal-Glass, Costa Mesa, California, 1000- or 2000-mL continuous extractor, or equivalent).

5.3.2.2 Round-bottom flask, 500-mL, with heating mantle.

5.3.2.3 Condenser, Graham, to fit extractor.

5.3.2.4 pH meter, with combination glass electrode.

5.3.2.5 pH paper, wide range (Hydrion Papers, or equivalent).

5.2.3 Separatory funnels: 250-, 500-, 1000-, and 2000-mL, with PTFE stopcocks.

5.3.4 Filtration apparatus.

- 5.3.4.1 Glass powder funnels: 125- to 250-mL.
 - 5.3.4.2 Filter paper for above (Whitman 41, or equivalent).
- 5.3.5 Beakers.
 - 5.3.5.1 1.5- to 2-L, calibrated to 1 L.
 - 5.3.5.2 400- to 500-mL.
- 5.3.6 Spatulas: Stainless steel or PTFE.
- 5.3.7 Drying column: 400 mm long by 15 to 20 mm ID Pyrex chromatographic column equipped with coarse glass frit or glass wool plug.
 - 5.3.7.1 Pyrex glass wool: Extracted with solvent or baked at 450°C for 1 hour minimum.
- 5.4 Evaporation/concentration apparatus.
 - 5.4.1 Kuderna-Danish (K-D) apparatus.
 - 5.4.1.1 Evaporation flask: 500-mL (Kontes K-570001-0500, or equivalent), attached to concentrator tube with springs (Kontes K-662750-0012).
 - 5.4.1.2 Concentrator tube: 10-mL, graduated (Kontes K-570050-1025, or equivalent) with calibration verified. Ground-glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.
 - 5.4.1.3 Snyder column: Three-ball macro (Kontes K-503000-0232, or equivalent).
 - 5.4.1.4 Snyder column: Two-ball micro (Kontes K-469002-0219, or equivalent).
 - 5.4.1.5 Boiling chips.
 - 5.4.1.5.1 Glass or silicon carbide: Approximately 10/40 mesh, extracted with methylene chloride and baked at 450°C for 1 hour minimum.
 - 5.4.1.5.2 PTFE (optional): Extracted with methylene chloride.
 - 5.4.2 Water bath: Heated, with concentric ring cover, capable of temperature control ($\pm 2^{\circ}\text{C}$), installed in a fume hood.
 - 5.4.3 Nitrogen evaporation device: Equipped with heated bath that can be maintained at 35 to 40°C (N-Evap, Organomation Associates, Inc., or equivalent).
 - 5.4.4 Sample vials: Amber glass, 1- to 5-mL with PTFE-lined screw- or crimp-cap, to fit GC autosampler.
- 5.5 Balances.
 - 5.5.1 Analytical: Capable of weighing 0.1 mg.
 - 5.5.2 Top loading: Capable of weighing 10 mg.
- 5.6 Apparatus for sample cleanup.
 - 5.6.1 Automated gel permeation chromatograph (Analytical Biochemical Labs, Inc., Columbia, MO, Model GPC Autoprep 1002, or equivalent).
 - 5.6.1.1 Column: 600 to 700 mm long by 25 mm ID, packed with 70 g of SX-3 Bio-beads (Bio-Rad Laboratories, Richmond, CA, or equivalent).

- 5.6.1.2 Syringe, 10-mL, with Luer fitting.
- 5.6.1.3 Syringe-filter holder, stainless steel, and glass fiber or PTFE filters (Gelman Acrodisc-CR, 1 to 5 micron, or equivalent).
- 5.6.1.4 UV detector: 254-nm, preparative or semi-prep flow cell: (Isco, Inc., Type 6; Schmadzu, 5 mm path length; Beckman-Altex 152W, 8 μ L micro-prep flow cell, 2 mm path; Pharmacia UV-1, 3 mm flow cell; LDC Milton-Roy UV-3, monitor #1203; or equivalent).
- 5.6.2 Vacuum system and cartridges for solid-phase extraction (SPE).
 - 5.6.2.1 Vacuum system: Capable of achieving 0.1 bar (house vacuum, vacuum pump, or water aspirator), with vacuum gauge.
 - 5.6.2.2 VacElute Manifold (Analytichem International, or equivalent).
 - 5.6.2.3 Vacuum trap: Made from 500-mL sidearm flask fitted with single-hole rubber stopper and glass tubing.
 - 5.6.2.4 Rack for holding 50-mL volumetric flasks in the manifold.
 - 5.6.2.5 Column: Mega Bond Elut, Non-polar, C18 Octadecyl, 10 g/60 mL (Analytichem International Cat. No. 607H060, or equivalent).
- 5.6.3 Chromatographic column: 400 mm long by 22 mm ID, with PTFE stopcock and coarse frit (Kontes K-42054, or equivalent).
- 5.6.4 Sulfur removal tubes: 40- to 50-mL bottle or test tube with PTFE-lined screw-cap.
- 5.7 Centrifuge apparatus.
 - 5.7.1 Centrifuge: Capable of rotating 500-mL centrifuge bottles or 15-mL centrifuge tubes at 5,000 rpm minimum.
 - 5.7.2 Centrifuge bottles: 500-mL, with screw-caps, to fit centrifuge.
 - 5.7.3 Centrifuge tubes: 12- to 15-mL, with screw-caps, to fit centrifuge.
 - 5.7.3 Funnel, Buchner, 15 cm.
 - 5.7.3.1 Flask, filter, for use with Buchner funnel.
 - 5.7.3.2 Filter paper, 15 cm (Whatman #41, or equivalent).
- 5.8 Miscellaneous glassware.
 - 5.8.1 Pipettes, glass, volumetric, 1.00-, 5.00-, and 10.0-mL.
 - 5.8.2 Syringes, glass, with Luerlok tip, 0.1-, 1.0- and 5.0-mL. Needles for syringes, 2-inch, 22-gauge.
 - 5.8.3 Volumetric flasks, 10.0-, 25.0-, and 50.0-mL.
 - 5.8.4 Scintillation vials, glass, 20- to 50-mL, with PTFE-lined screw-caps.
- 5.9 Gas chromatograph: Shall have splitless or on-column simultaneous automated injection into separate capillary columns with a halide-specific detector at the end of each column, temperature program with isothermal holds, data system capable of recording simultaneous signals from the two detectors, and shall meet all of the performance specifications in Section 14.
 - 5.9.1 GC columns: Bonded-phase, fused-silica capillary.

- 5.9.1.1 Primary: 30 m (± 3 m) long by 0.5 mm (± 0.05 mm) ID DB-608 (or equivalent).
- 5.9.1.2 Confirmatory: DB-1701, or equivalent, with same dimensions as primary column.
- 5.9.2 Data system: Shall collect and record GC data, store GC runs on magnetic disk or tape, process GC data, compute peak areas, store calibration data including retention times and calibration factors, identify GC peaks through retention times, compute concentrations, and generate reports.
 - 5.9.2.1 Data acquisition: GC data shall be collected continuously throughout the analysis and stored on a mass storage device.
 - 5.9.2.2 Calibration factors and calibration curves: The data system shall be used to record and maintain lists of calibration factors, and multi-point calibration curves (Section 7). Computations of relative standard deviation (coefficient of variation) are used for testing calibration linearity. Statistics on initial (Section 8.2) and ongoing (Section 14.6) performance shall be computed and maintained.
 - 5.9.2.3 Data processing: The data system shall be used to search, locate, identify, and quantify the compounds of interest in each GC analysis. Software routines shall be employed to compute and record retention times and peak areas. Displays of chromatograms and library comparisons are required to verify results.
- 5.9.3 Halide-specific detector: Electron capture or electrolytic conductivity (Micoulometric, Hall, O.I., or equivalent), capable of detecting 8 pg of aldrin under the analysis conditions given in Table 2.

6. REAGENTS AND STANDARDS

- 6.1 Sample preservation: Sodium thiosulfate (ACS), granular.
- 6.2 pH adjustment.
 - 6.2.1 Sodium hydroxide: Reagent grade.
 - 6.2.1.1 Concentrated solution (10N): Dissolve 40 g NaOH in 100 mL reagent water.
 - 6.2.1.2 Dilute solution (0.1M): Dissolve 4 g NaOH in 1 L of reagent water.
 - 6.2.2 Sulfuric acid (1 + 1): Reagent grade, 6N in reagent water. Slowly add 50 mL H₂SO₄ (specific gravity 1.84) to 50 mL reagent water.
 - 6.2.3 Potassium hydroxide: 37 % (W/V). Dissolve 37 g KOH in 100 mL reagent water.
- 6.3 Solution drying and back-extraction.
 - 6.3.1 Sodium sulfate, reagent grade, granular anhydrous (Baker 3375, or equivalent), rinsed with methylene chloride (20 mL/g), baked at 450°C for 1 hour minimum, cooled in a desiccator, and stored in a pre-cleaned glass bottle with screw-cap which prevents moisture from entering.

- 6.3.2** Sodium sulfate solution: 2% (W/V) in reagent water, pH-adjusted to 8.5 to 9.0 with KOH or H₂SO₄.
- 6.4** Solvents: Methylene chloride, hexane, ethyl ether, acetone, acetonitrile, isooctane, and methanol; pesticide-quality; lot-certified to be free of interferences.
 - 6.4.1** Ethyl ether must be shown to be free of peroxides before it is used, as indicated by EM Laboratories Quant Test Strips (Scientific Products P1126-8, or equivalent). Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL of ethyl alcohol is added to each liter of ether as a preservative.
- 6.5** GPC calibration solution: Solution containing 300 mg/mL corn oil, 15 mg/mL bis (2-ethylhexyl) phthalate, 1.4 mg/mL pentachlorophenol, 0.1 mg/mL perylene, and 0.5 mg/mL sulfur.
- 6.6** Sample cleanup.
 - 6.6.1** Florisil: PR grade, 60/100 mesh, activated at 650 to 700°C, stored in the dark in glass container with PTFE-lined screw-cap. Activate at 130°C for 16 hours minimum immediately prior to use. Alternatively, 500-mg cartridges (J.T. Baker, or equivalent) may be used.
 - 6.6.2** Solid-phase extraction.
 - 6.6.2.1** SPE cartridge calibration solution: 2,4,6-trichlorophenol, 0.1 ug/mL in acetone.
 - 6.6.2.2** SPE elution solvent: Methylene chloride:acetonitrile:hexane (50:3:47).
 - 6.6.3** Alumina, neutral, Brockman Activity I, 80 to 200 mesh (Fisher Scientific Certified, or equivalent). Heat for 16 hours at 400 to 450°C. Seal and cool to room temperature. Add 7% (W/W) reagent water and mix for 10 to 12 hours. Keep bottle tightly sealed.
 - 6.6.4** Silicic acid, 100 mesh.
 - 6.6.5** Sulfur removal: Mercury (triple-distilled), copper powder (bright, non-oxidized), or TBA sodium sulfite. If mercury is used, observe the handling precautions in Section 4.
- 6.7** Reagent water: Water in which the compounds of interest and interfering compounds are not detected by this method.
- 6.8** High-solids reference matrix: Playground sand or similar material in which the compounds of interest and interfering compounds are not detected by this method. May be prepared by extraction with methylene chloride and/or baking at 450°C for 4 hours minimum.
- 6.9** Standard solutions: Purchased as solutions or mixtures with certification to their purity, concentration, and authenticity, or prepared from materials of known purity and composition. If compound purity is 96% or greater, the weight may be used without correction to compute the concentration of the standard. When not being used, standards are stored in the dark at -20 to -10°C in screw-capped vials with PTFE-lined lids. A mark is placed on the vial at the level of the solution so that solvent evaporation loss can be detected. The vials are brought to room temperature prior to use. Any precipitate is redissolved and solvent is added if solvent loss has occurred.
- 6.10** Preparation of stock solutions: Prepare in isooctane per the steps below. Observe the safety precautions in Section 4.

- 6.10.1** Dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 10 mg aldrin in a 10-mL ground-glass stoppered volumetric flask and fill to the mark with isooctane. After the aldrin is completely dissolved, transfer the solution to a 15-mL vial with PTFE-lined cap.
- 6.10.2** Stock solutions should be checked for signs of degradation prior to the preparation of calibration or performance test standards.
- 6.10.3** Stock solutions shall be replaced after 6 months, or sooner if comparison with quality control check standards indicates a change in concentration.
- 6.11** Secondary mixtures: Using stock solutions (Section 6.10), prepare mixtures at the levels shown in Table 3 for calibration and calibration verification (Sections 7.3 and 14.5), for initial and ongoing precision and recovery (Sections 8.2 and 14.6), and for spiking into the sample matrix (Section 8.4).
- 6.12** Surrogate spiking solution: Prepare dibutyl chlorendate (OBC) at a concentration of 2 ng/mL in acetone.

NOTE: If DBC is not available, compounds such as tetrachloro-m-xylene or decachlorobiphenyl may be used provided that the laboratory performs the tests described in Section 8.2 using these compounds.

- 6.13** DDT and endrin decomposition solution: Prepare a solution containing endrin at a concentration of 1 µg/mL and DDT at a concentration of 2 µg/mL.
- 6.14** Stability of solutions: All standard solutions (Sections 6.9 through 6.13) shall be analyzed within 48 hours of preparation and on a monthly basis thereafter for signs of degradation. Standards will remain acceptable if the peak area remains within $\pm 15\%$ of the area obtained in the initial analysis of the standard.

7. SETUP AND CALIBRATION

- 7.1** Configure the GC system as given in Section 5.9 and establish the operating conditions in Table 2.
- 7.2** Attainment of method detection limit (MDL) and DDT/Endrin decomposition requirements: Determine that each column/detector system meets the MDLs (Table 2), and the DDT and Endrin decomposition test (Section 13.4).
- 7.3** Calibration.
- 7.3.1** Injection of calibration solutions.
- 7.3.1.1** Compounds with calibration data in Table 3: The compounds in each calibration group in Table 3 were chosen so that each compound would be separated from the others by approximately 1 minute on the primary column. The concentrations were chosen to bracket the working range of either the ECD or the ELCD. However, because the response of the ELCD is less for some compounds than that of the ECD, it may be necessary to inject a larger volume of calibration solution when the ELCD is used.

7.3.1.2 Compounds without calibration data in Table 3: Prepare calibration standards at a minimum of three concentration levels. One of these concentrations should be near, but above, the MDL (Table 2) and the other concentrations should define the working range of the detectors.

7.3.1.3 Set the automatic injector to inject a constant volume in the range of 0.5 to 5.0 μL of each calibration solution into the GC column/detector pairs, beginning with the lowest level mixture and proceeding to the highest. For each compound, compute and store, as a function of the concentration injected, the retention time and peak area on each column/detector system (primary and confirmatory). For the multi-component analytes (PCBs, toxaphene), store the retention time and peak area for the five largest peaks.

7.3.2 Retention time: The polar nature of some analytes causes the retention time to decrease as the quantity injected increases. To compensate this effect, the retention time for compound identification is correlated with the analyte level.

7.3.2.1 If the difference between the maximum and minimum retention times for any compound is less than 5 seconds over the calibration range, the retention time for that compound can be considered constant and an average retention time may be used for compound identification.

7.3.2.2 Retention time calibration curve (retention time vs. amount): If the retention time for a compound in the lowest level standard is more than 5 seconds greater than the retention time for the compound in the highest level standard, a retention time calibration curve shall be used for identification of that compound.

7.3.3 Calibration factor (ratio of area to amount injected).

7.3.3.1 Compute the coefficient of variation (relative standard deviation) of the calibration factor over the calibration range for each compound on each column/detector system.

7.3.3.2 Linearity: If the calibration factor for any compound is constant ($C_v < 20\%$) over the calibration range, an average calibration factor may be used for that compound; otherwise, the complete calibration curve (area vs. amount) for that compound shall be used.

7.4 Combined QC standards: To preclude periodic analysis of all of the individual calibration groups of compounds (Table 3), the GC systems are calibrated with combined solutions as a final step. Not all of the compounds in these standards will be separated by the GC columns used in this method. Retention times and calibration factors are verified for the compounds that are resolved, and calibration factors are obtained for the unresolved peaks.

7.4.1 Analyze the combined QC standard on each column/detector pair.

7.4.1.1 For those compounds that exhibit a single, resolved GC peak, the retention time shall be within ± 5 seconds of the retention time of the peak in the medium level calibration standard (Table 3), and the calibration factor using the primary column shall be within $\pm 20\%$ of the calibration factor in the medium level standard (Table 3).

7.4.1.2 For the peaks containing two or more compounds, compute and store the retention times at the peak maxima on both columns (primary and confirmatory), and also compute and store the calibration factors on both columns. These results will be used for calibration verification (Section 13.2 and 13.5) and for precision and recovery studies (Sections 8.2 and 13.6).

7.5 Florisil calibration: The cleanup procedure in Section 11 utilizes Florisil column chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil that is used, the use of the lauric acid value (Reference 4) is suggested. The referenced procedure determines the adsorption of lauric acid (in milligrams per gram of Florisil) from hexane solution. The amount of Florisil to be used for each column is calculated by dividing 110 by this ratio and multiplying by 20 g.

8. QUALITY CONTROL

8.1 Each laboratory that uses this method is required to operate a formal quality control program (Reference 5). The minimum requirements of this program consist of an initial demonstration of laboratory capability, an ongoing analysis of standards and blanks as tests of continued performance, and analysis of spiked samples to assess accuracy. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method. If the method is to be applied routinely to samples containing high solids with very little moisture (e.g., soils, compost), the high-solids reference matrix (Section 6.8) is substituted for reagent water (Section 6.7) in all performance tests, and the high-solids method (Section 10) is used for these tests.

8.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 The analyst is permitted to modify this method to improve separations or lower the costs of measurements, provided all performance requirements are met. Each time a modification is made to the method or a cleanup procedure is added, the analyst is required to repeat the procedure in Section 8.2 to demonstrate method performance. If detection limits will be affected by the modification, the analyst is required to repeat the demonstration of detection limits (Section 7.2).

8.1.3 The laboratory shall spike all samples with at least one surrogate compound to monitor method performance. This test is described in Section 8.3. When results of these spikes indicate atypical method performance for samples, the samples are diluted to bring method performance within acceptable limits (Section 16).

8.1.4 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and the analysis of the combined QC standard (Section 6.11) that the analysis system is in control. These procedures are described in Sections 13.1, 13.5, and 13.6.

8.1.5 The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Section 8.4.

8.1.6 Analyses of blanks are required to demonstrate freedom from contamination. The procedures and criteria for analysis of a blank are described in Section 8.5.

- 8.1.7** Other analytes may be determined by this method. The procedure for establishing a preliminary quality control limit for a new analyte is given in Section 8.6.
- 8.2** Initial precision and recovery: To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations.
- 8.2.1** For analysis of samples containing low solids (aqueous samples), extract, concentrate, and analyze one set of four 1-L aliquots of reagent water spiked with the combined QC standard (Section 6.11) according to the procedure in Section 10. Alternatively, sets of four replicates of the individual calibration groups (Table 3) may be used. For samples containing high solids, a set of four 30-g aliquots of the high-solids reference matrix are used.
- 8.2.2** Using results of the set of four analyses, compute the average percent recovery (\bar{X}) and the coefficient of variation (C_v) of percent recovery (s) for each compound.
- 8.2.3** For each compound, compare s and \bar{X} with the corresponding limits for initial precision and accuracy in Table 4. For coeluting compounds, use the coeluted compound with the least restrictive specification (largest C_v and widest range). If s and \bar{X} for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any individual s exceeds the precision limit or any individual \bar{X} falls outside the range for accuracy, system performance is unacceptable for that compound. In this case, correct the problem and repeat the test.
- 8.3** The laboratory shall spike all samples with at least one surrogate compound to assess method performance on the sample matrix.
- 8.3.1** Analyze each sample according to the method beginning in Section 10.
- 8.3.2** Compute the percent recovery (P) of the DBC or other surrogate.
- 8.3.3** The surrogate recovery shall be 40 to 120%. If the recovery of the surrogate falls outside of these limits, method performance is unacceptable for that sample, and the sample is complex. Water samples are diluted, and smaller amounts of soils, sludges, and sediments are reanalyzed per Section 16.
- 8.4** Method accuracy: The laboratory shall spike (matrix spike) at least 10% of the samples from a given site type (e.g., influent to treatment, treated effluent, produced water, river sediment). If only one sample from a given site type is analyzed, a separate aliquot of that sample shall be spiked.
- 8.4.1** The concentration of the matrix spike shall be determined as follows:
- 8.4.1.1** If, as in compliance monitoring, the concentration of a specific analyte in the sample is being checked against a regulatory concentration limit, the matrix spike shall be at that limit or at 1 to 5 times higher than the background concentration determined in Section 8.4.2, whichever concentration is larger.
- 8.4.1.2** If the concentration of an analyte in the sample is not being checked against a limit specific to that analyte, the matrix spike shall be at the concentration of the combined QC standard (Table 3) or at 1 to 5 times higher than the background concentration, whichever concentration is larger.
- 8.4.1.3** If it is impractical to determine the background concentration before spiking (e.g., maximum holding times will be exceeded), the matrix spike

concentration shall be the regulatory concentration limit, if any; otherwise, the larger of either 5 times the expected background concentration or at the concentration of the combined QC standard (Table 3).

- 8.4.2** Analyze one sample aliquot to determine the background concentration (B) of each analyte. If necessary, prepare a standard solution appropriate to produce a level in the sample 1 to 5 times the background concentration. Spike a second sample aliquot with the standard solution and analyze it to determine the concentration after spiking (A) of each analyte. Calculate the percent recovery (P) of each analyte:

Equation 1

$$P = \frac{100(A-B)}{T}$$

where

T = True value of the spike

- 8.4.3** Compare the percent recovery for each analyte with the corresponding QC acceptance criteria in Table 4. If any analyte fails the acceptance criteria for recovery, the sample is complex and must be diluted and reanalyzed per Section 16.
- 8.4.4** As part of the QC program for the laboratory, method accuracy for samples shall be assessed and records shall be maintained. After the analysis of five spiked samples of a given matrix type (water, soil, sludge, sediment) in which the analytes pass the tests in Section 8.4.3, compute the average percent recovery (P) and the standard deviation of the percent recovery (s_p) for each compound (or coeluting compound group). Express the accuracy assessment as a percent recovery interval from $P - 2s_p$ to $P + 2s_p$ for each matrix. For example, if $P=90\%$ and $s_p=10\%$ for five analyses of compost, the accuracy interval is expressed as 70 to 110%. Update the accuracy assessment for each compound in each matrix on a regular basis (e.g., after each five to ten new accuracy measurements).
- 8.5** Blanks: Reagent water and high-solids reference matrix blanks are analyzed to demonstrate freedom from contamination.
- 8.5.1** Extract and concentrate a 1-L reagent water blank or a 30-g high-solids reference matrix blank with each sample batch (samples started through the extraction process on the same 8-hour shift, to a maximum of 20 samples). Analyze the blank immediately after analysis of the combined QC standard (Section 13.6) to demonstrate freedom from contamination.
- 8.5.2** If any of the compounds of interest (Table 1) or any potentially interfering compound is found in an aqueous blank at greater than $0.05 \mu\text{g/L}$, or in a high-solids reference matrix blank at greater than $1 \mu\text{g/kg}$ (assuming the same calibration factor as aldrin for compounds not listed in Table 1), analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level.
- 8.6** Other analytes may be determined by this method. To establish a quality control limit for an analyte, determine the precision and accuracy by analyzing four replicates of the analyte along with the combined QC standard per the procedure in Section 8.2. If the analyte coelutes with an

analyte in the QC standard, prepare a new QC standard without the coeluting component(s). Compute the average percent recovery (A) and the standard deviation of percent recovery (s_r) for the analyte, and measure the recovery and standard deviation of recovery for the other analytes. The data for the new analyte is assumed to be valid if the precision and recovery specifications for the other analytes are met; otherwise, the analytical problem is corrected and the test is repeated. Establish a preliminary quality control limit of $A \pm 2s_r$ for the new analyte and add the limit to Table 4.

- 8.7 The specifications contained in this method can be met if the apparatus used is calibrated properly, then maintained in a calibrated state. The standards used for calibration (Section 7), calibration verification (Section 13.5), and for initial (Section 8.2) and ongoing (Section 13.6) precision and recovery should be identical, so that the most precise results will be obtained. The GC instruments will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of the analytes given in this method.
- 8.8 Depending on specific program requirements, field replicates and field spikes of the analytes of interest into samples may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

9. **SAMPLE COLLECTION, PRESERVATION, AND HANDLING**

- 9.1 Collect samples in glass containers following conventional sampling practices (Reference 6), except that the bottle shall not be prerinsed with sample before collection. Aqueous samples which flow freely are collected in refrigerated bottles using automatic sampling equipment. Solid samples are collected as grab samples using wide-mouth jars.
- 9.2 Maintain samples at 0 to 4°C from the time of collection until extraction. If the samples will not be extracted within 72 hours of collection, adjust the sample to a pH of 5.0 to 9.0 using sodium hydroxide or sulfuric acid solution. Record the volume of acid or base used. If residual chlorine is present in aqueous samples, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine (Reference 7).
- 9.3 Begin sample extraction within 7 days of collection, and analyze all extracts within 40 days of extraction.

10. **SAMPLE EXTRACTION AND CONCENTRATION**

Samples containing 1% solids or less are extracted directly using continuous liquid-liquid extraction techniques (Section 10.2.1). Samples containing 1 to 30% solids are diluted to the 1% level with reagent water and extracted using continuous liquid-liquid extraction techniques (Section 10.2.2). Samples containing more than 30% solids are extracted using ultrasonic techniques (Section 10.2.5). Figure 1 outlines the extraction and concentration steps.

10.1 **Determination of percent solids.**

10.1.1 Weigh 5 to 10 g of sample into a tared beaker. Record the weight to three significant figures.

10.1.2 Dry overnight (12 hours minimum) at 110°C ($\pm 5^\circ\text{C}$), and cool in a dessicator.

10.1.3 Determine percent solids as follows:

Equation 2

$$\% \text{ solids} = \frac{\text{weight of dry sample}}{\text{weight of wet sample}} \times 100$$

10.2 Preparation of samples for extraction

10.2.1 Samples containing 1% solids or less: Extract the sample directly using continuous liquid-liquid extraction techniques.

10.2.1.1 Measure 1.00 L (± 0.01 L) of sample into a clean 1.5- to 2.0-L beaker.

10.2.1.2 Spike 0.5 mL of the surrogate spiking solution (Section 6.12) into the sample aliquot. Proceed to preparation of the QC aliquots for low solids samples (Section 10.2.3).

10.2.2 Samples containing 1 to 30% solids.

10.2.2.1 Mix sample thoroughly.

10.2.2.2 Using the percent solids found in Section 10.1.3, determine the weight of sample required to produce 1 L of solution containing 1% solids as follows:

Equation 3

$$\text{sample weight} = \frac{1000 \text{ g}}{\% \text{ solids}}$$

10.2.2.3 Place the weight determined in Section 10.2.2.2 in a clean 1.5- to 2.0-L beaker. Discard all sticks, rocks, leaves, and other foreign material prior to weighing.

10.2.2.4 Bring the volume of the sample aliquot(s) to 100 to 200 mL with reagent water.

10.2.2.5 Spike 0.5 mL of the surrogate spiking solution (Section 6.12) into each sample aliquot.

10.2.2.6 Using a clean metal spatula, break any solid portions of the sample into small pieces.

10.2.2.7 Place the $\frac{3}{4}$ -inch horn on the ultrasonic probe approximately $\frac{1}{2}$ inch below the surface of each sample aliquot and pulse at 50% for 3 minutes at full power. If necessary, remove the probe from the solution and break any large pieces using the metal spatula or a stirring rod and repeat the sonication. Clean the probe with methylene chloride:acetone (1:1) between samples to preclude cross-contamination.

10.2.2.8 Bring the sample volume to 1.0 L (± 0.1 L) with reagent water.

10.2.3 Preparation of QC aliquots for samples containing low solids (less than 30%).

10.2.3.1 For each sample or sample batch (to a maximum of 20) to be extracted at the same time, place two 1.0 L (± 0.01 L) aliquots of reagent water in clean 1.5- to 2.0-L beakers.

10.2.3.2 Blank: Spike 0.5 mL of the surrogate spiking solution (Section 6.12) into one reagent water aliquot.

10.2.3.3 Spike the combined QC standard (Section 7.4) into the remaining reagent water aliquot.

10.2.3.4 If a matrix spike is required, prepare an aliquot at the concentrations specified in Section 8.4.

10.2.4 Stir and equilibrate all sample and QC solutions for 1 to 2 hours. Extract the samples and QC aliquots per Section 10.3.

10.2.5 Samples containing 30% solids or more.

10.2.5.1 Mix the sample thoroughly.

10.2.5.2 Weigh 30 g (± 0.3 g) into a clean 400- to 500-mL beaker. Discard all sticks, rocks, leaves, and other foreign material prior to weighing.

10.2.5.3 Spike 0.5 mL of the surrogate spiking solution (Section 6.12) into the aliquot.

10.2.5.4 QC aliquot: For each sample or sample batch (to a maximum of 20) to be extracted at the same time, place 30 g (± 0.3 g) of the high-solids reference matrix in each of two clean 400- to 500-mL beakers.

10.2.5.5 Blank: Spike 0.5 mL of the surrogate spiking solution (Section 6.12) into one aliquot of the high-solids reference matrix.

10.2.5.6 Spike the combined QC standard (Section 6.11) into the remaining high-solids reference matrix aliquot. Extract the high-solids samples per Section 10.4.

10.3 Continuous extraction of low-solids (aqueous) samples: Place 100 to 150 mL methylene chloride in each continuous extractor and 200 to 300 mL in each distilling flask.

10.3.1 Pour the sample(s), blank, and standard aliquots into the extractors. Rinse the glass containers with 50 to 100 mL methylene chloride and add to the respective extractors. Include all solids in the extraction process.

10.3.2 Extraction: Adjust the pH of the waters in the extractors to 5 to 9 with NaOH or H_2SO_4 while monitoring with a pH meter. Caution: Some samples require acidification in a hood because of the potential for generating hydrogen sulfide.

10.3.3 Begin the extraction by heating the flask until the methylene chloride is boiling. When properly adjusted, one to two drops of methylene chloride per second will fall from the condenser tip into the water. Test and adjust the pH of the waters during the first 1 to 2 hours of extraction. Extract for 18 to 24 hours.

10.3.4 Remove the distilling flask, estimate and record the volume of extract (to the nearest 100 mL), and pour the contents through a prerinsed drying column containing 7 to 10 cm of anhydrous sodium sulfate. Rinse the distilling flask with 30 to 50 mL of methylene chloride and pour through the drying column. For extracts to be cleaned up

using GPC, collect the solution in a 500-mL K-D evaporator flask equipped with a 10-mL concentrator tube. Seal, label the pesticide and herbicide fractions, and concentrate per Sections 10.5 to 10.6.

10.4 Ultrasonic extraction of high solids samples: Procedures are provided for extraction of non-municipal sludge (Section 10.4.1) and municipal sludge samples (Section 10.4.2).

10.4.1 Ultrasonic extraction of non-municipal sludge high-solids aliquots.

10.4.1.1 Add 60 to 70 g of powdered sodium sulfate to the sample and QC aliquots. Mix each aliquot thoroughly. Some wet sludge samples may require more than 70 g for complete removal of water. All water must be removed prior to addition of organic solvent so that the extraction process is efficient.

10.4.1.2 Add 100 mL (± 10 mL) of acetone:methylene chloride (1:1) to each of the aliquots and mix thoroughly.

10.4.1.3 Place the $\frac{3}{4}$ -inch horn on the ultrasonic probe approximately $\frac{1}{2}$ inch below the surface of the solvent but above the solids layer and pulse at 50% for 3 minutes at full power. If necessary, remove the probe from the solution and break any large pieces using a metal spatula or a stirring rod and repeat the sonication.

10.4.1.4 Decant the extract through a prerinsed drying column containing 7 to 10 cm anhydrous sodium sulfate into a 500- to 1000-mL graduated cylinders.

10.4.1.5 Repeat the extraction steps (Sections 10.4.1.3 to 10.4.1.4) twice more for each sample and QC aliquot. On the final extraction, swirl the sample or QC aliquot, pour into its respective drying column, and rinse with acetone:methylene chloride. Record the total extract volume. If necessary, transfer the extract to a centrifuge tube and centrifuge for 10 minutes to settle fine particles.

10.4.2 Ultrasonic extraction of high-solids municipal sludge aliquots.

10.4.2.1 Add 100 mL (± 10 mL) of acetonitrile to each of the aliquots and mix thoroughly.

10.4.2.2 Place the $\frac{3}{4}$ -inch horn on the ultrasonic probe approximately $\frac{1}{2}$ inch below the surface of the solvent but above the solids layer and pulse at 50% for 3 minutes at full power. If necessary, remove the probe from the solution and break any large pieces using a metal spatula or a stirring rod and repeat the sonication.

10.4.2.3 Decant the extract through filter paper into a 1000- to 2000-mL separatory funnel.

10.4.2.4 Repeat the extraction and filtration steps (Sections 10.4.2.2 to 10.4.2.3) using a second 100 mL (± 10 mL) of acetonitrile.

10.4.2.5 Repeat the extraction step (Section 10.4.2.3) using 100 mL (± 10 mL) of methylene chloride. On this final extraction, swirl the sample or QC aliquot, pour into its respective filter paper, and rinse with methylene chloride. Record the total extract volume.

10.4.2.6 For each extract, prepare 1.5 to 2 L of reagent water containing 2% sodium sulfate. Adjust the pH of the water to 6.0 to 9.0 with NaOH or H₂SO₄.

10.4.2.7 Back-extract each extract three times sequentially with 500 mL of the aqueous sodium sulfate solution, returning the bottom (organic) layer to the separatory funnel the first two times while discarding the top (aqueous) layer. On the final back extraction, filter each pesticide extract through a prerinsed drying column containing 7 to 10 cm anhydrous sodium sulfate into a 500- to 1000-mL graduated cylinder. Record the final extract volume.

10.4.3 For extracts to be cleaned up using GPC, filter these extracts through Whatman #41 paper into a 500-mL K-D evaporator flask equipped with a 10-mL concentrator tube. Rinse the graduated cylinder or centrifuge tube with 30 to 50 mL of methylene chloride and pour through filter to complete the transfer. Seal and label the K-D flasks. Concentrate these fractions per Sections 10.5 through 10.8.

10.5 Macro concentration.

10.5.1 Concentrate the extracts in separate 500-mL K-D flasks equipped with 10-mL concentrator tubes. Add one to two clean boiling chips to the flask and attach a three-ball macro Snyder column. Prewet the column by adding approximately 1 mL of methylene chloride through the top. Place the K-D apparatus in a hot water bath so that the entire lower rounded surface of the flask is bathed with steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

10.5.2 When the liquid has reached an apparent volume of 1 mL, remove the K-D apparatus from the bath and allow the solvent to drain and cool for at least 10 minutes.

10.5.3 If the extract is to be cleaned up using GPC, remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommended for this operation. Adjust the final volume to 10 mL and proceed to GPC cleanup in Section 11.

10.6 Hexane exchange: Extracts to be subjected to Florisil or silica gel cleanup and extracts that have been cleaned up are exchanged into hexane.

10.6.1 Remove the Snyder column, add approximately 50 mL of hexane and a clean boiling chip, and reattach the Snyder column. Concentrate the extract as in Section 10.5 except use hexane to prewet the column. The elapsed time of the concentration should be 5 to 10 minutes.

10.6.2 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane. Adjust the final volume of extracts that have not been cleaned up by GPC to 10 mL and those that have been cleaned up by GPC to 5 mL (the difference accounts for the 50% loss in the GPC cleanup). Clean up the extracts using the Florisil, silica gel, and/or sulfur removal procedures in Section 11.

11. CLEANUP AND SEPARATION

11.1 Cleanup procedures may not be necessary for relatively clean samples (treated effluents, ground water, drinking water). If particular circumstances require the use of a cleanup procedure, the analyst may use any or all of the procedures below or any other appropriate procedure. However, the analyst shall first repeat the tests in Section 8.2 to demonstrate that the requirements of Section 8.2 can be met using the cleanup procedure(s) as an integral part of the method. Figure 1 outlines the cleanup steps.

11.1.1 Gel permeation chromatography (Section 11.2) removes many high molecular weight interferents that cause GC column performance to degrade. It is used for all soil and sediment extracts and may be used for water extracts that are expected to contain high molecular weight organic compounds (e.g., polymeric materials, humic acids).

11.1.2 The solid-phase extraction cartridge (Section 11.3) removes polar organic compounds such as phenols.

11.1.3 The Florisil column (Section 11.4) allows for selected fractionation of the organo-chlorine compounds and will also eliminate polar interferences.

11.1.4 Alumina column cleanup (Section 11.5) may also be used for cleanup of the organo-chlorine compounds.

11.1.5 Elemental sulfur, which interferes with the electron capture gas chromatography of some of the pesticides, is removed using GPC, mercury, or activated copper. Sulfur removal (Section 11.6) is required when sulfur is known or suspected to be present.

11.2 Gel permeation chromatography (GPC).

11.2.1 Column packing.

11.2.1.1 Place 70 to 75 g of SX-3 Bio-beads in a 400- to 500-mL beaker.

11.2.1.2 Cover the beads with methylene chloride and allow to swell overnight (12 hours minimum).

11.2.1.3 Transfer the swelled beads to the column and pump solvent through the column, from bottom to top, at 4.5 to 5.5 mL/min prior to connecting the column to the detector.

11.2.1.4 After purging the column with solvent for 1 to 2 hours, adjust the column head pressure to 7 to 10 psig, and purge for 4 to 5 hours to remove air. Maintain a head pressure of 7 to 10 psig. Connect the column to the detector.

11.2.2 Column calibration.

11.2.2.1 Load 5 mL of the calibration solution (Section 6.5) into the sample loop.

11.2.2.2 Inject the calibration solution and record the signal from the detector. The elution pattern will be corn oil, bis (2-ethylhexyl) phthalate, pentachlorophenol, perylene, and sulfur.

11.2.2.3 Set the "dump time" to allow greater than 85% removal of the corn oil and greater than 85% collection of the phthalate.

11.2.2.4 Set the "collect time" to the peak minimum between perylene and sulfur.

11.2.2.5 Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the pentachlorophenol is greater than 85%. If calibration is not verified, the system shall be recalibrated using the calibration solution, and the previous 20 samples shall be re-extracted and cleaned up using the calibrated GPC system.

11.2.3 Extract cleanup: GPC requires that the column not be overloaded. The column specified in this method is designed to handle a maximum of 0.5 g of high molecular weight material in a 5-mL extract. If the extract is known or expected to contain more than 0.5 g, the extract is split into fractions for GPC and the fractions are combined after elution from the column. The solids content of the extract may be obtained gravimetrically by evaporating the solvent from a 50- μ L aliquot.

11.2.3.1 Filter the extract or load through the filter holder to remove particulates. Load the 5.0-mL extract onto the column.

11.2.3.2 Elute the extract using the calibration data determined in Section 11.2.2. Collect the eluate in a clean 400- to 500-mL beaker.

11.2.3.3 Rinse the sample loading tube thoroughly with methylene chloride between extracts to prepare for the next sample.

11.2.3.4 If a particularly dirty extract is encountered, a 5.0-mL methylene chloride blank shall be run through the system to check for carry-over.

11.2.3.5 Concentrate the extract and exchange into hexane per Sections 10.5 and 10.6. Adjust the final volume to 5.0 mL.

11.3 Solid-phase extraction (SPE).

11.3.1 Setup.

11.3.1.1 Attach the Vac-elute manifold to a water aspirator or vacuum pump with the trap and gauge installed between the manifold and vacuum source.

11.3.1.2 Place the SPE cartridges in the manifold, turn on the vacuum source, and adjust the vacuum to 5 to 10 psia.

11.3.2 Cartridge washing: Pre-elute each cartridge prior to use sequentially with 10-mL portions each of hexane, methanol, and water using vacuum for 30 seconds after each eluant. Follow this pre-elution with 1 mL methylene chloride and three 10-mL portions of the elution solvent (Section 6.6.2.2) using vacuum for 5 minutes after each eluant. Tap the cartridge lightly while under vacuum to dry between eluants. The three portions of elution solvent may be collected and used as a blank if desired. Finally, elute the cartridge with 10 mL each of methanol and water, using the vacuum for 30 seconds after each eluant.

11.3.3 Cartridge certification: Each cartridge lot must be certified to ensure recovery of the compounds of interest and removal of 2,4,6-trichlorophenol.

11.3.3.1 To make the test mixture, add the trichlorophenol solution (Section 6.6.2.1) to the combined calibration standard (Section 6.11). Elute the mixture using the procedure in Section 11.3.4.

11.3.3.2 Concentrate the eluant to 1.0 mL and inject 1.0 μ L of the concentrated eluant into the GC using the procedure in Section 13. The recovery of all analytes (including the unresolved GC peaks) shall be within the ranges for recovery specified in Table 4, and the peak for trichlorophenol shall not be detectable; otherwise the SPE cartridge is not performing properly and the cartridge lot shall be rejected.

11.3.4 Extract cleanup.

11.3.4.1 After cartridge washing (Section 11.3.2), release the vacuum and place the rack containing the 50-mL volumetric flasks (Section 5.6.2.4) in the vacuum manifold. Re-establish the vacuum at 5 to 10 psia.

11.3.4.2 Using a pipette or a 1-mL syringe, transfer 1.0 mL of extract to the SPE cartridge. Apply vacuum for 5 minutes to dry the cartridge. Tap gently to aid in drying.

11.3.4.3 Elute each cartridge into its volumetric flask sequentially with three 10-mL portions of the elution solvent (Section 6.6.2.2), using vacuum for 5 minutes after each portion. Collect the eluants in the 50-mL volumetric flasks.

11.3.4.4 Release the vacuum and remove the 50-mL volumetric flasks.

11.3.4.5 Concentrate the eluted extracts to 1.0 mL using the nitrogen blow-down apparatus.

11.4 Florisil column.

11.4.1 Place a weight of Florisil (nominally 20 g) predetermined by calibration (Section 7.5) in a chromatographic column. Tap the column to settle the Florisil and add 1 to 2 cm of anhydrous sodium sulfate to the top.

11.4.2 Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate layer to the air, stop the elution of the hexane by closing the stopcock on the chromatographic column. Discard the eluate.

11.4.3 Transfer the concentrated extract (Section 10.6.2) onto the column. Complete the transfer with two 1-mL hexane rinses.

11.4.4 Place a clean 500-mL K-D flask and concentrator tube under the column. Drain the column into the flask until the sodium sulfate layer is nearly exposed. Elute Fraction 1 with 200 mL of 6% ethyl ether in hexane (V/V) at a rate of approximately 5 mL/min. Remove the K-D flask. Elute Fraction 2 with 200 mL of 15% ethyl ether in hexane (V/V) into a second K-D flask. Elute Fraction 3 with 200 mL of 50% ethyl ether in hexane (V/V).

11.4.5 Concentrate the fractions as in Section 10.6, except use hexane to prewet the column. Readjust the final volume to 5 or 10 mL as in Section 10.6, depending on whether the extract was subjected to GPC cleanup, and analyze by gas chromatography per the procedure in Section 12.

11.5 Alumina column.

11.5.1 Reduce the volume of the extract to 0.5 mL and bring to 1.0 mL with acetone.

- 11.5.2** Add 3 g of activity III neutral alumina to a 10-mL chromatographic column. Tap the column to settle the alumina.
- 11.5.3** Transfer the extract to the top of the column and collect the eluate in a clean 10-mL concentrator tube. Rinse the extract container with 1 to 2 mL portions of hexane (to a total volume of 9 mL) and add to the alumina column. Do not allow the column to go dry.
- 11.5.4** Concentrate the extract to 1.0 mL if sulfur is to be removed, or adjust the final volume to 5 or 10 mL as in Section 10.6, depending on whether the extract was subjected to GPC cleanup, and analyze by gas chromatography per Section 13.
- 11.6 Sulfur removal:** Elemental sulfur will usually elute entirely in Fraction 1 of the Florisil column cleanup.
- 11.6.1** Transfer the concentrated extract into a clean concentrator tube or PTFE-sealed vial. Add 1 to 2 drops of mercury or 100 mg of activated copper powder and seal (Reference 8). If TBA sulfite is used, add 1 mL of the TBA sulfite reagent and 2 mL of isopropanol.
- 11.6.2** Agitate the contents of the vial for 1 to 2 hours on a reciprocal shaker. If the mercury or copper appears shiny, or if precipitated sodium sulfite crystals from the TBA sulfite reagent are present, and if the color remains unchanged, all sulfur has been removed; if not, repeat the addition and shaking.
- 11.6.2.1** If mercury or copper is used, centrifuge and filter the extract to remove all residual mercury or copper. Dispose of the mercury waste properly. Bring the final volume to 1.0 mL and analyze by gas chromatography per the procedure in Section 13.
- 11.6.2.2** If TBA sulfite is used, add 5 mL of reagent water and shake for 1 to 2 minutes. Centrifuge and filter the extract to remove all precipitate. Transfer the hexane (top) layer to a sample vial and adjust the final volume to 5 or 10 mL as in Section 10.6, depending on whether the extract was subjected to GPC cleanup, and analyze by gas chromatography per Section 12.

12. GAS CHROMATOGRAPHY

Table 2 summarizes the recommended operating conditions for the gas chromatograph. Included in these tables are the retention times and minimum levels that can be achieved under these conditions. Examples of the separations achieved by the primary and confirmatory columns are shown in Figure 2.

- 12.1** Calibrate the system as described in Section 7.
- 12.2** Set the auto-sampler to inject the same volume that was chosen for calibration (Section 7.3.1.3) for all standards and extracts of blanks and samples.
- 12.3** Set the data system or GC control to start the temperature program upon sample injection, and begin data collection after the solvent peak elutes. Set the data system to stop data collection after the last analyte is expected to elute and to return the column to the initial temperature.

13. SYSTEM AND LABORATORY PERFORMANCE

- 13.1** At the beginning of each 8-hour shift during which analyses are performed, GC system performance and calibration are verified for all pollutants and surrogates on both column/detector systems. For these tests, analysis of the combined QC standard (Section 6.11) shall be used to verify all performance criteria. Adjustment and/or recalibration (per Section 7) shall be performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks, and precision and recovery standards be analyzed.
- 13.2** Retention times: The absolute retention times of the peak maxima shall be within ± 10 seconds of the retention times in the initial calibration (Section 7.4.1).
- 13.3** GC resolution: Resolution is acceptable if the valley height between two peaks (as measured from the baseline) is less than 10% of the taller of the two peaks.
- 13.3.1** Primary column (DB-608): DDT and endrin aldehyde.
- 13.3.2** Confirmatory column (DB-1701): Alpha and gamma chlordane.
- 13.4** Decomposition of DDT and endrin.
- 13.4.1** Analyze a total of 2 ng DDT and 1 ng endrin on each column using the analytical conditions specified in Table 2.
- 13.4.2** Measure the total area of all peaks in the chromatogram.
- 13.4.3** The area of peaks other than the sum of the areas of the DDT and endrin peaks shall be less than 20% the sum of the areas of these two peaks. If the area is greater than this sum, the system is not performing acceptably for DDT and endrin. In this case, the GC system that failed shall be repaired and the performance tests (Sections 13.1 to 13.4) shall be repeated until the specification is met.

NOTE: DDT and endrin decomposition are usually caused by accumulations of particulates in the injector and in the front end of the column. Cleaning and silanizing the injection port liner, and breaking off a short section of the front end of the column will usually eliminate the decomposition problem.

- 13.5** Calibration verification: Calibration is verified for the combined QC standard only.
- 13.5.1** Inject the combined QC standard (Section 6.11)
- 13.5.2** Compute the percent recovery of each compound or coeluting compounds, based on the calibration data (Section 7.4).
- 13.5.3** For each compound or coeluted compounds, compare this calibration verification recovery with the corresponding limits for ongoing accuracy in Table 4. For coeluting compounds, use the coeluted compound with the least restrictive specification (the widest range). If the recoveries for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any recovery falls outside the calibration verification range, system performance is unacceptable for that compound. In this case, correct the problem and repeat the test, or recalibrate (Section 7). If verification requirements are met, the calibration is assumed to be valid for the multicomponent analytes (PCBs and toxaphene).

13.6 Ongoing precision and recovery.

- 13.6.1 Analyze the extract of the precision and recovery standard extracted with each sample batch (Sections 10.2.3.3 and 10.2.5.7).
- 13.6.2 Compute the percent recovery of each analyte and for coeluting compounds.
- 13.6.3 For each compound or coeluted compound, compare the percent recovery with the limits for ongoing recovery in Table 4. For coeluted compounds, use the coeluted compound with the least restrictive specification (widest range). If all analytes pass, the extraction, concentration, and cleanup processes are in control and analysis of blanks and samples may proceed. If, however, any of the analytes fail, these processes are not in control. In this event, correct the problem, re-extract the sample batch, and repeat the ongoing precision and recovery test.
- 13.6.4 Add results which pass the specifications in Section 13.6.3 to initial and previous ongoing data. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory data quality for each analyte by calculating the average percent recovery (R) and the standard deviation of percent recovery s_r . Express the accuracy as a recovery interval from $R - 2s_r$ to $R + 2s_r$. For example, if $R=95\%$ and $s_r=5\%$, the accuracy is 85 to 105%.

14. QUALITATIVE DETERMINATION

- 14.1 Qualitative determination is accomplished by comparison of data from analysis of a sample or blank with data from analysis of the shift standard (Section 13.2), and with data stored in the retention-time and calibration libraries (Section 7.3.2 and 7.3.3.2). Identification is confirmed when retention time and amounts agree per the criteria below.
- 14.2 For each compound on each column/detector system, establish a retention-time window ± 20 seconds on either side of the retention time in the calibration data (Section 7.3.2). For compounds that have a retention-time curve (Section 7.3.2.2), establish this window as the minimum -20 seconds and maximum +20 seconds. For the multi-component analytes, use the retention times of the five largest peaks in the chromatogram from the calibration data (Section 7.3.2).
 - 14.2.1 Compounds not requiring a retention-time calibration curve: If a peak from the analysis of a sample or blank is within a window (as defined in Section 14.2) on the primary column/detector system, it is considered tentatively identified. A tentatively identified compound is confirmed when (1) the retention time for the compound on the confirmatory column/detector system is within the retention-time window on that system, and (2) the computed amounts (Section 15) on each system (primary and confirmatory) agree within a factor of three.
 - 14.2.2 Compounds requiring a retention-time calibration curve: If a peak from the analysis of a sample or blank is within a window (as defined in Section 14.2) on the primary column/detector system, it is considered tentatively identified. A tentatively identified compound is confirmed when (1) the retention times on both systems (primary and confirmatory) are within ± 30 seconds of the retention times for the computed amounts (Section 15), as determined by the retention-time calibration curve (Section 7.3.2.2), and (2) the computed amounts (Section 15) on each system (primary and confirmatory) agree within a factor of 3.

15. QUANTITATIVE DETERMINATION

- 15.1 Using the GC data system, compute the concentration of the analyte detected in the extract (in micrograms per milliliter) using the calibration factor or calibration curve (Section 7.3.3.2).
- 15.2 Liquid samples: Compute the concentration in the sample using the following equation:

Equation 4

$$C_s = 10 \frac{(C_{ex})}{(V_s)}$$

where

C_s = The concentration in the sample, in $\mu\text{g/L}$

10 = The extract total, in mL

C_{ex} = The concentration in the extract, in $\mu\text{g/mL}$

V_s = The sample extracted, in L

- 15.3 Solid samples: Compute the concentration in the solid phase of the sample using the following equation:

Equation 5

$$C_s = 10 \frac{(C_{ex})}{1000(W_s)(\text{solids})}$$

where

C_s = Concentration in the sample, in $\mu\text{g/kg}$

10 = Extract total, in mL

C_{ex} = Concentration in the extract, in $\mu\text{g/mL}$

1000 = Conversion factor, g to kg

W_s = Sample weight, in g

solids = Percent solids in Section 10.1.3 divided by 100

- 15.4 If the concentration of any analyte exceeds the calibration range of the system, the extract is diluted by a factor of 10, and a 1- μL aliquot of the diluted extract is analyzed.
- 15.5 Two or more PCBs in a given sample are quantitated and reported as total PCB.
- 15.6 Report results for all pollutants found in all standards, blanks, and samples to three significant figures. Results for samples that have been diluted are reported at the least dilute level at which the concentration is in the calibration range.

16. ANALYSIS OF COMPLEX SAMPLES

- 16.1 Some samples may contain high levels (greater than 1000 ng/L) of the compounds of interest, interfering compounds, and/or polymeric materials. Some samples may not concentrate to 10 mL (Section 10.6); others may overload the GC column and/or detector.

- 16.2** The analyst shall attempt to clean up all samples using GPC (Section 11.2), the SPE cartridge (Section 11.3), by Florisil (Section 11.4) or Alumina (11.5), and sulfur removal (Section 11.6). If these techniques do not remove the interfering compounds, the extract is diluted by a factor of 10 and reanalyzed (Section 15.4).
- 16.3** Recovery of surrogate: In most samples, surrogate recoveries will be similar to those from reagent water or from the high solids reference matrix. If the surrogate recovery is outside the range specified in Section 8.3.3, the sample shall be reextracted and reanalyzed. If the surrogate recovery is still outside this range, the extract is diluted by a factor of 10 and reanalyzed (Section 15.4).
- 16.4** Recovery of matrix spikes: In most samples, matrix spike recoveries will be similar to those from reagent water or from the high-solids reference matrix. If the matrix spike recovery is outside the range specified in Table 4, the sample shall be diluted by a factor of 10, respiked, and reanalyzed. If the matrix spike recovery is still outside the range, the method may not apply to the sample being analyzed and the result may not be reported for regulatory compliance purposes.

17. METHOD PERFORMANCE

- 17.1** Development of this method is detailed in References 9 and 10.

References

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Table 1. Organo-Halide Pesticides Determined by Large-Bore, Fused-Silica Capillary Column Gas Chromatography with Halide-Specific Detector

EPA EGD	Compound	CAS Registry
	Acephate	30560-19-1
	Acifluorfen	50594-66-6
	Alachlor	15972-60-8
089	Aldrin	309-00-2
	Atrazine	1912-24-9
	Benfluralin (Benefin)	1861-40-1
102	α -BHC	319-84-6
103	β -BHC	319-85-7
104	δ -BHC (Lindane)	58-89-9
105	γ -BHC	319-86-8
	Bromacil	314-40-9
	Bromoxynil octanoate	1689-99-2
	Butachlor	23184-66-9
434	Captafol	2425-06-1
433	Captan	133-06-2
441	Carbophenothion (Trithion)	786-19-6
	α -Chlordane (cis-Chlordane)	5103-71-9
091	γ -Chlordane (trans-Chlordane)	5566-34-7
431	Chlorobenzilate	510-15-6
	Chloroneb (Terraneb)	2675-77-6
	Chloropropylate (Acaralate)	5836-10-2
	Chlorothalonil	1897-45-6
	DBCP (Dibromochloropropane)	96-12-8
	DCPA (Dacthal)	1861-32-1
094	4,4'-DDD (TDE)	72-54-8
093	4,4'-DDE	72-55-9
092	4,4'-DDT	50-29-3
432	Diallate (Avadex)	2303-16-4

Table 1. Organo-Halide Pesticides Determined by Large-Bore, Fused-Silica Capillary Column Gas Chromatography with Halide-Specific Detector, cont.

EPA EGD	Compound	CAS Registry
478	Dichlone	117-80-6
	Dicofol	115-32-2
090	Dieldrin	60-57-1
095	Endosulfan I	959-98-8
096	Endosulfan II	33213-65-9
097	Endosulfan sulfate	1031-07-8
098	Endrin	72-20-8
099	Endrin aldehyde	7421-93-4
435	Endrin ketone	53494-70-5
	Ethalfuralin (Sonalan)	55283-68-6
	Etridiazole	2593-15-9
	Fenarimol (Rubigan)	60168-88-9
100	Heptachlor	76-44-8
101	Heptachlor epoxide	1024-57-3
437	Isodrin	465-73-6
	Isopropalin (Paarlan)	33820-53-0
439	Kepone	143-50-0
430	Methoxychlor	72-43-5
	Metribuzin	21087-64-9
438	Mirex	2385-85-5
436	Nitrofen (TOK)	1836-75-5
	Norfluorazon	27314-13-2
112	PCB-1016	12674-11-2
108	PCB-1221	11104-28-2
109	PCB-1232	11141-16-5
106	PCB-1242	53469-21-9
110	PCB-1248	12672-29-6

Table 1. Organo-Halide Pesticides Determined by Large-Bore, Fused-Silica Capillary Column Gas Chromatography with Halide-Specific Detector, cont.

<i>EPA EGD</i>	<i>Compound</i>	<i>CAS Registry</i>
107	PCB-1254	11097-69-1
111	PCB-1260	11096-82-5
440	PCNB (pentachloronitrobenzene)	82-68-8
	Pendamethalin (Prowl)	40487-42-1
	cis-Permethrin	61949-76-6
	trans-Permethrin	61949-77-7
	Perthane (Ethylan)	72-56-0
	Propachlor	1918-16-7
	Propanil	709-98-8
	Propazine	139-40-2
	Simazine	122-34-9
	Strobane	8001-50-1
	Terbacil	5902-51-2
	Terbuthylazine	5915-41-3
113	Toxaphene	8001-35-2
	Triadimefon (Bayleton)	43121-43-3
442	Trifluralin	1582-09-8

Table 2. Gas Chromatography of Organo-Halide Pesticides

EPA EGD	Compound	Retention Time (min)(1)		Method Detection Limit(2) (ng/L)	
		DB-608	DB-1701		
	Acephate	5.03	(3)	2000	est (ECD)
442	Trifluralin	5.16	6.79	50	est
	Ethalfuralin	5.28	6.49	5	est (ECD)
	Benfluralin	5.53	6.87	20	est (ECD)
432	Diallate-A	7.15	6.23	45	
	Diallate-B	7.42	6.77	32	
102	α -BHC	8.14	7.44	6	
440	PCNB	9.03	7.58	6	
	Simazine	9.06	9.29	400	est (ECD)
	Atrazine	9.12	9.12	500	est (ECD)
	Terbuthylazine	9.17	9.46	300	est (ECD)
104	δ -BHC (Lindane)	9.52	9.91	11	
103	β -BHC	9.86	11.90	7	
100	Heptachlor	10.66	10.55	5	
	Chlorothalonil	10.66	10.96	15	est (ECD)
478	Dichlone	10.80	(3)	(4)	
	Terbacil	11.11	12.63	200	est (ECD)
105	γ -BHC	11.20	12.98	5	
	Alachlor	11.57	11.06	20	est (ECD)
	Propanil	11.60	14.10		
089	Aldrin	11.84	11.46	8	
	DCPA	12.18	12.09	3	est (ECD)
	Metribuzin	12.80	11.68	5	est (ECD)
	Triadimefon	12.99	13.57	50	est (ECD)
	Isopropalin	13.06	13.37	20	est (ECD)
437	Isodrin	13.47	11.12	13	
101	Heptachlor epoxide	13.97	12.56	12	
	Pendamethalin	14.21	13.46	30	

Table 2. Gas Chromatography of Organo-Halide Pesticides, cont.

EPA EGD	Compound	Retention Time (min)(1)		Method Detection Limit(2) (ng/L)
		DB-608	DB-1701	
	Bromacil	14.39	(3)	70 est (ECD)
	δ -Chlordane	14.63	14.20	9
	Butachlor	15.03	15.69	30 est (ECD)
091	α -Chlordane	15.24	14.36	8
095	Endosulfan I	15.25	13.87	11
093	4,4'-DDE	16.34	14.84	10
090	Dieldrin	16.41	15.25	6
433	Captan	16.83	15.43	100 est (ECD)
431	Chlorobenzilate	17.58	17.28	25
098	Endrin	17.80	15.86	4
436	Nitrofen (TOK)	17.86	17.47	13
439	Kepone	17.92	24.03	100 est (ECD)
094	4,4'-DDD	18.43	17.77	5
096	Endosulfan II	18.45	18.57	8
	Bromoxynil octanoate	18.85	18.57	30 est (ECD)
092	4,4'-DDT	19.48	18.32	12
441	Carbophenothion	19.65	18.21	50
099	Endrin aldehyde	19.72	19.18	11
097	Endosulfan sulfate	20.21	20.37	7
434	Captafol	22.51	21.22	100 est (ECD)
	Norfluorazon	20.68	22.01	50 est (ECD)
438	Mirex	22.75	19.79	4
430	Methoxychlor	22.80	20.68	30
435	Endrin ketone	23.00	21.79	8
	Fenarimol	24.53	23.79	20 est (ECD)
	cis-Permethrin	25.00	23.59	200 est (ECD)
	trans-Permethrin	25.62	23.92	200 est (ECD)
106	PCB-1242			150 est

Table 2. Gas Chromatography of Organo-Halide Pesticides, cont.

EPA EGD	Compound	Retention Time (min)(1)		Method Detection Limit(2) (ng/L)
		DB-608	DB-1701	
109	PCB-1232			150 est
112	PCB-1016			150 est
108	PCB-1221			150 est
110	PCB-1248			150 est
107	PCB-1254			150 est
111	PCB-1260	15.44	14.64	140
		15.73	15.36	
		16.94	16.53	
		17.28	18.70	
		19.17	19.92	
113	Toxaphene	16.60	16.60	910
		17.37	17.52	
		18.11	17.92	
		19.46	18.73	
		19.69	19.00	

Notes:

1. Columns: 30 m long by 0.53 mm ID; DB-608: 0.83 μ ; DB-1701: 1.0 μ . Conditions suggested to meet retention times shown: 150°C for 0.5 minutes, 150 to 270° at 5°C/min, 270°C until trans-permethrin elutes. Carrier gas flow rates approximately 7 mL/min.
2. 40 CFR Part 136, Appendix B (49 FR 43234). MDL's were obtained with an electrolytic conductivity detector, except as noted. MDL's for soils (in ng/kg) are estimated to be 30 to 100 times this level.
3. Does not elute from DB-1701 column at level tested.
4. Not recovered from water at the levels tested.

Table 3. Concentrations of Calibration Solutions for Electron Capture Detector and Suggested Calibration Groups

EPA EGD	Compound	Concentration (ng/mL)		
		Low	Medium	High
	Calibration group 1			
	Acephate	2000	10000	40000
	Alachlor	20	100	400
	Atrazine	1000	5000	20000
103	β -BHC	10	50	200
	Bromoxynil octanoate	50	250	1000
434	Captafol	200	1000	4000
432	Diallate	200	1000	4000
097	Endosulfan sulfate	10	50	200
098	Endrin	20	100	400
437	Isodrin	10	50	200
	Pendimethalin (Prowl)	50	250	1000
	trans-Permethrin	200	1000	4000
	Calibration group 2			
102	α -BHC	5.0	25	100
	DCPA	5.0	25	100
093	4,4'-DDE	10	50	200
092	4,4'-DDT	10	50	200
478	Dichlone	20	100	400
	Ethalfuralin	10	50	200
	Fenarimol	20	100	400
430	Methoxychlor	20	100	400
	Metribuzin	10	50	200
	Calibration group 3			
105	γ -BHC	5	25	100
091	δ -Chlordane	5	25	100
435	Endrin ketone	10	50	200
101	Heptachlor epoxide	5	25	100

Table 3. Concentrations of Calibration Solutions for Electron Capture Detector and Suggested Calibration Groups, cont.

EPA EGD	Compound	Concentration (ng/mL)		
		Low	Medium	High
	Isopropalin	20	100	400
436	Nitrofen (TOK)	20	100	400
440	PCNB	5	25	100
	cis-Permethrin	200	1000	4000
442	Trifluralin	10	50	200
	Calibration group 4			
	Benfluralin	20	100	400
431	Chlorobenzilate	50	500	5000
090	Dieldrin	5	20	100
095	Endosulfan I	10	50	200
438	Mirex	20	100	400
	Terbacil	200	1000	4000
	Terbuthylazine	500	2500	10000
	Triadimefon	100	500	2000
	Calibration group 5			
	α -Chlordane	10	50	200
433	Captan	100	500	2000
	Chlorothalonil	20	100	400
094	4,4'-DDD	20	100	400
	Norfluorazon	100	500	2000
	Simazine	800	4000	20000
	Calibration group 6			
089	Aldrin	20	100	400
104	δ -BHC (Lindane)	5	25	100
	Bromacil	100	500	2000
	Butachlor	50	250	1000
096	Endosulfan II	100	500	2000

Table 3. Concentrations of Calibration Solutions for Electron Capture Detector and Suggested Calibration Groups, cont.

EPA EGD	Compound	Concentration (ng/mL)		
		Low	Medium	High
100	Heptachlor	10	50	200
439	Kepone	100	500	2000

Table 4. Acceptance Criteria for Performance Tests for Organo-Halide Compounds

EGD No.	Compound	Acceptance Criteria				
		Spike Level (ng/L)	Initial Precision and Accuracy		Calibration Verifi- cation*	Recovery Ongoing Accuracy
			s	X	(%)	R (%)
	Acephate	100000	94	0 - 195	6 - 194	0 - 209
	Alachlor	1000	20	26 - 100	80 - 120	23 - 101
089	Aldrin	1000	12	82 - 108	79 - 113	76 - 114
	Atrazine	50000	26	35 - 129	74 - 126	31 - 132
	Benfluralin	1000	22	45 - 125	78 - 122	42 - 128
102	α -BHC	250	10	57 - 135	69 - 108	38 - 154
103	β -BHC	500	10	66 - 130	85 - 102	50 - 146
105	δ -BHC	250	24	60 - 122	79 - 103	45 - 136
104	γ -BHC (Lindane)	250	10	66 - 112	75 - 119	55 - 123
	Bromacil	5000	84	0 - 263	16 - 184	0 - 275
	Bromoxynil octanoate	2500	28	31 - 131	72 - 128	27 - 135
	Butachlor	2500	32	21 - 137	68 - 132	17 - 141
434	Captafol	10000	76	0 - 221	24 - 176	0 - 232
433	Captan	5000	32	28 - 144	49 - 114	24 - 148
441	Carbophenothion	1000	10	63 - 141	79 - 102	43 - 161
091	Chlordane- α	500	10	79 - 122	73 - 102	69 - 133
	Chlordane- δ	250	13	32 - 140	79 - 113	4 - 169
431	Chlorobenzilate	500	19	58 - 118	54 - 129	43 - 133
	Chlorothalonil	1000	20	37 - 109	80 - 120	34 - 112
	DCPA	250	20	57 - 129	80 - 120	54 - 132
094	4,4'-DDD	1000	12	69 - 117	77 - 109	57 - 129
093	4,4'-DDE	500	13	66 - 114	81 - 121	54 - 126
092	4,4'-DDT	500	19	86 - 112	77 - 118	79 - 119
432	Diallate	1000	16	44 - 120	70 - 124	24 - 139
478	Dichlone	1000	20	45 - 117	79 - 110	42 - 120
090	Dieldrin	500	11	66 - 140	48 - 115	48 - 158

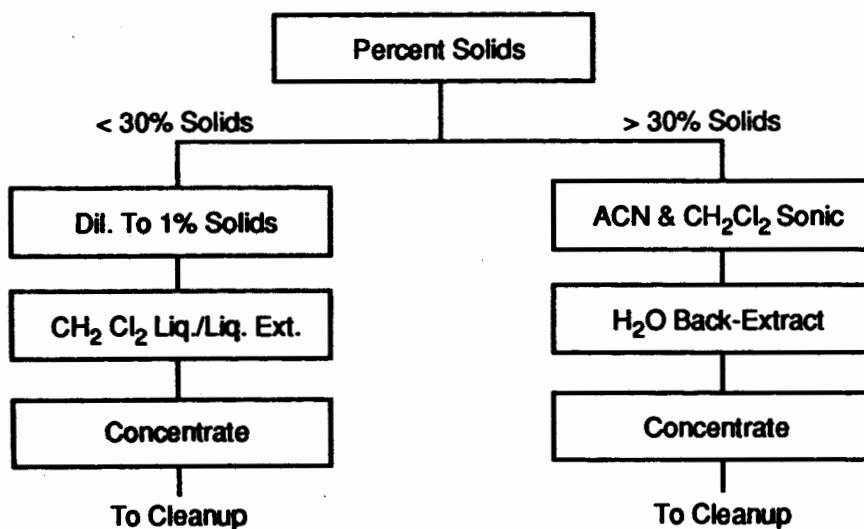
Table 4. Acceptance Criteria for Performance Tests for Organo-Halide Compounds, cont.

EGD No.	Compound	Acceptance Criteria				
		Spike Level (ng/L)	Initial Precision and Accuracy		Calibration Verifi- cation*	Recovery Ongoing Accuracy
			s	X	(%)	R (%)
095	Endosulfan I	500	14	41 - 133	78 - 119	18 - 156
096	Endosulfan II	500	19	78 - 142	76 - 119	62 - 158
097	Endosulfan sulfate	500	17	50 - 130	70 - 109	31 - 149
098	Endrin	1000	13	17 - 149	5 - 117	0 - 182
099	Endrin aldehyde	1000	13	0 - 149	86 - 117	0 - 190
435	Endrin ketone	500	25	36 - 126	68 - 135	14 - 148
	Ethalfuralin	500	24	46 - 132	76 - 124	42 - 136
	Fenarimol	1000	26	46 - 140	74 - 126	42 - 144
100	Heptachlor	500	12	78 - 104	80 - 114	71 - 111
101	Heptachlor epoxide	250	13	63 - 117	79 - 117	49 - 131
437	Isodrin	500	15	69 - 113	71 - 126	45 - 127
	Isopropalin	1000	20	47 - 129	80 - 120	54 - 132
439	Kepone	5000	46	31 - 197	47 - 134	25 - 203
430	Methoxychlor	200	19	50 - 136	47 - 128	28 - 158
	Metribuzin	500	24	54 - 140	76 - 124	50 - 155
438	Mirex	1000	23	25 - 155	78 - 114	0 - 188
436	Nitrofen (TOK)	1000	22	15 - 139	59 - 142	0 - 170
	Norfluorazon	5000	20	71 - 143	80 - 120	68 - 146
112	PCB-1016					
108	PCB-1221					
109	PCB-1232					
106	PCB-1242					
110	PCB-1248					
107	PCB-1254					
111	PCB-1260	1000	20	82 - 112	79 - 126	75 - 119

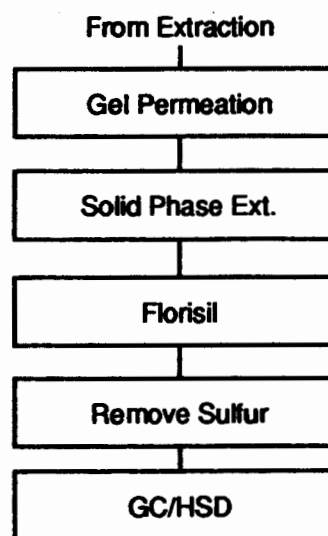
Table 4. Acceptance Criteria for Performance Tests for Organo-Halide Compounds, cont.

EGD No.	Compound	Acceptance Criteria				
		<i>Spike Level (ng/L)</i>	<i>Initial Precision and Accuracy</i>		<i>Calibration Verifi- cation*</i>	<i>Recovery Ongoing Accuracy</i>
			<i>s</i>	<i>X</i>	<i>(%)</i>	<i>R (%)</i>
440	PCNB	250	11	49 - 129	78 - 101	29 - 149
	Pendamethalin	2500	24	32 - 118	76 - 124	28 - 122
	cis-Permethrin	10000	30	45 - 153	70 - 130	41 - 157
	trans-Permethrin	10000	20	59 - 131	80 - 120	56 - 134
	Simazine	40000	20	16 - 100	80 - 120	13 - 101
	Terbacil	10000	82	0 - 217	18 - 182	0 - 228
	Terbuthylazine	25000	20	32 - 104	80 - 120	29 - 107
113	Toxaphene	5000	20	82 - 112	68 - 134	76 - 122
	Triadimefon	5000	54	32 - 104	80 - 120	0 - 107
442	Trifluralin	500	12	32 - 148	47 - 134	3 - 177

* Verified at the level of the median standard in Table 3.



Extraction and Concentration Steps



Cleanup and Analysis Steps

A52-002-84

Figure 1. Extraction, Cleanup, Derivatization, and Analysis

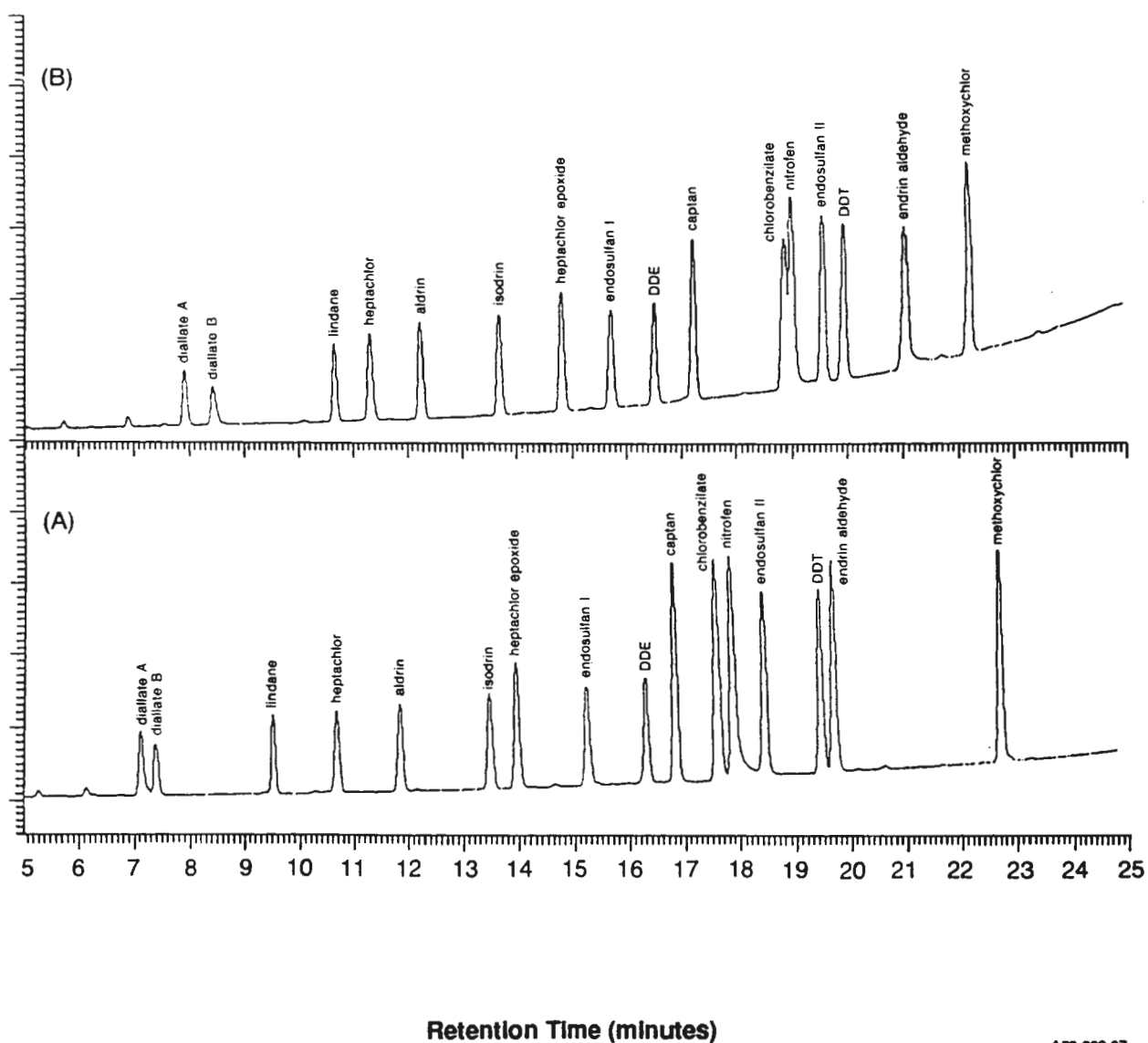


Figure 2. Gas Chromatogram of Selected Organo-Chlorine Compounds.

Method 1657
The Determination of
Organo-Phosphorus Pesticides
in Municipal and Industrial
Wastewater

Method 1657

The Determination of Organo-Phosphorus Pesticides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method is designed to meet the survey requirements of the Environmental Protection Agency (EPA). It is used to determine the (1) organo-phosphorus pesticides associated with the Clean Water Act, the Resource Conservation and Recovery Act, and the Comprehensive Environmental Response, Compensation and Liability Act; and (2) other compounds amenable to extraction and analysis by automated, wide-bore capillary column gas chromatography (GC) with a flame photometric detector.
- 1.2 The compounds listed in Table 1 may be determined in waters, soils, sediments, and sludges by this method. The method is a consolidation of several EPA methods. For waters, the sample extraction and concentration steps are essentially the same as in these methods. However, the extraction and concentration steps have been extended to other sample matrices. The method may be applicable to other phosphorus containing pesticides. The quality assurance/quality control requirements in this method give the steps necessary to determine this applicability.
- 1.3 This method is applicable to a large number of compounds. Calibrating the GC systems for all compounds is time-consuming. If only a single compound or small number of compounds is to be tested for, it is necessary to calibrate the GC systems and meet the performance specifications in this method for these compounds only. In addition, the GC conditions can be optimized for these compounds provided that all performance specifications in this method are met.
- 1.4 When this method is applied to analysis of unfamiliar samples, compound identity must be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Gas chromatography/mass spectrometry (GC/MS) can be used to confirm compounds in extracts produced by this method when analyte levels are sufficient.
- 1.5 The detection limits of this method are usually dependent on the level of interferences rather than instrumental limitations. The limits in Table 2 typify the minimum quantities that can be detected with no interferences present.
- 1.6 This method is for use by or under the supervision of analysts experienced in the use of a gas chromatograph and in the interpretation of gas chromatographic data. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 8.2.

2. SUMMARY OF METHOD

2.1 Extraction.

2.1.1 The percent solids content of a sample is determined.

2.1.2 Aqueous samples containing less than or equal to 1% solids.

- 3.1** Solvents, reagents, glassware, and other sample-processing hardware may yield artifacts and/or elevated baselines causing misinterpretation of chromatograms. All materials used in the analysis shall be demonstrated to be free from interferences under the conditions of analysis by running method blanks as described in Section 8.5.
- 3.2** Glassware and, where possible, reagents are cleaned by rinsing with solvent and baking at 450°C for 1 hour minimum in a muffle furnace or kiln. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment and thorough rinsing with acetone and pesticide-quality hexane may be required.

- 3.3 Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.
- 3.4 Interferences coextracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled. The cleanup procedures given in this method can be used to overcome many of these interferences, but unique samples may require additional cleanup to achieve the minimum levels given in Table 2.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 1 to 3.
- 4.2 Unknown samples may contain high concentrations of volatile toxic compounds. Sample containers should be opened in a hood and handled with gloves that will prevent exposure. The oven used for sample drying to determine percent moisture should be located in a hood so that vapors from samples do not create a health hazard in the laboratory.

5. APPARATUS AND MATERIALS

NOTE: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance meeting the requirements of this method is the responsibility of the laboratory.

5.1 Sampling equipment for discrete or composite sampling.

5.1.1 Sample bottles and caps.

- 5.1.1.1 Liquid samples (waters, sludges and similar materials that contain less than 5% solids): Sample bottle, amber glass, 1-L or 1-quart, with screw-cap.
- 5.1.1.2 Solid samples (soils, sediments, sludges, filter cake, compost, and similar materials that contain greater than 5% solids): Sample bottle, wide-mouth, amber glass, 500-mL minimum.
- 5.1.1.3 If amber bottles are not available, samples shall be protected from light.
- 5.1.1.4 Bottle caps: Threaded to fit sample bottles. Caps shall be lined with PTFE.
- 5.1.1.5 Cleaning.
- 5.1.1.5.1 Bottles are detergent-water washed, then rinsed with solvent or baked at 450°C for 1 hour minimum before use.

5.1.1.5.2 Liners are detergent water washed, then rinsed with reagent water and solvent, and baked at approximately 200°C for 1 hour minimum prior to use.

5.1.2 Compositing equipment: Automatic or manual compositing system incorporating glass containers cleaned per bottle cleaning procedure above. Sample containers are kept at 0 to 4°C during sampling. Glass or PTFE tubing only shall be used. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, the tubing shall be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.

5.2 Equipment for determining percent moisture.

5.2.1 Oven, capable of maintaining a temperature of 110°C ($\pm 5^\circ\text{C}$).

5.2.2 Dessicator.

5.2.3 Crucibles, porcelain.

5.2.4 Weighing pans, aluminum.

5.3 Extraction equipment.

5.3.1 Equipment for ultrasonic extraction.

5.3.1.1 Sonic disruptor: 375 watt with pulsing capability and ½- or ¾-inch disruptor horn (Ultrasonics, Inc, Model 375C, or equivalent).

5.3.1.2 Sonabox (or equivalent): For use with disruptor.

5.3.2 Equipment for liquid-liquid extraction.

5.3.2.1 Continuous liquid-liquid extractor: PTFE or glass connecting joints and stopcocks without lubrication, 1.5- to 2-L capacity (Hershberg-Wolf Extractor, Cal-Glass, Costa Mesa, California, 1000- or 2000-mL continuous extractor, or equivalent).

5.3.2.2 Round-bottom flask: 500-mL, with heating mantle.

5.3.2.3 Condenser: Graham, to fit extractor.

5.3.2.4 pH meter: With combination glass electrode.

5.3.2.5 pH paper: Wide-range (Hydrion Papers, or equivalent).

5.3.3 Separatory funnels: 250-, 500-, 1000-, and 2000-mL, with PTFE stopcocks.

5.3.4 Filtration apparatus.

5.3.4.1 Glass powder funnels: 125- to 250-mL.

5.3.4.2 Filter paper for above (Whatman 41, or equivalent).

5.3.5 Beakers.

5.3.5.1 1.5- to 2-L, calibrated to 1 L.

5.3.5.2 400- to 500-mL.

5.3.6 Spatulas: stainless steel or PTFE.

5.3.7 Drying column: 400 mm long by 15 to 20 mm ID Pyrex chromatographic column equipped with coarse glass frit or glass wool plug.

5.3.7.1 Pyrex glass wool: Extracted with solvent or baked at 450°C for 1 hour minimum.

5.4 Evaporation/concentration apparatus.

5.4.1 Kuderna-Danish (K-D) apparatus.

5.4.1.1 Evaporation flask: 500-mL (Kontes K-570001-0500, or equivalent), attached to concentrator tube with springs (Kontes K-662750-0012).

5.4.1.2 Concentrator tube: 10-mL, graduated (Kontes K-570050-1025, or equivalent) with calibration verified. Ground-glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.

5.4.1.3 Snyder column: Three-ball macro (Kontes K-503000-0232, or equivalent).

5.4.1.4 Snyder column: Two-ball micro (Kontes K-469002-0219, or equivalent).

5.4.1.5 Boiling chips.

5.4.1.5.1 Glass or silicon carbide: Approximately 10/40 mesh, extracted with methylene chloride and baked at 450°C for 1 hour minimum.

5.4.1.5.2 PTFE (optional): Extracted with methylene chloride.

5.4.2 Water bath: Heated, with concentric ring cover, capable of temperature control ($\pm 2^\circ\text{C}$), installed in a fume hood.

5.4.3 Nitrogen-evaporation device: Equipped with heated bath that can be maintained at 35 to 40°C (N-Evap, Organomation Associates, Inc., or equivalent).

5.4.4 Sample vials: Amber glass, 1- to 5-mL with PTFE-lined screw- or crimp-cap, to fit GC autosampler.

5.5 Balances

5.5.1 Analytical: Capable of weighing 0.1 mg.

5.5.2 Top loading: Capable of weighing 10 mg.

5.6 Apparatus for sample cleanup.

5.6.1 Automated gel permeation chromatograph (Analytical Biochemical Labs, Inc, Columbia, MO, Model GPC Autoprep 1002, or equivalent).

5.6.1.1 Column: 600 to 700 mm long by 25 mm ID, packed with 70 g of SX-3 Bio-beads (Bio-Rad Laboratories, Richmond, CA, or equivalent).

5.6.1.2 Syringe: 10-mL, with Luer fitting.

5.6.1.3 Syringe-filter holder: Stainless steel. Glass fiber or PTFE filters (Gelman Acrodisc-CR, 1 to 5 micron, or equivalent).

5.6.1.4 UV detectors: 254-mu, preparative or semi-prep flow cell (Isco, Inc., Type 6; Schmadzu, 5 mm path length; Beckman-Altex 152W, 8 μL micro-prep flow cell, 2 mm path; Pharmacia UV-1, 3 mm flow cell; LDC Milton-Roy UV-3, monitor #1203; or equivalent).

- 5.6.2 Vacuum system and cartridges for solid-phase extraction (SPE).
 - 5.6.2.1 Vacuum system: Capable of achieving 0.1 bar (house vacuum, vacuum pump, or water aspirator), with vacuum gauge.
 - 5.6.2.2 VacElute Manifold (Analytichem International, or equivalent).
 - 5.6.2.3 Vacuum trap: Made from 500-mL sidearm flask fitted with single-hole rubber stopper and glass tubing.
 - 5.6.2.4 Rack: For holding 50-mL volumetric flasks in the manifold.
 - 5.6.2.5 Column: Mega Bond Elut, Non-polar, C18 Octadecyl, 10 g/60 mL (Analytichem International Cat. No. 607H060, or equivalent).
- 5.7 Centrifuge apparatus.
 - 5.7.1 Centrifuge: Capable of rotating 500-mL centrifuge bottles or 15-mL centrifuge tubes at 5,000 rpm minimum
 - 5.7.2 Centrifuge bottles: 500-mL, with screw-caps, to fit centrifuge.
 - 5.7.3 Centrifuge tubes: 12- to 15-mL, with screw-caps, to fit centrifuge.
 - 5.7.4 Funnel: Buchner, 15 cm.
 - 5.7.4.1 Flask: Filter, for use with Buchner funnel.
 - 5.7.4.2 Filter paper: 15 cm (Whatman #41, or equivalent).
- 5.8 Miscellaneous glassware.
 - 5.8.1 Pipettes: Glass, volumetric, 1-, 5-, and 10-mL.
 - 5.8.2 Syringes: Glass, with Luerlok tip, 0.1-, 1- and 5-mL. Needles for syringes, 2-inch, 22-gauge.
 - 5.8.3 Volumetric flasks: 10-, 25-, and 50-mL.
 - 5.8.4 Scintillation vials: Glass, 20- to 50-mL, with PTFE-lined screw-caps.
- 5.9 Gas chromatograph: Shall have splitless or on-column simultaneous automated injection into separate capillary columns with a flame photometric detector at the end of each column, temperature program with isothermal holds, data system capable of recording simultaneous signals from the two detectors, and shall meet all of the performance specifications in Section 14.
 - 5.9.1 GC columns: Bonded-phase fused silica capillary.
 - 5.9.1.1 Primary: 30 m (± 3 m) by 0.5 mm (± 0.05 mm) ID DB-1, or equivalent.
 - 5.9.1.2 Confirmatory: DB-1701, or equivalent, with same dimensions as primary column.
 - 5.9.2 Data system: Shall collect and record GC data, store GC runs on magnetic disk or tape, process GC data, compute peak areas, store calibration data including retention times and calibration factors, identify GC peaks through retention times, compute concentrations, and generate reports.
 - 5.9.2.1 Data acquisition: GC data shall be collected continuously throughout the analysis and stored on a mass storage device.
 - 5.9.2.2 Calibration factors and calibration curves: The data system shall be used to record and maintain lists of calibration factors, and multi-point calibration

curves (Section 7). Computations of relative standard deviation (coefficient of variation) are used for testing calibration linearity. Statistics on initial (Section 8.2) and ongoing (Section 13.6) performance shall be computed and maintained.

5.9.2.3 Data processing: The data system shall be used to search, locate, identify, and quantify the compounds of interest in each GC analysis. Software routines shall be employed to compute and record retention times and peak areas. Displays of chromatograms and library comparisons are required to verify results.

5.9.2.4 Flame photometric detector: Capable of detecting 11 pg of malathion under the analysis conditions given in Table 2.

6. REAGENTS AND STANDARDS

6.1 Sample preservation: Sodium thiosulfate (ACS), granular.

6.2 pH adjustment.

6.2.1 Sodium hydroxide: Reagent grade.

6.2.1.1 Concentrated solution (10N): Dissolve 40 g NaOH in 100 mL reagent water.

6.2.1.2 Dilute solution (0.1M): Dissolve 4 g NaOH in 1 L of reagent water.

6.2.2 Sulfuric acid (1 + 1): Reagent grade, 6N in reagent water. Slowly add 50 mL H₂SO₄ (specific gravity 1.84) to 50 mL reagent water.

6.2.3 Potassium hydroxide: 37% (W/V); dissolve 37 g KOH in 100 mL reagent water.

6.3 Solution drying and back-extraction.

6.3.1 Sodium sulfate: Reagent grade, granular anhydrous (Baker 3375, or equivalent), rinsed with methylene chloride (20 mL/g), baked at 450°C for 1 hour minimum, cooled in a dessicator, and stored in a pre-cleaned glass bottle with screw-cap which prevents moisture from entering.

6.3.2 Sodium sulfate solution: 2% (W/V) in reagent water, pH adjusted to 8.5 to 9.0 with KOH or H₂SO₄.

6.4 Solvents: Methylene chloride, hexane, acetone, acetonitrile, isooctane, and methanol; pesticide-quality; lot-certified to be free of interferences.

6.5 GPC calibration solution: Solution containing 300 mg/mL corn oil, 15 mg/mL bis(2-ethylhexyl) phthalate, 1.4 mg/mL pentachlorophenol, 0.1 mg/mL perylene, and 0.5 mg/mL sulfur.

6.6 Sample cleanup.

6.6.1 Solid-phase extraction.

6.6.1.1 SPE cartridge calibration solution: 2,4,6-trichlorophenol, 0.1 µg/mL in acetone.

6.6.1.2 SPE elution solvent: methylene chloride:acetonitrile:hexane (50:3:47).

6.7 Reagent water: Water in which the compounds of interest and interfering compounds are not detected by this method.

- 6.8 High-solids reference matrix:** Playground sand or similar material in which the compounds of interest and interfering compounds are not detected by this method. May be prepared by extraction with methylene chloride and/or baking at 450°C for 4 hours minimum.
- 6.9 Standard solutions:** Purchased as solutions or mixtures with certification to their purity, concentration, and authenticity, or prepared from materials of known purity and composition. If compound purity is 96% or greater, the weight may be used without correction to compute the concentration of the standard. When not being used, standards are stored in the dark at -20 to -10°C in screw-capped vials with PTFE-lined lids. A mark is placed on the vial at the level of the solution so that solvent evaporation loss can be detected. The vials are brought to room temperature prior to use. Any precipitate is redissolved and solvent is added if solvent loss has occurred.
- 6.10 Preparation of stock solutions:** Prepare in isooctane per the steps below. Observe the safety precautions in Section 4.
- 6.10.1** Dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 10 mg of malathion in a 10-mL ground-glass stoppered volumetric flask and fill to the mark with isooctane. After the malathion is completely dissolved, transfer the solution to a 15-mL vial with PTFE-lined cap.
- 6.10.2** Stock solutions should be checked for signs of degradation prior to the preparation of calibration or performance test standards.
- 6.10.3** Stock standard solutions shall be replaced after 6 months, or sooner if comparison with quality control check standards indicates a change in concentration.
- 6.11 Secondary mixtures:** Using stock solutions (Section 6.10), prepare mixtures at the levels shown in Table 3 for calibration and calibration verification (Sections 7.3 and 13.5), for initial and ongoing precision and recovery (Sections 8.2 and 13.6), and for spiking into the sample matrix (Section 8.4).
- 6.12 Surrogate spiking solution:** Prepare tributyl phosphate and triphenyl phosphate each at a concentration of 2 ng/mL in acetone.
- 6.13 Stability of solutions:** All standard solutions (Sections 6.9 to 6.12) shall be analyzed within 48 hours of preparation and on a monthly basis thereafter for signs of degradation. Standards will remain acceptable if the peak area remains within $\pm 15\%$ of the area obtained in the initial analysis of the standard.

7. SETUP AND CALIBRATION

- 7.1** Configure the GC system as given in Section 5.9 and establish the operating conditions in Table 2.
- 7.2** Attainment of method detection limit (MDL): Determine that each column/detector system meets the MDL's in Table 2.
- 7.3** Calibration.
- 7.3.1** Injection of calibration solutions.
- 7.3.1.1** Compounds with calibration data in Table 3: The compounds in each calibration group in Table 3 were chosen so that each compound would be separated from the others by approximately 1 minute on the primary column.

The concentrations were chosen to bracket the working range of the FPD. However, because the response of some models of FPD are greater than others, it may be necessary to inject a larger volume of calibration solution for these detectors.

7.3.1.2 Compounds without calibration data in Table 3: Prepare calibration standards at a minimum of three concentration levels. One of these concentrations should be near, but above, the MDL (Table 2) and the other concentrations should define the working range of the detectors.

7.3.1.3 Set the automatic injector to inject a constant volume in the range of 0.5 to 5.0 μL of each calibration solution into the GC column/detector pairs, beginning with the lowest level mixture and proceeding to the highest. For each compound, compute and store, as a function of the concentration injected, the retention time and peak area on each column/detector system (primary and confirmatory).

7.3.2 Retention time: The polar nature of some analytes causes the retention time to decrease as the quantity injected increases. To compensate this effect, the retention time for compound identification is correlated with the analyte level.

7.3.2.1 If the difference between the maximum and minimum retention times for any compound is less than 5 seconds over the calibration range, the retention time for that compound can be considered constant and an average retention time may be used for compound identification.

7.3.2.2 Retention-time calibration curve (retention time vs. amount): If the retention time for a compound in the lowest level standard is more than 5 seconds greater than the retention time for the compound in the highest level standard, a retention-time calibration curve shall be used for identification of that compound.

7.3.3 Calibration factor (ratio of area to amount injected).

7.3.3.1 Compute the coefficient of variation (relative standard deviation) of the calibration factor over the calibration range for each compound on each column/detector system.

7.3.3.2 Linearity: If the calibration factor for any compound is constant ($C_v < 20\%$) over the calibration range, an average calibration factor may be used for that compound; otherwise, the complete calibration curve (area vs. amount) for that compound shall be used.

7.4 Combined QC standards: To preclude periodic analysis of all of the individual calibration groups of compounds (Table 3), the GC systems are calibrated with combined solutions as a final step. Not all of the compounds in these standards will be separated by the GC columns used in this method. Retention times and calibration factors are verified for the compounds that are resolved, and calibration factors are obtained for the unresolved peaks. These combined QC standards are prepared at the level the mid-range calibration standard (Table 3).

7.4.1 Analyze the combined QC standard on each column/detector pair.

7.4.1.1 For those compounds that exhibit a single, resolved GC peak, the retention time shall be within ± 5 seconds of the retention time of the peak in the medium level calibration standard (Section 7.3.1), and the calibration factor using the primary column shall be within $\pm 20\%$ of the calibration factor in the medium level standard (Table 3).

7.4.1.2 For the peaks containing two or more compounds, compute and store the retention times at the peak maxima on both columns (primary and confirmatory), and also compute and store the calibration factors on both columns. These results will be used for calibration verification (Section 13.2 and 13.5) and for precision and recovery studies (Sections 8.2 and 13.6).

8. QUALITY CONTROL

8.1 Each laboratory that uses this method is required to operate a formal quality control program (Reference 5). The minimum requirements of this program consist of an initial demonstration of laboratory capability, an ongoing analysis of standards and blanks as tests of continued performance, and analysis of spiked samples to assess accuracy. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method. If the method is to be applied routinely to samples containing high solids with very little moisture (e.g., soils, compost), the high-solids reference matrix (Section 6.8) is substituted for the reagent water (Section 6.8) in all performance tests, and the high-solids method (Section 10) is used for these tests.

8.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 The analyst is permitted to modify this method to improve separations or lower the costs of measurements, provided all performance requirements are met. Each time a modification is made to the method or a cleanup procedure is added, the analyst is required to repeat the procedure in Section 8.2 to demonstrate method performance. If detection limits will be affected by the modification, the analyst is required to repeat the demonstration of detection limits (Section 7.2).

8.1.3 The laboratory shall spike all samples with at least one surrogate compound to monitor method performance. This test is described in Section 8.3. When results of these spikes indicate atypical method performance for samples, the samples are diluted to bring method performance within acceptable limits (Section 16).

8.1.4 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and the analysis of the combined QC standard (Section 7.4) that the analysis system is in control. These procedures are described in Sections 13.1, 13.5, and 13.6.

8.1.5 The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Section 8.4.

8.1.6 Analyses of blanks are required to demonstrate freedom from contamination. The procedures and criteria for analysis of a blank are described in Section 8.5.

- 8.1.7** Other analytes may be determined by this method. The procedure for establishing a preliminary quality control limit for a new analyte is given in Section 8.6.
- 8.2** Initial precision and recovery: To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations.
- 8.2.1** For analysis of samples containing low solids (aqueous samples), extract, concentrate, and analyze one set of four 1-L aliquots of reagent water spiked with the combined QC standard (Section 7.4) according to the procedure in Section 10. Alternatively, sets of four replicates of the individual calibration groups (Section 7.3) may be used. For samples containing high solids, sets of four 30 g aliquots of the high-solids reference matrix are used.
- 8.2.2** Using results of the set of four analyses, compute the average percent recovery (\bar{X}) and the coefficient of variation (C_v) of percent recovery (s) for each compound.
- 8.2.3** For each compound, compare s and \bar{X} with the corresponding limits for initial precision and accuracy in Table 4. For coeluting compounds, use the coeluted compound with the least restrictive specification (largest C_v and widest range). If s and \bar{X} for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any individual s exceeds the precision limit or any individual \bar{X} falls outside the range for recovery, system performance is unacceptable for that compound. In this case, correct the problem and repeat the test.
- 8.3** The laboratory shall spike all samples with at least one surrogate compound to assess method performance on the sample matrix.
- 8.3.1** Analyze each sample according to the method beginning in Section 10.
- 8.3.2** Compute the percent recovery (P) of the surrogate compounds.
- 8.3.3** The recovery of the surrogate compounds shall be within the limits of 40 to 120%. If the recovery of any surrogate falls outside of these limits, method performance is unacceptable for that sample, and the sample is complex. Water samples are diluted, and smaller amounts of soils, sludges, and sediments are reanalyzed per Section 16.
- 8.4** Method accuracy: The laboratory shall spike (matrix spike) at least 10% of the samples from a given site type (e.g., influent to treatment, treated effluent, produced water, river sediment). If only one sample from a given site type is analyzed, a separate aliquot of that sample shall be spiked.
- 8.4.1** The concentration of the matrix spike shall be determined as follows.
- 8.4.1.1** If, as in compliance monitoring, the concentration of a specific analyte in the sample is being checked against a regulatory concentration limit, the matrix spike shall be at that limit or at 1 to 5 times higher than the background concentration determined in Section 8.4.2, whichever concentration is larger.
- 8.4.1.2** If the concentration of an analyte in the sample is not being checked against a limit specific to that analyte, the matrix spike shall be at the concentration of the combined QC standard (Section 7.4) or at 1 to 5 times higher than the background concentration, whichever concentration is larger.
- 8.4.1.3** If it is impractical to determine the background concentration before spiking (e.g., maximum holding times will be exceeded), the matrix spike

concentration shall be the regulatory concentration limit, if any; otherwise, the larger of either 5 times the expected background concentration or at the concentration of the combined QC standard (Section 7.4).

- 8.4.2** Analyze one sample aliquot to determine the background concentration (B) of each analyte. If necessary, prepare a standard solution appropriate to produce a level in the sample one to five times the background concentration. Spike a second sample aliquot with the standard solution and analyze it to determine the concentration after spiking (A) of each analyte. Calculate the percent recovery (P) of each analyte:

Equation 1

$$P = \frac{100(A-B)}{T}$$

where

T = True value of the spike

- 8.4.3** Compare the percent recovery for each analyte with the corresponding QC acceptance criteria in Table 4. If any analyte fails the acceptance criteria for recovery, the sample is complex and must be diluted and reanalyzed per Section 16.
- 8.4.4** As part of the QC program for the laboratory, method accuracy for samples shall be assessed and records shall be maintained. After the analysis of five spiked samples of a given matrix type (water, soil, sludge, sediment) in which the analytes pass the tests in Section 8.4, compute the average percent recovery (P) and the standard deviation of the percent recovery (s_p) for each compound (or coeluting compound group). Express the accuracy assessment as a percent recovery interval from $P - 2s_p$ to $P + 2s_p$ for each matrix. For example, if $P = 90\%$ and $s_p = 10\%$ for five analyses of compost, the accuracy interval is expressed as 70 to 110%. Update the accuracy assessment for each compound in each matrix on a regular basis (e.g., after each five to ten new accuracy measurements).
- 8.5** **Blanks:** Reagent water and high-solids reference matrix blanks are analyzed to demonstrate freedom from contamination.
- 8.5.1** Extract and concentrate a 1 L reagent water blank or a 30 g high-solids reference matrix blank with each sample batch (samples started through the extraction process on the same 8 hour shift, to a maximum of 20 samples). Analyze the blank immediately after analysis of the combined QC standard (Section 13.6) to demonstrate freedom from contamination.
- 8.5.2** If any of the compounds of interest (Table 1) or any potentially interfering compound is found in an aqueous blank at greater than 0.05 $\mu\text{g/L}$, or in a high-solids reference matrix blank at greater than 1 $\mu\text{g/kg}$ (assuming the same calibration factor as malathion for compounds not listed in Table 1), analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level.
- 8.6** Other analytes may be determined by this method. To establish a quality control limit for an analyte, determine the precision and accuracy by analyzing four replicates of the analyte along with the combined QC standard per the procedure in Section 8.2. If the analyte coelutes with an

analyte in the QC standard, prepare a new QC standard without the coeluting component(s). Compute the average percent recovery (A) and the standard deviation of percent recovery (s_p) for the analyte, and measure the recovery and standard deviation of recovery for the other analytes. The data for the new analyte is assumed to be valid if the precision and recovery specifications for the other analytes are met; otherwise, the analytical problem is corrected and the test is repeated. Establish a preliminary quality control limit of $A \pm 2s_p$ for the new analyte and add the limit to Table 4.

- 8.7 The specifications contained in this method can be met if the apparatus used is calibrated properly, then maintained in a calibrated state. The standards used for calibration (Section 7), calibration verification (Section 13.5), and for initial (Section 8.2) and ongoing (Section 13.6) precision and recovery should be identical, so that the most precise results will be obtained. The GC instruments will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of the analytes given in this method.
- 8.8 Depending on specific program requirements, field replicates and field spikes of the analytes of interest into samples may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

9. **SAMPLE COLLECTION, PRESERVATION, AND HANDLING**

- 9.1 Collect samples in glass containers following conventional sampling practices (Reference 6), except that the bottle shall not be prerinsed with sample before collection. Aqueous samples which flow freely are collected in refrigerated bottles using automatic sampling equipment. Solid samples are collected as grab samples using wide-mouth jars.
- 9.2 Maintain samples at 0 to 4°C from the time of collection until extraction. If the samples will not be extracted within 72 hours of collection, adjust the sample to a pH of 5.0 to 9.0 using sodium hydroxide or sulfuric acid solution. Record the volume of acid or base used. If residual chlorine is present in aqueous samples, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine (Reference 7).
- 9.3 Begin sample extraction within 7 days of collection, and analyze all extracts within 40 days of extraction.

10. **SAMPLE EXTRACTION AND CONCENTRATION**

Samples containing 1% solids or less are extracted directly using continuous liquid-liquid extraction techniques (Section 10.2.1). Samples containing 1 to 30% solids are diluted to the 1% level with reagent water (Section 10.2.2) and extracted using continuous liquid-liquid extraction techniques. Samples containing greater than 30% solids are extracted using ultrasonic techniques (Section 10.2.5). For highly water soluble compounds such as methamidophos, samples are salted and extracted using a chloroform:acetone azeotrope (Section 10.2.6). Figure 1 outlines the extraction and concentration steps.

10.1 Determination of percent solids.

10.1.1 Weigh 5 to 10 g of sample into a tared beaker. Record the weight to three significant figures.

10.1.2 Dry overnight (12 hours minimum) at 110°C ($\pm 5^\circ\text{C}$), and cool in a dessicator.

10.1.3 Determine percent solids as follows:

Equation 2

$$\% \text{ solids} = \frac{\text{weight of dry sample}}{\text{weight of wet sample}} \times 100$$

10.2 Preparation of samples for extraction.

10.2.1 Aqueous samples containing 1% solids or less: Extract the sample directly using continuous liquid-liquid extraction techniques.

10.2.1.1 Measure 1 L (± 0.01 L) of sample into a clean 1.5- to 2-L beaker.

10.2.1.2 Spike 0.5 mL of the surrogate spiking solution (Section 6.12) into the sample aliquot. Proceed to preparation of the QC aliquots for low-solids samples (Section 10.2.3).

10.2.2 Samples containing 1 to 30% solids.

10.2.2.1 Mix sample thoroughly.

10.2.2.2 Using the percent solids found in Section 10.1.3, determine the weight of sample required to produce 1 L of solution containing 1% solids as follows:

Equation 3

$$\text{sample weight} = \frac{1000 \text{ g}}{\% \text{ solids}}$$

10.2.2.3 Place the weight determined in Section 10.2.2.2 in a clean 1.5-L to 2.0-L beaker. Discard all sticks, rocks, leaves, and other foreign material prior to weighing.

10.2.2.4 Bring the volume of the sample aliquot(s) to 100 to 200 mL with reagent water.

10.2.2.5 Spike 0.5 mL of the appropriate surrogate spiking solution (Section 6.12) into each sample aliquot.

10.2.2.6 Using a clean metal spatula, break any solid portions of the sample into small pieces.

10.2.2.7 Place the $\frac{3}{4}$ -inch horn on the ultrasonic probe approximately $\frac{1}{2}$ inch below the surface of each sample aliquot and pulse at 50% for 3 minutes at full power. If necessary, remove the probe from the solution and break any large pieces using the metal spatula or a stirring rod and repeat the sonication. Clean the probe with methylene chloride:acetone (1:1) between samples to preclude cross-contamination.

10.2.2.8 Bring the sample volume to 1.0 L (± 0.1 L) with reagent water.

10.2.3 Preparation of QC aliquots for samples containing low solids (less than 30%).

- 10.2.2.5** Spike 0.5 mL of the appropriate surrogate spiking solution (Section 6.12) into each sample aliquot.
- 10.2.2.6** Using a clean metal spatula, break any solid portions of the sample into small pieces.
- 10.2.2.7** Place the $\frac{3}{4}$ -inch horn on the ultrasonic probe approximately $\frac{1}{2}$ inch below the surface of each sample aliquot and pulse at 50% for 3 minutes at full power. If necessary, remove the probe from the solution and break any large pieces using the metal spatula or a stirring rod and repeat the sonication. Clean the probe with methylene chloride:acetone (1:1) between samples to preclude cross-contamination.
- 10.2.2.8** Bring the sample volume to 1.0 L (± 0.1 L) with reagent water.
- 10.2.3** Preparation of QC aliquots for samples containing low solids (less than 30%).
 - 10.2.3.1** For each sample or sample batch (to a maximum of 20) to be extracted at the same time, place two 1.0 L (± 0.01 L) aliquots of reagent water in clean 1.5- to 2.0-L beakers.
 - 10.2.3.2** Blank: Spike 0.5 mL of the pesticide surrogate spiking solution (Section 6.12) into one reagent water aliquot.
 - 10.2.3.3** Spike the combined QC standard (Section 7.4) into a reagent water aliquot.
 - 10.2.3.4** If a matrix spike is required, prepare an aliquot at the concentrations specified in Section 8.4.
- 10.2.4** Stir and equilibrate all sample and QC solutions for 1 to 2 hours. Extract the samples and QC aliquots per Section 10.3.
- 10.2.5** Samples containing 30% solids or greater.
 - 10.2.5.1** Mix the sample thoroughly.
 - 10.2.5.2** Weigh 30 g (± 0.3 g) into a clean 400- to 500-mL beaker. Discard all sticks, rocks, leaves, and other foreign material prior to weighing.
 - 10.2.5.3** Spike 0.5 mL of the surrogate spiking solution (Section 6.12) into the sample aliquot.
 - 10.2.5.4** QC aliquots: For each sample or sample batch (to a maximum of 20) to be extracted at the same time, place two 30 g (± 0.3 g) aliquots of the high-solids reference matrix in clean 400- to 500-mL beakers.
 - 10.2.5.5** Blank: Spike 0.5 mL of the surrogate spiking solution (Section 6.12) into an aliquot of the high-solids reference matrix.
 - 10.2.5.6** Spike the combined QC standard (Section 7.4) into a high-solids reference matrix aliquot. Extract the high-solids samples per Section 10.4.
- 10.2.6** Samples containing methamidophos and other highly water-soluble compounds: Prepare samples containing less than 30% solids per Sections 10.2.6.1 to 10.2.6.5; prepare samples containing greater than 30% solids per Section 10.2.5.
 - 10.2.6.1** Interferences: If interferences are expected, aqueous samples can be pre-extracted with methylene chloride to remove these interferences. This

extract can be used for determination of insoluble or slightly soluble compounds and the surrogates. Methamidophos is only slightly soluble in methylene chloride and will not be in this extract unless carried by polar species in the sample matrix. If compounds other than methamidophos are not to be determined, the methylene chloride extract can be discarded.

- 10.2.6.2 Determine the percent solids and prepare a 1-L sample aliquot and the QC aliquots per Sections 10.2.2 and 10.2.4 or 10.2.3 and 10.2.4, except do not spike the surrogate into the sample aliquot if the methylene chloride extract will be discarded (Section 10.2.6.1).
- 10.2.6.3 Extract the aliquots per Section 10.3 using methylene chloride to remove interferences.
- 10.2.6.4 After extraction, remove the water and methylene chloride from the extractor. Decant the aqueous portion into a beaker and combine the remaining methylene chloride with the extract in the distilling flask. If the methylene chloride extract is to be used for determination of other analytes and the surrogate, proceed to Section 10.5 with that extract.
- 10.2.6.5 Saturate the water sample with sodium chloride. Approximately 350 g will be required.
- 10.2.6.6 If the methylene chloride extract was discarded, spike the surrogates into the sample aliquot.
- 10.2.6.7 Extract the sample per Section 10.3 except use a chloroform:acetone azeotrope (2:1 V/V or 4:1 W/W) for the extraction.

NOTE: *Note: As a result of the increased density of the water caused by saturation with salt, the sample may sink to where the water enters the siphon tube of the continuous extractor. To prevent this from occurring, use a smaller volume of water (e.g., 800 mL) in the extractor. Correct for this adjustment in the calculation of the concentration of the pollutants in the extract (Section 15).*

- 10.3 Continuous extraction of low-solids (aqueous) samples: Place 100 to 150 mL methylene chloride in each continuous extractor and 200 to 300 mL in each distilling flask.
 - 10.3.1 Pour the sample(s), blank, and standard aliquots into the extractors. Rinse the glass containers with 50 to 100 mL methylene chloride and add to the respective extractors. Include all solids in the extraction process.
 - 10.3.2 Extraction: Adjust the pH of the waters in the extractors to 5 to 9 with NaOH or H₂SO₄ while monitoring with a pH meter.

NOTE: *Caution: Some samples require acidification in a hood because of the potential for generating hydrogen sulfide.*

- 10.3.3** Begin the extraction by heating the flask until the methylene chloride is boiling. When properly adjusted, one to two drops of methylene chloride per second will fall from the condenser tip into the water. Test and adjust the pH of the waters during the first 1 to 2 hours of extraction. Extract for 18 to 24 hours.
- 10.3.4** Remove the distilling flask, estimate and record the volume of extract (to the nearest 100 mL), and pour the contents through a prerinsed drying column containing 7 to 10 cm of anhydrous sodium sulfate. Rinse the distilling flask with 30 to 50 mL of methylene chloride and pour through the drying column. For extracts to be cleaned up using GPC, collect the solution in a 500-mL K-D evaporator flask equipped with a 10-mL concentrator tube. Seal, label, and concentrate per Sections 10.5 to 10.6.
- 10.4** Ultrasonic extraction of high-solids samples: Procedures are provided for extraction of non-municipal sludge (Section 10.4.1) and municipal sludge samples (Section 10.4.2).
- 10.4.1** Ultrasonic extraction of non-municipal sludge high-solids aliquots.
- 10.4.1.1** Add 60 to 70 g of powdered sodium sulfate to the sample and QC aliquots. Mix each aliquot thoroughly. Some wet sludge samples may require more than 70 g for complete removal of water. All water must be removed prior to addition of organic solvent so that the extraction process is efficient.
- 10.4.1.2** Add 100 mL (± 10 mL) of acetone:methylene chloride (1:1) to each of the aliquots and mix thoroughly.
- 10.4.1.3** Place the $\frac{3}{4}$ -inch horn on the ultrasonic probe approximately $\frac{1}{2}$ inch below the surface of the solvent but above the solids layer and pulse at 50% for 3 minutes at full power. If necessary, remove the probe from the solution and break any large pieces using a metal spatula or a stirring rod and repeat the sonication.
- 10.4.1.4** Decant the pesticide extracts through a prerinsed drying column containing 7 to 10 cm anhydrous sodium sulfate into 500- to 1000-mL graduated cylinders.
- 10.4.1.5** Repeat the extraction steps (Sections 10.4.1.2 to 10.4.1.4) twice more for each sample and QC aliquot. On the final extraction, swirl the sample or QC aliquot, pour into its respective drying column, and rinse with acetone:methylene chloride. Record the total extract volume. If necessary, transfer the extract to a centrifuge tube and centrifuge for 10 minutes to settle fine particles.
- 10.4.2** Ultrasonic extraction of high solids municipal sludge aliquots.
- 10.4.2.1** Add 100 mL (± 10 mL) of acetonitrile to each of the aliquots and mix thoroughly.
- 10.4.2.2** Place the $\frac{3}{4}$ -inch horn on the ultrasonic probe approximately $\frac{1}{2}$ inch below the surface of the solvent but above the solids layer and pulse at 50% for 3 minutes at full power. If necessary, remove the probe from the solution and break any large pieces using a metal spatula or a stirring rod and repeat the sonication.

- 10.4.2.3** Decant the extract through filter paper into a 1000- to 2000-mL separatory funnel.
- 10.4.2.4** Repeat the extraction and filtration steps (Sections 10.4.2.1 to 10.4.2.3) using a second 100 mL (± 10 mL) of acetonitrile.
- 10.4.2.5** Repeat the extraction step (Sections 10.4.2.1 and 10.4.2.2) using 100 mL (± 10 mL) of methylene chloride. On this final extraction, swirl the sample or QC aliquot, pour into its respective filter paper, and rinse with methylene chloride. Record the total extract volume.
- 10.4.2.6** For each extract, prepare 1.5 to 2 L of reagent water containing 2% sodium sulfate. Adjust the pH of the water to 6.0 to 9.0 with NaOH or H₂SO₄.
- 10.4.2.7** Back-extract each extract three times sequentially with 500 mL of the aqueous sodium sulfate solution, returning the bottom (organic) layer to the separatory funnel the first two times while discarding the top (aqueous) layer. On the final back-extraction, filter each pesticide extract through a prerinsed drying column containing 7 to 10 cm anhydrous sodium sulfate into a 500- to 1000-mL graduated cylinder. Record the final extract volume.
- 10.4.3** For extracts to be cleaned up using GPC, filter these extracts through Whatman #41 paper into a 500-mL K-D evaporator flask equipped with a 10-mL concentrator tube. Rinse the graduated cylinder or centrifuge tube with 30 to 50 mL of methylene chloride and pour through filter to complete the transfer. Seal and label the K-D flask. Concentrate these fractions per Sections 10.5 through 10.8.
- 10.5 Macro concentration.**
 - 10.5.1** Concentrate the extracts in separate 500-mL K-D flasks equipped with 10-mL concentrator tubes. Add one to two clean boiling chips to the flask and attach a three-ball macro Snyder column. Prewet the column by adding approximately one mL of methylene chloride through the top. Place the K-D apparatus in a hot water bath so that the entire lower rounded surface of the flask is bathed with steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.
 - 10.5.2** When the liquid has reached an apparent volume of 1 mL, remove the K-D apparatus from the bath and allow the solvent to drain and cool for at least 10 minutes.
 - 10.5.3** If the extract is to be cleaned up using GPC, remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommended for this operation. Adjust the final volume to 10 mL and proceed to GPC cleanup in Section 11.
- 10.6 Hexane exchange:** Extracts that have been cleaned up are exchanged into hexane.
 - 10.6.1** Remove the Snyder column, add approximately 50 mL of hexane and a clean boiling chip, and reattach the Snyder column. Concentrate the extract as in Section 10.5 except use hexane to prewet the column. The elapsed time of the concentration should be 5 to 10 minutes.

- 10.6.2** Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane. Adjust the final volume of extracts that have not been cleaned up by GPC to 10 mL and those that have been cleaned up by GPC to 5 mL (the difference accounts for the 50% loss in the GPC cleanup).

11. CLEANUP AND SEPARATION

- 11.1** Cleanup procedures may not be necessary for relatively clean samples (treated effluents, ground water, drinking water). If particular circumstances require the use of a cleanup procedure, the analyst may use any or all of the procedures below or any other appropriate procedure. However, the analyst shall first repeat the tests in Section 8.2 to demonstrate that the requirements of Section 8.2 can be met using the cleanup procedure(s) as an integral part of the method. Figure 1 outlines the cleanup steps.
- 11.1.1** Gel permeation chromatography (Section 11.2) removes many high molecular weight interferents that cause GC column performance to degrade. It is used for all soil and sediment extracts and may be used for water extracts that are expected to contain high molecular weight organic compounds (e.g., polymeric materials, humic acids).
- 11.1.2** The solid-phase extraction cartridge (Section 11.3) removes polar organic compounds such as phenols. It is used for cleanup of organo-chlorine and organo-phosphate extracts.
- 11.2** Gel permeation chromatography (GPC).
- 11.2.1** Column packing.
- 11.2.1.1** Place 70 to 75 g of SX-3 Bio-beads in a 400- to 500-mL beaker.
- 11.2.1.2** Cover the beads with methylene chloride and allow to swell overnight (12 hours minimum).
- 11.2.1.3** Transfer the swelled beads to the column and pump solvent through the column, from bottom to top, at 4.5 to 5.5 mL/min prior to connecting the column to the detector.
- 11.2.1.4** After purging the column with solvent for 1 to 2 hours, adjust the column head pressure to 7 to 10 psig, and purge for 4 to 5 hours to remove air. Maintain a head pressure of 7 to 10 psig. Connect the column to the detector.
- 11.2.2** Column calibration.
- 11.2.2.1** Load 5 mL of the calibration solution (Section 6.5) into the sample loop.
- 11.2.2.2** Inject the calibration solution and record the signal from the detector. The elution pattern will be corn oil, bis(2-ethylhexyl) phthalate, pentachlorophenol, perylene, and sulfur.
- 11.2.2.3** Set the "dump time" to allow >85% removal of the corn oil and >85% collection of the phthalate.
- 11.2.2.4** Set the "collect time" to the peak minimum between perylene and sulfur.
- 11.2.2.5** Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the pentachlorophenol is greater than 85%. If calibration is not verified, the system shall be recalibrated using the

calibration solution, and the previous 20 samples shall be re-extracted and cleaned up using the calibrated GPC system.

11.2.3 Extract cleanup: GPC requires that the column not be overloaded. The column specified in this method is designed to handle a maximum of 0.5 g of high molecular weight material in a 5 mL extract. If the extract is known or expected to contain more than 0.5 g, the extract is split into fractions for GPC and the fractions are combined after elution from the column. The solids content of the extract may be obtained gravimetrically by evaporating the solvent from a 50- μ L aliquot.

11.2.3.1 Filter the extract or load through the filter holder to remove particulates. Load the 5.0 mL extract onto the column.

11.2.3.2 Elute the extract using the calibration data determined in Section 11.2.2. Collect the eluate in a clean 400- to 500-mL beaker.

11.2.3.3 Rinse the sample loading tube thoroughly with methylene chloride between extracts to prepare for the next sample.

11.2.3.4 If a particularly dirty extract is encountered, a 5.0 mL methylene chloride blank shall be run through the system to check for carry-over.

11.2.3.5 Concentrate the extract and exchange into hexane per Sections 10.5 and 10.6. Adjust the final volume to 5.0 mL.

11.3 Solid-phase extraction (SPE).

11.3.1 Setup.

11.3.1.1 Attach the Vac-elute manifold to a water aspirator or vacuum pump with the trap and gauge installed between the manifold and vacuum source.

11.3.1.2 Place the SPE cartridges in the manifold, turn on the vacuum source, and adjust the vacuum to 5 to 10 psia.

11.3.2 Cartridge washing: Pre-elute each cartridge prior to use sequentially with 10 mL portions each of hexane, methanol, and water using vacuum for 30 seconds after each eluant. Follow this pre-elution with 1 mL methylene chloride and three 10 mL portions of the elution solvent (Section 6.6.2.2) using vacuum for 5 minutes after each eluant. Tap the cartridge lightly while under vacuum to dry between eluants. The three portions of elution solvent may be collected and used as a blank if desired. Finally, elute the cartridge with 10 mL each of methanol and water, using the vacuum for 30 seconds after each eluant.

11.3.3 Cartridge certification: Each cartridge lot must be certified to ensure recovery of the compounds of interest and removal of 2,4,6-trichlorophenol.

11.3.3.1 To make the test mixture, add the trichlorophenol solution (Section 6.6.2.1) to the combined calibration standard (Section 7.4). Elute the mixture using the procedure in Section 11.3.4.

11.3.3.2 Concentrate the eluant to 1.0 mL and inject 1.0 μ L of the concentrated eluant into the GC using the procedure in Section 13. The recovery of all analytes

(including the unresolved GC peaks) shall be within the ranges for recovery specified in Table 4, and the peak for trichlorophenol shall not be detectable; otherwise the SPE cartridge is not performing properly and the cartridge lot shall be rejected.

11.3.4 Extract cleanup.

11.3.4.1 After cartridge washing (Section 11.3.2), release the vacuum and place the rack containing the 50-mL volumetric flasks (Section 5.6.2.4) in the vacuum manifold. Reestablish the vacuum at 5 to 10 psia.

11.3.4.2 Using a pipette or a 1-mL syringe, transfer 1.0 mL of extract to the SPE cartridge. Apply vacuum for 5 minutes to dry the cartridge. Tap gently to aid in drying.

11.3.4.3 Elute each cartridge into its volumetric flask sequentially with three 10-mL portions of the elution solvent (Section 6.6.2.2), using vacuum for 5 minutes after each portion. Collect the eluants in the 50-mL volumetric flasks.

11.3.4.4 Release the vacuum and remove the 50-mL volumetric flasks.

11.3.4.5 Concentrate the eluted extracts to approximately 0.5 mL using the nitrogen blow-down apparatus. Adjust the final volume to 1.0 mL (per Section 10.6) and proceed to Section 13 for GC analysis.

12. GAS CHROMATOGRAPHY

Table 2 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are the retention times and estimated detection limits that can be achieved under these conditions. Examples of the separations achieved by the primary and confirmatory columns are shown in Figure 2.

12.1 Calibrate the system as described in Section 7.

12.2 Set the autosampler to inject the same volume that was chosen for calibration (Section 7.3.1.3) for all standards and extracts of blanks and samples.

12.3 Set the data system or GC control to start the temperature program upon sample injection, and begin data collection after the solvent peak elutes. Set the data system to stop data collection after the last analyte is expected to elute and to return the column to the initial temperature.

13. SYSTEM AND LABORATORY PERFORMANCE

13.1 At the beginning of each 8-hour shift during which analyses are performed, GC system performance and calibration are verified for all pollutants and surrogates on both column/detector systems. For these tests, analysis of the combined QC standard (Section 7.4) shall be used to verify all performance criteria. Adjustment and/or recalibration (per Section 7) shall be performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks, and precision and recovery standards be analyzed.

13.2 Retention times: The absolute retention times of the peak maxima shall be within ± 10 seconds of the retention times in the initial calibration (Section 7.4.1).

- 13.3 GC resolution:** Resolution is acceptable if the valley height between two peaks (as measured from the baseline) is less than 10% of the taller of the two peaks.
- 13.3.1 Primary column (DB-1):** Malathion and ethyl parathion.
- 13.3.2 Confirmatory column (DB-1701):** Terbufos and diazinon.
- 13.4 Calibration verification:** Calibration is verified for the combined QC standard only.
- 13.4.1 Inject the combined QC standard (Section 7.4)**
- 13.4.2 Compute the percent recovery of each compound or coeluting compounds, based on the calibration data (Section 7.4).**
- 13.4.3 For each compound or coeluted compounds, compare this calibration verification recovery with the corresponding limits for ongoing accuracy in Table 4. For coeluting compounds, use the coeluted compound with the least restrictive specification (the widest range). If the recoveries for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any recovery falls outside the calibration verification range, system performance is unacceptable for that compound. In this case, correct the problem and repeat the test, or recalibrate (Section 7).**
- 13.5 Ongoing precision and recovery.**
- 13.5.1 Analyze the extract of the precision and recovery standard extracted with each sample batch (Sections 10.2.3.3 and 10.2.5.7).**
- 13.5.2 Compute the percent recovery of each analyte and coeluting compounds.**
- 13.5.3 For each compound or coeluted compounds, compare the percent recovery with the limits for ongoing recovery in Table 4. For coeluted compounds, use the coeluted compound with the least restrictive specification (widest range). If all analytes pass, the extraction, concentration, and cleanup processes are in control and analysis of blanks and samples may proceed. If, however, any of the analytes fail, these processes are not in control. In this event, correct the problem, re-extract the sample lot, and repeat the ongoing precision and recovery test.**
- 13.5.4 Add results which pass the specifications in Section 13.6.3 to initial and previous ongoing data. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory data quality for each analyte by calculating the average percent recovery (R) and the standard deviation of percent recovery s_r . Express the accuracy as a recovery interval from $R - 2s_r$ to $R + 2s_r$. For example, if $R = 95\%$ and $s_r = 5\%$, the accuracy is 85 to 105%.**

14. QUALITATIVE DETERMINATION

- 14.1 Qualitative determination is accomplished by comparison of data from analysis of a sample or blank with data from analysis of the shift standard (Section 14.2), and with data stored in the retention-time and calibration libraries (Sections 7.3.2 and 7.3.3.2). Identification is confirmed when retention time and amounts agree per the criteria below.**

14.2 For each compound on each column/detector system, establish a retention-time window ± 20 seconds on either side of the retention time in the calibration data (Section 7.3.2). For compounds that have a retention-time curve (Section 7.3.2.2), establish this window as the minimum -20 seconds and maximum +20 seconds.

14.2.1 Compounds not requiring a retention-time calibration curve: If a peak from the analysis of a sample or blank is within a window (as defined in Section 14.2) on the primary column/detector system, it is considered tentatively identified. A tentatively identified compound is confirmed when (1) the retention time for the compound on the confirmatory column/detector system is within the retention-time window on that system, and (2) the computed amounts (Section 16) on each system (primary and confirmatory) agree within a factor of 3.

14.2.2 Compounds requiring a retention-time calibration curve: If a peak from the analysis of a sample or blank is within a window (as defined in Section 14.2) on the primary column/detector system, it is considered tentatively identified. A tentatively identified compound is confirmed when (1) the retention times on both systems (primary and confirmatory) are within ± 30 seconds of the retention times for the computed amounts (Section 15), as determined by the retention-time calibration curve (Section 7.3.2.2), and (2) the computed amounts (Section 15) on each system (primary and confirmatory) agree within a factor of 3.

15. QUANTITATIVE DETERMINATION

15.1 Using the GC data system, compute the concentration of the analyte detected in the extract (in micrograms per milliliter) using the calibration factor or calibration curve (Section 7.3.3.2).

15.2 Liquid samples: Compute the concentration in the sample using the following equation:

Equation 4

$$C_s = 10 \frac{(C_{ex})}{(V_s)}$$

where

C_s = The concentration in the sample, in $\mu\text{g/L}$

10 = The extract total, in mL

C_{ex} = The concentration in the extract, in $\mu\text{g/mL}$

V_s = The sample extracted, in L

- 15.3 Solid samples:** Compute the concentration in the solid phase of the sample using the following equation:

Equation 5

$$C_s = 10 \frac{(C_e)}{1000(W_s)(\text{solids})}$$

where

- C_s = Concentration in the sample, in $\mu\text{g/kg}$
10 = Extract total, in mL
 C_e = Concentration in the extract, in $\mu\text{g/mL}$
1000 = Conversion factor, g to kg
 W_s = Sample weight, in g
solids = Percent solids in Section 10.1.3 divided by 100
-

- 15.4** If the concentration of any analyte exceeds the calibration range of the system, the extract is diluted by a factor of 10, and a 1- μL aliquot of the diluted extract is analyzed.
- 15.5** Report results for all pollutants found in all standards, blanks, and samples to three significant figures. Results for samples that have been diluted are reported at the least dilute level at which the concentration is in the calibration range.

16. ANALYSIS OF COMPLEX SAMPLES

- 16.1** Some samples may contain high levels ($> 1000 \text{ ng/L}$) of the compounds of interest, interfering compounds, and/or polymeric materials. Some samples may not concentrate to 10 mL (Section 10.6); others may overload the GC column and/or detector.
- 16.2** The analyst shall attempt to clean up all samples using GPC (Section 11.2), and the SPE cartridge (Section 11.3). If these techniques do not remove the interfering compounds, the extract is diluted by a factor of 10 and reanalyzed (Section 16.4).
- 16.3** Recovery of surrogates: In most samples, surrogate recoveries will be similar to those from reagent water or from the high solids reference matrix. If the surrogate recovery is outside the range specified in Section 8.3, the sample shall be re-extracted and reanalyzed. If the surrogate recovery is still outside this range, the sample is diluted by a factor of 10 and reanalyzed (Section 15.4).
- 16.4** Recovery of matrix spikes: In most samples, matrix spike recoveries will be similar to those from reagent water or from the high solids reference matrix. If the matrix spike recovery is outside the range specified in Table 4, the sample shall be diluted by a factor of 10, respiked, and reanalyzed. If the matrix spike recovery is still outside the range, the method may not apply to the sample being analyzed and the result may not be reported for regulatory compliance purposes.

17. METHOD PERFORMANCE

- 17.1** Development of this method is detailed in References 8 and 9.

References

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4. Mills, P. A., "Variation of Florisil Activity: Simple Method for Measuring Adsorbent Capacity and Its Use in Standardizing Florisil Columns," *Journal of the Association of Official Analytical Chemists*, 51, 29: 1968.
5. "Handbook of Quality Control in Wastewater Laboratories," U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH,: EPA-600/4-79-019, March 1979.
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8. "Consolidated GC Method for the Determination of ITD/RCRA Pesticides using Selective GC Detectors," S-CUBED, A Division of Maxwell Laboratories, Inc., La Jolla, CA: Ref. 32145-01, Document R70, September 1986.
9. "Method Development and Validation, EPA Method 1618," Pesticide Center, Department of Environmental Health, Colorado State University: November 1988, January 1989, and March 1992.

Table 1. Organo-Phosphorus Pesticides Determined by Large-Bore, Fused-Silica Capillary Column Gas Chromatography with Flame Photometric Detector

EPA EGD	Compound	CAS Registry
	Acephate	30560-19-1
468	Azinphos ethyl	2642-71-9
453	Azinphos methyl	86-50-0
461	Chlorfevinphos	470-90-6
469	Chlorpyrifos	2921-88-2
443	Coumaphos	56-72-4
479	Crotoxyphos	7700-17-6
	DEF	78-48-8
471	Demeton	8065-48-3
460	Diazinon	333-41-5
	Dichlofenthion	97-17-6
450	Dichlorvos	62-73-7
455	Dicrotophos	141-66-2
449	Dimethoate	60-51-5
452	Dioxathion	78-34-2
458	Disulfoton	298-04-4
467	EPN	2104-64-5
463	Ethion	563-12-2
	Ethoprop	13194-48-4
446	Famphur	52-85-7
454	Fensulfothion	115-90-2
447	Fenthion	55-38-9
464	Hexamethylphosphoramide	680-31-9
474	Leptophos	21609-90-5
475	Malathion	121-75-5
	Merphos	150-50-5
	Methamidophos	10265-92-6
	Methyl chlorpyrifos	5598-13-0
456	Methyl parathion	298-00-0
	Methyl trithion	953-17-3
444	Mevinphos	7786-34-7
470	Monocrotophos	6923-22-4
459	Naled	300-76-5
448	Parathion (ethyl)	56-38-2
457	Phorate	298-02-2
465	Phosmet	732-11-6
473	Phosphamidon	13171-21-6
	Ronnel	299-84-3

Table 1. Organo-Phosphorus Pesticides Determined by Large-Bore, Fused-Silica Capillary Column Gas Chromatography with Flame Photometric Detector (cont.)

EPA		
EGD	Compound	CAS Registry
477	Sulfotepp	3689-24-5
	Sulprofos (Bolstar)	35400-43-2
476	TEPP	107-49-3
472	Terbufos	13071-79-9
466	Tetrachlorvinphos	961-11-5
	Tokuthion	34643-46-4
445	Trichlorfon	52-68-6
	Trichloronate	327-98-0
451	Tricresylphosphate	78-30-8
462	Trimethylphosphate	512-56-1

Table 2. Gas Chromatography of Organo-Phosphorus Pesticides

EPA EGD	Compound	Retention Time ¹ (min)		MDL ² (ng/L)
		DB-1	DB-1701	
450	Dichlorvos	6.56	9.22	4
444	Mevinphos	11.85	16.20	74
	Acephate	12.60	17.40	500
445	Trichlorofon	12.69	18.85	150 ³
	Methamidophos	15.10	19.20	100
471	Demeton-A	17.70	20.57	19
	Ethoprop	18.49	21.43	7
459	Naled	18.92	23.00	18
455	Dicrotophos	19.33	26.30	81
470	Monocrotophos	19.62	29.24	85
477	Sulfotepp	20.04	23.68	6
457	Phorate	20.12	23.08	10
449	Dimethoate	20.59	29.29	27
	Demeton-B	21.40	25.52	21
452	Dioxathion	22.24	26.70	121
472	Terbufos	22.97	24.55	26
473	Phosphamidon-E	23.70	29.89	28
458	Disulfoton	23.89	27.01	32
460	Diazinon	24.03	26.10	38
	Tributyl phosphate (surr)	24.50	17.20	-
	Phosphamidon-Z	25.88	32.62	116
456	Methyl parathion	25.98	32.12	18
	Dichlorofenthion	26.11	28.66	6
	Methyl chlorpyrifos	26.29	29.53	13
	Ronnel	27.33	30.09	11
475	Malathion	28.87	33.49	11
447	Fenthion	29.14	32.16	22
448	Parathion	29.29	34.61	10
469	Chlorpyrifos	29.48	32.15	4
	Trichloronate	30.44	32.12	14
461	Chlorfevinphos	32.05	36.08	2
479	Crotoxypfos	32.65	37.58	81
	Tokuthion	33.30	37.17	2
466	Tetrachlorvinphos	33.40	37.85	12
	DEF	34.05	37.50	50
	Merphos-B	35.16	37.37	18
454	Fensulfothion	36.58	43.86	104
	Methyl trithion	36.62	40.52	10
463	Ethion	37.61	41.67	13

Table 2. Gas Chromatography of Organo-Phosphorus Pesticides (cont.)

<i>EPA EGD</i>	<i>Compound</i>	<i>Retention Time¹ (min)</i>		<i>MDL² (ng/L)</i>
		<i>DB-1</i>	<i>DB-1701</i>	
	Sulprofos	38.10	41.74	6
446	Famphur	38.24	46.37	27
465	Phosmet	41.24	48.22	14
467	EPN	41.94	47.52	9
453	Azinphos methyl	43.33	50.26	9
474	Leptophos	44.32	47.36	14
468	Azinphos ethyl	45.55	51.88	22
	Triphenyl phosphate (surr)	47.68	40.43	-
443	Coumaphos	48.02	56.44	24

Notes:

1. Columns: 30 m long by 0.53 mm ID; DB-1: 1.5 μ ; DB-1701: 1.0 μ . Conditions suggested to meet retention times shown: 110°C for 0.5 min, 110 to 250° at 3°C/min, 250°C until coumaphos elutes. Carrier gas flow rate approximately 7 mL/min.
2. 40 CFR Part 136, Appendix B (49 FR 43234).
3. Estimated. Detection limits for soils (in ng/kg) are estimated to be 30 to 100 times this level.

Table 3. Concentrations of Calibration Solutions

EPA EGD	Compound	Concentration($\mu\text{g/mL}$)		
		Low	Medium	High
	Calibration group 1			
453	Azinphos methyl	0.1	0.5	2.0
450	Dichlorvos	0.5	2.5	10.0
458	Disulfoton	0.2	1.0	4.0
447	Fenthion	0.2	1.0	4.0
	Merphos-A	0.2	1.0	4.0
	Merphos-B	0.2	1.0	4.0
	Methyl trithion	0.5	2.5	10.0
	Ronnel	0.2	1.0	4.0
	Sulprofos	0.2	1.0	4.0
	Calibration group 2			
461	Chlorfevinphos	0.2	1.0	4.0
469	Chlorpyrifos	0.2	1.0	4.0
471	Demeton-A	0.2	1.0	4.0
	Demeton-B	0.2	1.0	4.0
	Dichlofenthion	0.2	1.0	4.0
449	Dimethoate	0.1	0.5	2.0
446	Famphur	0.5	2.5	10.0
474	Leptophos	0.2	1.0	4.0
456	Methyl parathion	0.2	1.0	4.0
445	Trichlorofon	0.5	2.5	10.0
451	Tricresylphosphate	1.0	5	20.0
	Calibration group 3			
468	Azinphos ethyl	0.2	1.0	4.0
479	Crotoxyphos	0.5	2.5	10.00
	DEF	0.2	1.0	4.0
454	Fensulfothion	0.5	2.5	10.0
	Methyl chlorpyrifos	0.2	1.0	4.0
444	Mevinphos	0.5	2.5	10.0
459	Naled	0.5	2.5	10.0
448	Parathion	0.2	1.0	4.0
465	Phosmet	0.5	2.5	10.0
473	Phosphamidon-E	0.5	2.5	10.0
	Phosphamidon-Z	0.5	2.5	10.0
477	Sulfotepp	0.2	1.0	4.0
472	Terbufos	0.2	1.0	4.0
	Calibration group 4			
443	Coumaphos	0.5	2.5	10.0
460	Diazinon	0.2	1.0	4.0
467	EPN	0.2	1.0	4.0

Table 3. Concentrations of Calibration Solutions (cont.)

<i>EPA EGD</i>	<i>Compound</i>	<i>Concentration(µg/mL)</i>		
		<i>Low</i>	<i>Medium</i>	<i>High</i>
463	Ethion	0.2	1.0	4.0
	Ethoprop	0.2	1.0	4.0
475	Malathion	0.2	1.0	4.0
457	Phorate	0.2	1.0	4.0
466	Tetrachlorvinphos	0.2	1.0	4.0
	Trichloronate	0.2	1.0	4.0

Table 4. Acceptance Criteria for Performance Tests for Organo-Phosphorus Compounds

EGD No.	Compound	Spike Level (ng/L)	Acceptance Criteria			
			Initial Precision and Accuracy (%)		Calibration Verification	Recovery Ongoing Accuracy
			s	x	(µg/mL)	R (%)
	Acephate	50000	25	32 - 122	68 - 132	28 - 126
468	Azinphos ethyl	200	10	71 - 117	77 - 127	59 - 129
453	Azinphos methyl	100	10	52 - 112	83 - 119	37 - 127
461	Chlorfevinphos	200	11	56 - 132	83 - 114	37 - 151
469	Chlorpyrifos	200	10	61 - 112	80 - 119	48 - 125
443	Coumaphos	500	10	78 - 104	82 - 120	72 - 110
479	Crotoxyphos	500	46	28 - 116	68 - 136	6 - 138
	DEF	1500	31	45 - 107	68 - 132	42 - 110
471	Demeton	200	23	33 - 101	64 - 123	16 - 118
460	Diazinon	200	10	70 - 110	86 - 114	60 - 120
	Dichlofenthion	200	10	75 - 115	80 - 110	65 - 125
450	Dichlorvos	500	18	52 - 106	77 - 103	39 - 119
455	Dicrotophos		not recovered		78 - 122	
449	Dimethoate	100	89	27 - 100	73 - 127	22 - 100
452	Dioxathion	600	22	59 - 101	79 - 121	49 - 111
458	Disulfoton	200	30	46 - 98	70 - 118	33 - 111
467	EPN	200	13	74 - 124	81 - 108	62 - 136
463	Ethion	200	11	72 - 134	70 - 118	47 - 149
	Ethoprop	200	14	79 - 103	84 - 108	73 - 109
446	Famphur	500	12	81 - 101	81 - 113	76 - 106
454	Fensulfothion	500	65	13 - 115	42 - 139	0 - 141
447	Fenthion	200	13	69 - 101	73 - 137	61 - 109
464	Hexamethylphosphoramide		not recovered		70 - 130	
474	Leptophos	200	10	85 - 105	85 - 112	80 - 110
475	Malathion	200	10	75 - 109	82 - 108	66 - 118
	Merphos-B	200	10	68 - 102	72 - 118	59 - 111
	Methamidophos ¹	10000	33	66 - 132	70 - 128	63 - 135
	Methyl chlorpyrifos	200	10	88 - 108	81 - 114	83 - 113
456	Methyl parathion	200	15	72 - 112	89 - 114	61 - 123
	Methyl trithion	500	20	21 - 137	78 - 122	0 - 166
444	Mevinphos	500	23	24 - 100	73 - 135	7 - 107
470	Monocrotophos		not recovered		19 - 206	
459	Naled	500	10	0 - 148	77 - 114	0 - 176
448	Parathion	200	10	71 - 111	79 - 110	61 - 121
457	Phorate	200	19	54 - 100	70 - 118	43 - 109
465	Phosmet	500	39	44 - 119	61 - 159	25 - 138

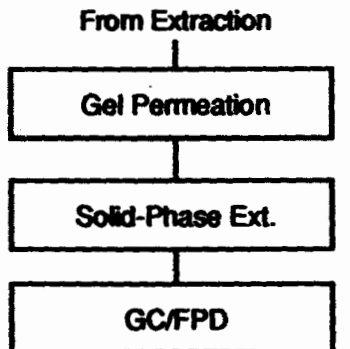
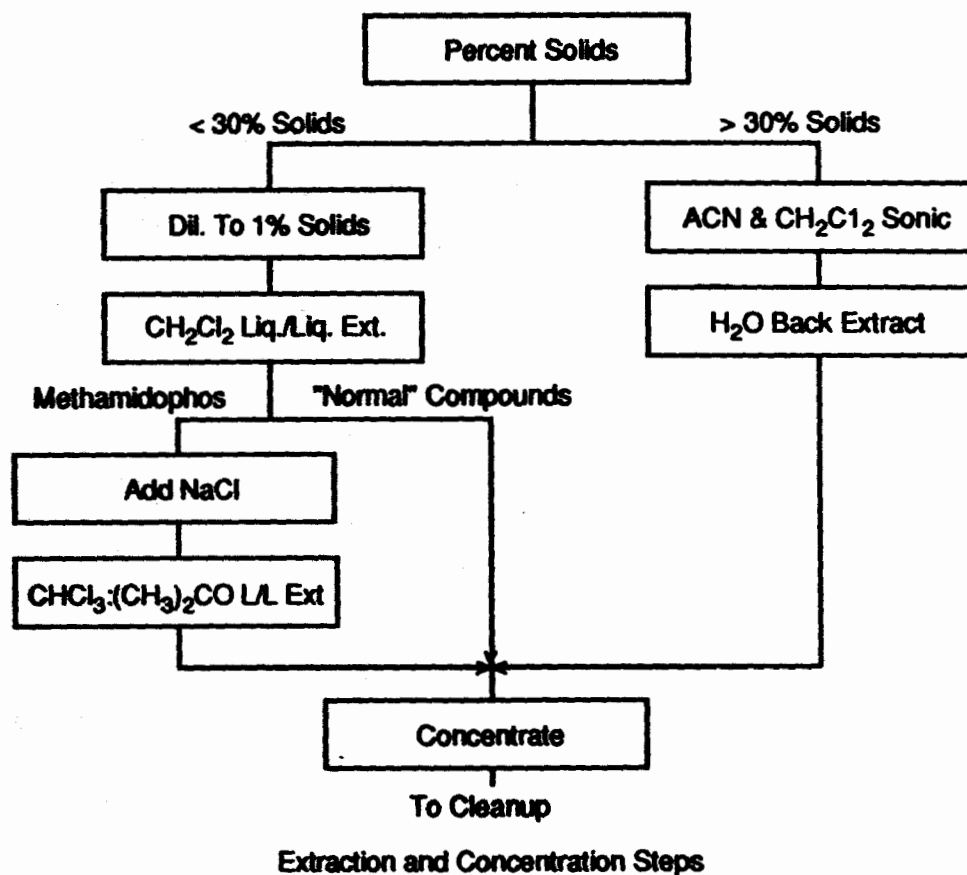
Table 4. Acceptance Criteria for Performance Tests for Organo-Phosphorus Compounds (cont.)

EGD No.	Compound	Spike Level (ng/L)	Acceptance Criteria			
			Initial Precision and Accuracy (%)		Calibration Verification	Recovery Ongoing Accuracy
			s	x	(µg/mL)	R (%)
473	Phosphamidon-Z	500	45	0 - 100	81 - 102	0 - 100
	Ronnel	200	10	79 - 111	78 - 113	71 - 119
477	Sulfotepp	200	10	70 - 120	75 - 115	58 - 132
	Sulprofos	200	10	75 - 100	81 - 118	70 - 100
476	TEPP		not recovered		70 - 130	
472	Terbufos	200	23	60 - 110	82 - 111	47 - 123
466	Tetrachlorvinphos	200	11	48 - 110	73 - 119	32 - 126
	Tokuthion	100	17	73 - 105	70 - 130	65 - 113
445	Trichlorofon ²	50000	42	43 - 195	58 - 142	37 - 201
	Trichloronate	200	10	82 - 102	80 - 113	77 - 107
451	Tricresylphosphate	1000	10	81 - 101	70 - 130	74 - 114
462	Trimethylphosphate		not recovered		70 - 130	

Notes:

¹With salt and azeotropic extraction

²With salt



AS2-002-83

Figure 1. Extraction, Cleanup, Derivatization, and Analysis

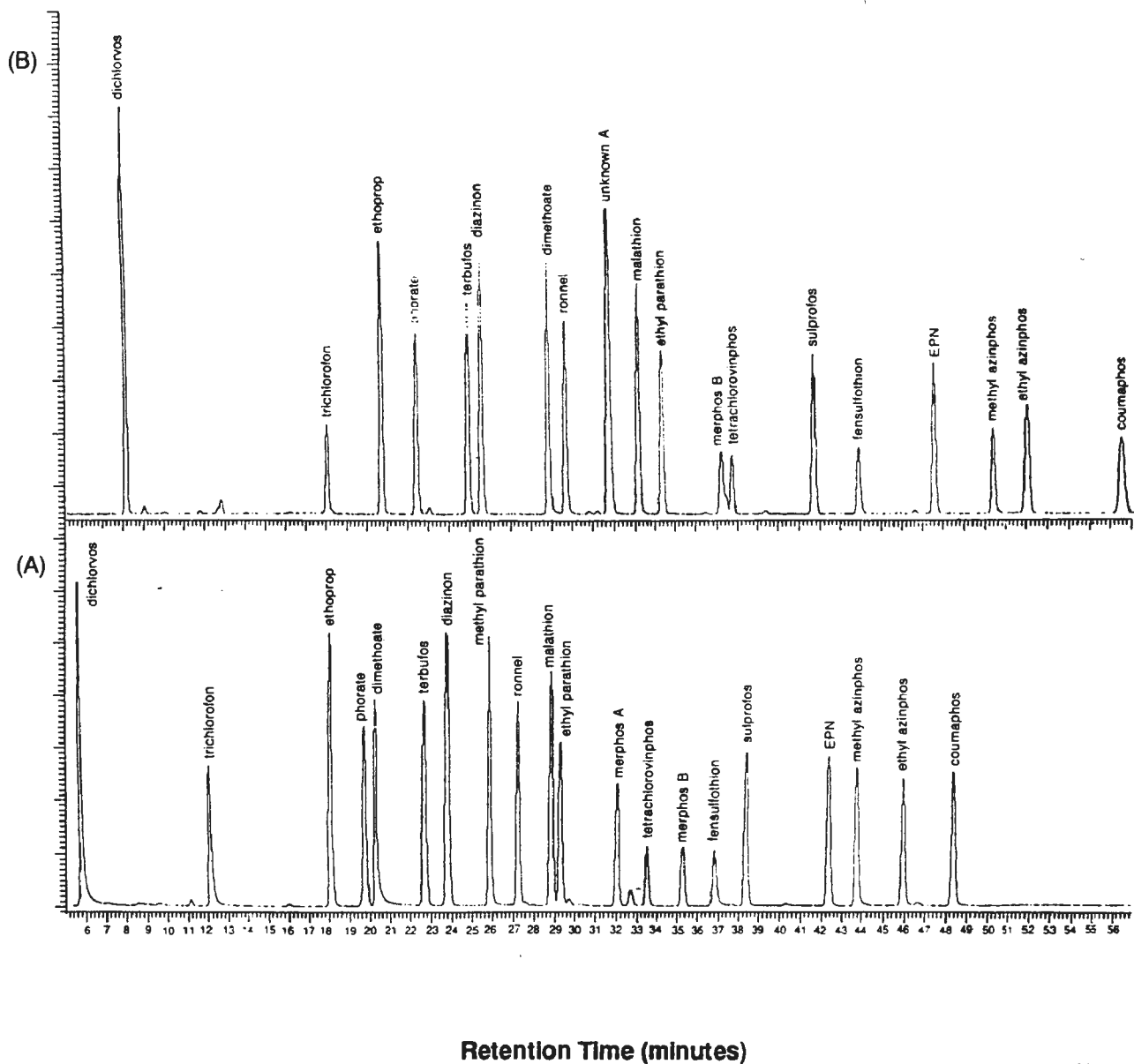


Figure 2. Gas Chromatogram of Selected Organo-Phosphorus Compounds.

Method 1658
The Determination of
Phenoxy-Acid Herbicides in
Municipal and Industrial
Wastewater

Method 1658

The Determination of Phenoxy-Acid Herbicides in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method is designed to meet the survey requirements of the Environmental Protection Agency (EPA). It is used to determine (1) the phenoxy-acid herbicides and herbicide esters associated with the Clean Water Act, the Resource Conservation and Recovery Act, and the Comprehensive Environmental Response, Compensation and Liability Act; and (2) other compounds amenable to extraction and analysis by automated, wide-bore capillary column gas chromatography (GC) with electron capture or halogen-selective detectors.
- 1.2 The chemical compounds listed in Table 1 may be determined in waters, soils, sediments, and sludges by this method. This method should be applicable to other herbicides. The quality assurance/quality control requirements in this method give the steps necessary to determine this applicability.
- 1.3 When this method is applied to analysis of unfamiliar samples, compound identity must be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Gas chromatography mass spectrometry (GC/MS) can be used to confirm compounds in extracts produced by this method when analyte levels are sufficient.
- 1.4 The detection limit of this method is usually dependent on the level of interferences rather than instrumental limitations. The limits in Table 2 typify the minimum quantity that can be detected with no interferences present.
- 1.5 This method is for use by or under the supervision of analysts experienced in the use of a gas chromatograph and in the interpretation of gas chromatographic data. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 8.2.

2. SUMMARY OF METHOD

2.1 Extraction.

2.1.1 The percent solids content of a sample is determined.

2.1.2 Samples containing low solids: If the solids content is less than or equal to 1%, the sample is extracted directly using continuous extraction techniques. The pH of a 1-L sample raised to 12 to 13 to hydrolyze acid esters, and the sample is extracted with methylene chloride to remove interferences. The pH is lowered to less than 2 and the free acids are extracted with methylene chloride.

- 2.1.3 Samples containing greater than 1% solids.**
- 2.1.3.1 Solids content 1 to 30%:** The sample is diluted to 1% solids with reagent water, homogenized ultrasonically, and extracted as a low-solids sample (Section 2.1.2).
- 2.1.3.2 Solids content greater than 30%:** The sample is placed in an extraction bottle and approximately 1-L of basic (pH 12-13) water is added. The bottle is tumbled for 18 hours. The water is removed and extracted as a low-solids sample (Section 2.1.2).
- 2.2 Concentration and cleanup:** The extract is dried over sodium sulfate, concentrated using a Kuderna-Danish evaporator, cleaned up (if necessary) using gel permeation chromatography (GPC) and concentrated to 5 or 10 mL (depending upon whether GPC was or was not used).
- 2.3 Derivatization and cleanup:** The acids in the extract are derivatized to form the methyl esters. The solution containing the methyl esters is cleaned up (if necessary) using solid-phase extraction (SPE) and/or adsorption chromatography and reconstituted to 5 or 10 mL.
- 2.4 Gas chromatography:** A 1- μ L aliquot of the extract is injected into the gas chromatograph (GC). The derivatized acids are separated on a wide-bore, fused-silica capillary column and are detected by an electron capture, microcoulometric, or electrolytic conductivity detector.
- 2.5 Identification of a pollutant (qualitative analysis)** is performed by comparing the GC retention times of the compound on two dissimilar columns with the respective retention times of an authentic standard. Compound identity is confirmed when the retention times agree within their respective windows.
- 2.6 Quantitative analysis** is performed by using an authentic standard to produce a calibration factor or calibration curve, and using the calibration data to determine the concentration of a pollutant in the extract. The concentration in the sample is calculated using the sample weight or volume and the extract volume.
- 2.7 Quality** is assured through reproducible calibration and testing of the extraction and GC systems.

3. CONTAMINATION AND INTERFERENCES

- 3.1 Solvents, reagents, glassware, and other sample-processing hardware** may yield artifacts and/or elevated baselines causing misinterpretation of chromatograms. All materials used in the analysis shall be demonstrated to be free from interferences under the conditions of analysis by running method blanks as described in Section 8.5.
- 3.2 Glassware and, where possible, reagents** are cleaned by solvent rinse and baking at 450°C for 1 hour minimum in a muffle furnace or kiln. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment and thorough rinsing with acetone and pesticide-quality hexane may be required.
- 3.3 Specific selection of reagents and purification of solvents** by distillation in all-glass systems may be required.
- 3.4 Interference by phthalate esters** can pose a major problem in herbicide analysis when using the electron capture detector. Phthalates usually appear in the chromatogram as large, late-eluting peaks. Phthalates may be leached from common flexible plastic tubing and other plastic materials during the extraction and clean-up processes. Cross-contamination of clean glassware routinely

occurs when plastics are handled during extraction, especially when solvent-wetted surfaces are handled. Interferences from phthalates can best be minimized by avoiding the use of plastics in the laboratory, or by using a microcoulometric or electrolytic conductivity detector.

- 3.5 The acid forms of the herbicides are strong acids that react readily with alkaline substances and can be lost during analysis. Glassware, glass wool, and all other apparatuses should be rinsed with dilute hydrochloric or sulfuric acid prior to use. Sodium sulfate and other reagents that can be acidified should be acidified to preclude the herbicides from being adsorbed by these reagents.
- 3.6 Organic acids and phenols cause the most direct interference with the herbicides. Alkaline hydrolysis and subsequent extraction of the basic solution can remove many hydrocarbons and esters that may interfere with the herbicide analysis.
- 3.7 Interferences coextracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled. The cleanup procedures given in this method can be used to overcome many of these interferences, but unique samples may require additional cleanup to achieve the minimum levels given in Table 2.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 1 through 3.
- 4.2 Primary standards of hazardous compounds shall be prepared in a hood, and a NIOSH/MESA approved toxic gas respirator should be worn when high concentrations are handled.
- 4.3 Diazomethane is a toxic carcinogen which can decompose or explode under certain conditions. Solutions decompose rapidly in the presence of solid materials such as copper powder, calcium chloride, and boiling chips. The following operations may cause explosion: heating above 90°C; use of grinding surfaces such as ground-glass joints, sleeve bearings, and glass stirrers; and storage near alkali metals. Diazomethane shall be used only behind a safety screen in a well ventilated hood and should be pipetted with mechanical devices only.
- 4.4 Unknown samples may contain high concentrations of volatile toxic compounds. Sample containers should be opened in a hood and handled with gloves that will prevent exposure. The oven used for sample drying to determine percent moisture should be located in a hood so that vapors from samples do not create a health hazard in the laboratory.

5. APPARATUS AND MATERIALS

NOTE: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance meeting requirements of this method is the responsibility of the laboratory.

5.1 Sampling equipment for discrete or composite sampling.

5.1.1 Sample bottles and caps.

5.1.1.1 Liquid samples (waters, sludges and similar materials that contain less than 5% solids): Sample bottle, amber glass, 1-L or 1-quart, with screw-cap.

5.1.1.2 Solid samples (soils, sediments, sludges, filter cake, compost, and similar materials that contain more than 5% solids): Sample bottle, wide-mouth, amber glass, 500-mL minimum.

5.1.1.3 If amber bottles are not available, samples shall be protected from light.

5.1.1.4 Bottle caps: Threaded to fit sample bottles. Caps shall be lined with PTFE.

5.1.1.5 Cleaning.

5.1.1.5.1 Bottles are detergent-water washed, then rinsed with solvent rinsed or baked at 450°C for 1 hour minimum before use.

5.1.1.5.2 Liners are detergent-water washed, then rinsed with reagent water and solvent, and baked at approximately 200°C for 1 hour minimum prior to use.

5.1.2 Compositing equipment: Automatic or manual compositing system incorporating glass containers cleaned per bottle cleaning procedure above. Sample containers are kept at 0 to 4°C during sampling. Glass or PTFE tubing only shall be used. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, the tubing shall be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.

5.2 Equipment for determining percent moisture.

5.2.1 Oven, capable of maintaining a temperature of 110°C ($\pm 5^\circ\text{C}$).

5.2.2 Dessicator.

5.2.3 Crucibles, porcelain.

5.2.4 Weighing pans, aluminum.

5.3 Extraction equipment.

5.3.1 Equipment for ultrasonic extraction.

5.3.1.1 Sonic disruptor: 375 watt with pulsing capability and ½- or ¾-inch. disruptor horn (Ultrasonics, Inc, Model 375C, or equivalent).

5.3.1.2 Sonabox (or equivalent), for use with disruptor.

5.3.2 Equipment for liquid-liquid extraction.

5.3.2.1 Continuous liquid-liquid extractor: PTFE or glass connecting joints and stopcocks without lubrication, 1.5- to 2-L (Hershberg-Wolf Extractor, Cal-Glass, Costa Mesa, California, 1000- or 2000-mL continuous extractor, or equivalent).

5.3.2.2 Round-bottom flask, 500-mL, with heating mantle.

- 5.3.2.3 Condenser, Graham, to fit extractor.
- 5.3.2.4 pH meter, with combination glass electrode.
- 5.3.2.5 pH paper, wide range (Hydriion Papers, or equivalent).
- 5.3.3 Separatory funnels: 250-, 500-, 1000-, and 2000-mL, with PTFE stopcocks.
- 5.3.4 Filtration apparatus.
 - 5.3.4.1 Glass powder funnels: 125- to 250-mL.
 - 5.3.4.2 Filter paper for above (Whatman 41, or equivalent).
- 5.3.5 Beakers.
 - 5.3.5.1 1.5- to 2-L, calibrated to 1 L.
 - 5.3.5.2 400- to 500-mL.
- 5.3.6 Spatulas: Stainless steel or PTFE.
- 5.3.7 Drying column: 400 mm long by 15 to 20 mm ID, Pyrex chromatographic column equipped with coarse glass frit or glass wool plug.
 - 5.3.7.1 Pyrex glass wool: Extracted with solvent or baked at 450°C for 1 hour minimum.
- 5.3.8 TLCP extractor.
 - 5.3.8.1 Rotary agitation apparatus: Capable of rotating the extraction vessel in an end over end fashion at 30 rpm (± 2 rpm) (Associated Design and Manufacturing Co, or equivalent).
 - 5.3.8.2 Bottle, polyethylene or polypropylene, 1- to 4-L, with screw-cap with PTFE-lined lid, to fit extractor.
- 5.4 Evaporation/concentration apparatus.
 - 5.4.1 Kuderna-Danish (K-D) apparatus.
 - 5.4.1.1 Evaporation flask: 500-mL (Kontes K-570001-0500, or equivalent), attached to concentrator tube with springs (Kontes K-662750-0012).
 - 5.4.1.2 Concentrator tube: 10-mL, graduated (Kontes K-570050-1025, or equivalent) with calibration verified. Ground-glass stopper (size 19/22 joint) is used to prevent evaporation of extracts.
 - 5.4.1.3 Snyder column: Three-ball macro (Kontes K-503000-0232, or equivalent).
 - 5.4.1.4 Snyder column: Two-ball micro (Kontes K-469002-0219, or equivalent).
 - 5.4.1.5 Boiling chips.
 - 5.4.1.5.1 Glass or silicon carbide: Approximately 10/40 mesh, extracted with methylene chloride and baked at 450°C for 1 hour minimum.
 - 5.4.1.5.2 PTFE (optional): Extracted with methylene chloride.
 - 5.4.2 Water bath: Heated, with concentric ring cover, capable of temperature control ($\pm 2^\circ\text{C}$), installed in a fume hood.

- 5.4.3 Nitrogen evaporation device: Equipped with heated bath that can be maintained at 35 to 40°C (N-Evap, Organomation Associates, Inc., or equivalent).
- 5.4.4 Sample vials: Amber glass, 1- to 5-mL with PTFE-lined screw- or crimp-cap, to fit GC auto-sampler.
- 5.5 Balances.
 - 5.5.1 Analytical: Capable of weighing 0.1 mg.
 - 5.5.2 Top loading: Capable of weighing 10 mg.
- 5.6 Apparatus for sample cleanup.
 - 5.6.1 Automated gel permeation chromatograph (Analytical Biochemical Labs, Inc, Columbia, MO, Model GPC Autoprep 1002, or equivalent).
 - 5.6.1.1 Column: 600 to 700 mm long by 25 mm ID, packed with 70 g of SX-3 Bio-beads (Bio-Rad Laboratories, Richmond, CA, or equivalent).
 - 5.6.1.2 Syringe, 10-mL, with Luer fitting.
 - 5.6.1.3 Syringe-filter holder, stainless steel, and glass fiber or PTFE filters (Gelman Acrodisc-CR, 1 to 5 micron, or equivalent).
 - 5.6.1.4 UV detectors: 254-mu, preparative or semi-prep flow cell: (Isco, Inc., Type 6; Schmadzu, 5 mm path length; Beckman-Altex 152W, 8-μL micro-prep flow cell, 2-mm path; Pharmacia UV-1, 3-mm flow cell; LDC Milton-Roy UV-3, monitor #1203; or equivalent).
 - 5.6.2 Vacuum system and cartridges for solid phase extraction (SPE).
 - 5.6.2.1 Vacuum system: Capable of achieving 0.1 bar (house vacuum, vacuum pump, or water aspirator), with vacuum gauge.
 - 5.6.2.2 VacElute Manifold (Analytichem International, or equivalent).
 - 5.6.2.3 Vacuum trap: Made from 500-mL sidearm flask fitted with single-hole rubber stopper and glass tubing.
 - 5.6.2.4 Rack for holding 50-mL volumetric flasks in the manifold.
 - 5.6.2.5 Column: Mega Bond Elut, Non-polar, C18 Octadecyl, 10 g/60 mL (Analytichem International Cat. No. 607H060, or equivalent).
 - 5.6.3 Chromatographic column: 400 mm long by 22 mm ID, with PTFE stopcock and coarse frit (Kontes K-42054, or equivalent).
- 5.7 Centrifuge apparatus.
 - 5.7.1 Centrifuge: Capable of rotating 500-mL centrifuge bottles or 15-mL centrifuge tubes at 5,000 rpm minimum.
 - 5.7.2 Centrifuge bottles: 500-mL, with screw-caps, to fit centrifuge.
 - 5.7.3 Centrifuge tubes: 12- to 15-mL, with screw-caps, to fit centrifuge.
 - 5.7.4 Funnel, Buchner, 15 cm.
 - 5.7.4.1 Flask, filter, for use with Buchner funnel.
 - 5.7.4.2 Filter paper, 15 cm (Whatman #41, or equivalent).

- 5.8** Derivatization apparatus: Diazald kit with clear seal joints for generation of diazomethane (Aldrich Chemical Co. Z10,025-0, or equivalent).
- 5.9** Miscellaneous glassware.
- 5.9.1** Pipettes, glass, volumetric, 1.00-, 5.00-, and 10.0-mL.
- 5.9.2** Syringes, glass, with Luerlok tip, 0.1-, 1.0- and 5.0-mL. Needles for syringes, 2-inch, 22-gauge.
- 5.9.3** Volumetric flasks, 10.0-, 25.0-, and 50.0-mL.
- 5.9.4** Scintillation vials, glass, 20- to 50-mL, with PTFE-lined screw-caps.
- 5.10** Gas chromatograph: Shall have splitless or on-column simultaneous automated injection into separate capillary columns with an electron capture or halide-specific detector at the end of each column, temperature program with isothermal holds, data system capable of recording simultaneous signals from the two detectors, and shall meet all of the performance specifications in Section 14.
- 5.10.1** GC columns: Bonded-phase fused-silica capillary.
- 5.10.1.1** Primary: 30 m (± 3 m) long by 0.5 mm (± 0.05 mm) ID (DB-608, or equivalent).
- 5.10.1.2** Confirmatory: DB-1701, or equivalent, with same dimensions as primary column.
- 5.10.2** Data system shall collect and record GC data, store GC runs on magnetic disk or tape, process GC data, compute peak areas, store calibration data including retention times and calibration factors, identify GC peaks through retention times, compute concentrations, and generate reports.
- 5.10.2.1** Data acquisition: GC data shall be collected continuously throughout the analysis and stored on a mass storage device.
- 5.10.2.2** Calibration factors and calibration curves: The data system shall be used to record and maintain lists of calibration factors, and multi-point calibration curves (Section 7). Computations of relative standard deviation (coefficient of variation) are used for testing calibration linearity. Statistics on initial (Section 8.2) and ongoing (Section 14.6) performance shall be computed and maintained.
- 5.10.2.3** Data processing: The data system shall be used to search, locate, identify, and quantify the compounds of interest in each GC analysis. Software routines shall be employed to compute and record retention times and peak areas. Displays of chromatograms and library comparisons are required to verify results.
- 5.10.3** Detectors.
- 5.10.3.1** Halide-specific: Electron capture or electrolytic conductivity (Micoulometric, Hall, O.I., or equivalent), capable of detecting 100 pg of 2,4-D under the analysis conditions given in Table 2.

6. REAGENTS AND STANDARDS

6.1 Sample preservation: Sodium thiosulfate (ACS), granular.

6.2 pH adjustment.

6.2.1 Sodium hydroxide: Reagent grade.

6.2.1.1 Concentrated solution (10N): Dissolve 40 g NaOH in 100 mL reagent water.

6.2.1.2 Dilute solution (0.1M): Dissolve 4 g NaOH in 1 L of reagent water.

6.2.2 Sulfuric acid (1 + 1): Reagent grade, 6N in reagent water. Slowly add 50 mL H₂SO₄ (specific gravity 1.84) to 50 mL reagent water.

6.2.3 Potassium hydroxide: 37% (W/V). Dissolve 37 g KOH in 100 mL reagent water.

6.3 Acidified sodium sulfate: Add 0.5 mL H₂SO₄ and 30 mL ethyl ether to 100 g sodium sulfate. Mix thoroughly. Allow the ether to evaporate completely. Transfer the mixture to a clean container and store at 110°C (±5°C).

6.4 Solvents: Methylene chloride, hexane, ethyl ether, acetone, acetonitrile, isooctane, and methanol; pesticide-quality; lot-certified to be free of interferences.

6.4.1 Ethyl ether must be shown to be free of peroxides before it is used, as indicated by EM Laboratories Quant test strips (Scientific Products P1126-8, or equivalent). Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL of ethyl alcohol is added to each liter of ether as a preservative.

6.5 GPC calibration solution: Solution containing 300 mg/mL corn oil, 15-mg/mL bis(2-ethylhexyl) phthalate, 1.4 mg/mL pentachlorophenol, 0.1 mg/mL perylene, and 0.5 mg/mL sulfur.

6.6 Sample cleanup.

6.6.1 Florisil: PR grade, 60/100 mesh, activated at 650 to 700°C, stored in the dark in glass container with PTFE-lined screw-cap. Activate at 130°C for 16 hours minimum immediately prior to use. Alternatively, 500-mg cartridges (J.T. Baker, or equivalent) may be used.

6.6.2 Solid-phase extraction.

6.6.2.1 SPE cartridge calibration solution: 2,4,6-trichlorophenol, 0.1 µg/mL in acetone.

6.6.2.2 SPE elution solvent: Methylene chloride: acetonitrile: hexane (50:3:47).

6.7 Derivatization: Diazald reagent [N-methyl-(N-nitroso-p-toluenesulfanamide)], fresh and high-purity (Aldrich Chemical Co.).

6.8 Reference matrices.

6.8.1 Reagent water: Water in which the compounds of interest and interfering compounds are not detected by this method.

6.8.2 High-solids reference matrix: Playground sand or similar material in which the compounds of interest and interfering compounds are not detected by this method. May be prepared by extraction with methylene chloride and/or baking at 450°C for 4 hours minimum.

- 6.9 Standard solutions:** Purchased as solutions or mixtures with certification to their purity, concentration, and authenticity, or prepared from materials of known purity and composition. If compound purity is 96% or greater, the weight may be used without correction to compute the concentration of the standard. When not being used, standards are stored in the dark at -20 to -10°C in screw-capped vials with PTFE-lined lids. A mark is placed on the vial at the level of the solution so that solvent evaporation loss can be detected. The vials are brought to room temperature prior to use. Any precipitate is redissolved and solvent is added if solvent loss has occurred.
- 6.10 Preparation of stock solutions:** Prepare in isooctane per the steps below. Observe the safety precautions in Section 4.
- 6.10.1** Dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 10 mg 2,4-D in a 10-mL ground- glass stoppered volumetric flask and fill to the mark with isooctane. After the 2,4-D is completely dissolved, transfer the solution to a 15-mL vial with PTFE-lined cap.
- 6.10.2** Stock standard solutions should be checked for signs of degradation prior to the preparation of calibration or performance test standards.
- 6.10.3** Stock standard solutions shall be replaced after 6 months, or sooner if comparison with quality control check standards indicates a change in concentration.
- 6.11 Secondary mixtures:** Combine stock solutions (Section 6.10) into a secondary mixture at the highest level required for required for calibration (Table 3). Derivatize the acids in this solution using the procedure in Section 12. After derivatization, prepare the solutions for calibration and calibration verification (Table 3), for initial and ongoing precision and recovery (Sections 8.2 and 14.6), and for spiking into the sample matrix (Section 8.4).
- 6.12 Surrogate spiking solution:** Prepare 2,4-dichlorophenylacetic acid at a concentration of 2 ng/mL in acetone.
- 6.13 Stability of solutions:** All standard solutions (Sections 6.9 - 6.12) shall be analyzed within 48 hours of preparation and on a monthly basis thereafter for signs of degradation. Standards will remain acceptable if the peak area remains within $\pm 15\%$ of the area obtained in the initial analysis of the standard.

7. SETUP AND CALIBRATION

- 7.1** Configure the GC system as given in Section 5.10 and establish the operating conditions in Table 2.
- 7.2** Attainment of method detection limit (MDL): Determine that the MDLs in Table 2 can be met on each column/detector system.
- 7.3** Calibration: Inject the calibration solutions into each GC column/detector pair, beginning with the lowest level mixture and proceeding to the highest. For each compound, compute and store, as a function of the concentration injected, the retention time, and the peak area on each column/detector system (primary and confirmatory).
- 7.3.1** Retention time: The polar nature of some analytes causes the retention time to decrease as the quantity injected increases. To compensate this effect, the retention time for compound identification is correlated with the analyte level.

- 7.3.1.1 If the difference between the maximum and minimum retention times for any compound is less than 5 seconds over the calibration range, the retention time for that compound can be considered constant and an average retention-time may be used for compound identification.
- 7.3.1.2 Retention time calibration curve (retention time vs. amount): If the retention time for a compound in the lowest level standard is more than 5 seconds greater than the retention time for the compound in the highest level standard, a retention time calibration curve shall be used for identification of that compound.
- 7.3.2 Calibration factor (ratio of area to amount injected).
 - 7.3.2.1 Compute the coefficient of variation (relative standard deviation) of the calibration factor over the calibration range for each compound on each column/detector system.
 - 7.3.2.2 Linearity: If the calibration factor for any compound is constant ($C_v < 20\%$) over the calibration range, an average calibration factor may be used for that compound; otherwise, the complete calibration curve (area vs. amount) for that compound shall be used.
- 7.4 Combined QC standards: To preclude periodic analysis of all of the individual calibration groups of compounds (Section 7.3.1), the GC systems are calibrated with combined solutions as a final step. Not all of the compounds in these standards will be separated by the GC columns used in this method. Retention times and calibration factors are verified for the compounds that are resolved, and calibration factors are obtained for the unresolved peaks. These combined QC standards are prepared at the level the mid-range calibration standard (Table 3).
 - 7.4.1 Analyze the combined QC standards on their respective column/detector pairs.
 - 7.4.1.1 For those compounds that exhibit a single, resolved GC peak, the retention time shall be within ± 5 seconds of the retention time of the peak in the medium level calibration standard (Table 3), and the calibration factor using the primary column shall be within $\pm 20\%$ of the calibration factor in the medium level standard (Table 3).
 - 7.4.1.2 For the peaks containing two or more compounds, compute and store the retention times at the peak maxima on both columns (primary and confirmatory), and also compute and store the calibration factors on both columns. These results will be used for calibration verification (Section 14.2 and 14.5) and for precision and recovery studies (Sections 8.2 and 14.6).
- 7.5 Florisil calibration: The cleanup procedure in Section 11 utilizes Florisil column chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil that is used, the use of the lauric acid value (Reference 4) is suggested. The referenced procedure determines the adsorption of lauric acid (in milligrams per gram Florisil) from hexane solution. The amount of Florisil to be used for each column is calculated by dividing 110 by this ratio and multiplying by 20 g.

8. QUALITY CONTROL

- 8.1** Each laboratory that uses this method is required to operate a formal quality control program (Reference 5). The minimum requirements of this program consist of an initial demonstration of laboratory capability, an ongoing analysis of standards and blanks as tests of continued performance, and analysis of spiked samples to assess accuracy. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method. If the method is to be applied routinely to samples containing high solids with very little moisture (e.g., soils, compost), the high-solids reference matrix (Section 6.8.2) is substituted for the reagent water (Section 6.8.1) in all performance tests, and the high-solids method (Section 10) is used for these tests.
- 8.1.1** The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2** The analyst is permitted to modify this method to improve separations or lower the costs of measurements, provided all performance requirements are met. Each time a modification is made to the method or a cleanup procedure is added, the analyst is required to repeat the procedure in Section 8.2 to demonstrate method performance. If detection limits will be affected by the modification, the analyst is required to repeat the demonstration of detection limits (Section 7.2).
- 8.1.3** The laboratory shall spike all samples with at least one surrogate compound to monitor method performance. This test is described in Section 8.3. When results of these spikes indicate a typical method performance for samples, the samples are diluted to bring method performance within acceptable limits (Section 17).
- 8.1.4** The laboratory shall, on an ongoing basis, demonstrate through calibration verification and the analysis of the combined QC standard (Section 7.4) that the analysis system is in control. These procedures are described in Sections 14.1, 14.5, and 14.6.
- 8.1.5** The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Section 8.4.
- 8.1.6** Analyses of blanks are required to demonstrate freedom from contamination. The procedures and criteria for analysis of a blank are described in Section 8.5.
- 8.1.7** Other analytes may be determined by this method. The procedure for establishing a preliminary quality control limit for a new analyte is given in Section 8.6.
- 8.2** Initial precision and recovery: To establish the ability to generate acceptable precision and recovery, the analyst shall perform the following operations.
- 8.2.1** For analysis of samples containing low solids (aqueous samples), extract, concentrate, and analyze one set of four 1-L aliquots of reagent water spiked with the combined QC standard (Section 7.4) according to the procedure in Section 10. Alternatively, sets of four replicates of the individual calibration groups (Section 7.3) may be used. For samples containing high-solids, sets of four 30-g aliquots of the high-solids reference matrix are used.
- 8.2.2** Using results of the set of four analyses, compute the average percent recovery (\bar{X}) and the coefficient of variation (C_v) of percent recovery (s) for each compound.

- 8.2.3** For each compound, compare *s* and *X* with the corresponding limits for initial precision and accuracy in Table 4. For coeluting compounds, use the coeluted compound with the least restrictive specification (largest *C_s* and widest range). If *s* and *X* for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any individual *s* exceeds the precision limit or any individual *X* falls outside the range for accuracy, system performance is unacceptable for that compound. In this case, correct the problem and repeat the test.
- 8.3** The laboratory shall spike all samples with at least one surrogate compound to assess method performance on the sample matrix.
- 8.3.1** Analyze each sample according to the method beginning in Section 10.
- 8.3.2** Compute the percent recovery (*P*) of the surrogate compound(s).
- 8.3.3** The recovery of the surrogate compound shall be within the limits of 40 to 120%. If the recovery of any surrogate falls outside of these limits, method performance is unacceptable for that sample, and the sample is complex. Water samples are diluted, and smaller amounts of soils, sludges, and sediments are reanalyzed per Section 17.
- 8.4** Method accuracy: The laboratory shall spike (matrix spike) at least 10% of the samples from a given site type (e.g., influent to treatment, treated effluent, produced water, river sediment). If only one sample from a given site type is analyzed, that sample shall be spiked.
- 8.4.1** The concentration of the matrix spike shall be determined as follows.
- 8.4.1.1** If, as in compliance monitoring, the concentration of a specific analyte in the sample is being checked against a regulatory concentration limit, the matrix spike shall be at that limit or at 1 to 5 times higher than the background concentration determined in Section 8.4.2, whichever concentration is larger.
- 8.4.1.2** If the concentration of an analyte in the sample is not being checked against a limit specific to that analyte, the matrix spike shall be at the concentration of the combined QC standard (Section 7.4) or at 1 to 5 times higher than the background concentration, whichever concentration is larger.
- 8.4.1.3** If it is impractical to determine the background concentration before spiking (e.g., maximum holding times will be exceeded), the matrix spike concentration shall be the regulatory concentration limit, if any; otherwise, the larger of either 5 times the expected background concentration or at the concentration of the combined QC standard (Section 7.4).
- 8.4.2** Analyze one sample aliquot to determine the background concentration (*B*) of each analyte. If necessary, prepare a standard solution appropriate to produce a level in the sample one to five times the background concentration. Spike a second sample aliquot with the standard solution and analyze it to determine the concentration after spiking (*A*) of each analyte. Calculate the percent recovery (*P*) of each analyte:

Equation 1

$$P = \frac{100(A-B)}{T}$$

where

T = True value of the spike

- 8.4.3** Compare the percent recovery for each analyte with the corresponding QC acceptance criteria in Table 4. If any analyte fails the acceptance criteria for recovery, the sample is complex and must be diluted and reanalyzed per Section 17.
- 8.4.4** As part of the QC program for the laboratory, method accuracy for samples shall be assessed and records shall be maintained. After the analysis of five spiked samples of a given matrix type (water, soil, sludge, sediment) in which the analytes pass the tests in Section 8.4.3, compute the average percent recovery (*P*) and the standard deviation of the percent recovery (*s_p*) for each compound (or coeluting compound group). Express the accuracy assessment as a percent recovery interval from *P* - 2*s_p* to *P* + 2*s_p* for each matrix. For example, if *P* = 90% and *s_p* = 10% for five analyses of compost, the accuracy interval is expressed as 70 to 110%. Update the accuracy assessment for each compound in each matrix on a regular basis (e.g., after each five to ten new accuracy measurements).
- 8.5** Blanks: Reagent water and high-solids reference matrix blanks are analyzed to demonstrate freedom from contamination.
- 8.5.1** Extract and concentrate a 1-L reagent water blank or a 30-g high-solids reference matrix blank with each sample lot (samples started through the extraction process on the same 8-hour shift, to a maximum of 20 samples). Analyze the blank immediately after analysis of the combined QC standard (Section 14.6) to demonstrate freedom from contamination.
- 8.5.2** If any of the compounds of interest (Table 1) or any potentially interfering compound is found in an aqueous blank at greater than 0.05 µg/L, or in a high-solids reference matrix blank at greater than 1 µg/kg (assuming the same calibration factor as 2,4-D for compounds not listed in Table 1), analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level.
- 8.6** Other analytes may be determined by this method. To establish a quality control limit for an analyte, determine the precision and accuracy by analyzing four replicates of the analyte along with the combined QC standard per the procedure in Section 8.2. If the analyte coelutes with an analyte in the QC standard, prepare a new QC standard without the coeluting component(s). Compute the average percent recovery (*A*) and the standard deviation of percent recovery (*s_n*) for the analyte, and measure the recovery and standard deviation of recovery for the other analytes. The data for the new analyte is assumed to be valid if the precision and recovery specifications for the other analytes are met; otherwise, the analytical problem is corrected and the test is repeated. Establish a preliminary quality control limit of *A* ± 2*s_n* for the new analyte and add the limit to Table 4.

- 8.7 The specifications contained in this method can be met if the apparatus used is calibrated properly, then maintained in a calibrated state. The standards used for calibration (Section 7), calibration verification (Section 14.5), and for initial (Section 8.2) and ongoing (Section 14.6) precision and recovery should be identical, so that the most precise results will be obtained. The GC instruments will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of the analytes given in this method.
- 8.8 Depending on specific program requirements, field replicates and field spikes of the analytes of interest into samples may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

9. **SAMPLE COLLECTION, PRESERVATION, AND HANDLING**

- 9.1 Collect samples in glass containers following conventional sampling practices, except that the bottle shall not be prerinsed with sample before collection. Aqueous samples which flow freely are collected in refrigerated bottles using automatic sampling equipment. Solid samples are collected as grab samples using wide-mouth jars.
- 9.2 Maintain samples at 0 to 4°C from the time of collection until extraction. If the samples will not be extracted within 72 hours of collection, adjust the sample to a pH of less than 2 using sulfuric acid solution. Record the volume of acid used. Caution: some samples require acidification in a hood because of the potential for generating hydrogen sulfide.
- 9.3 If residual chlorine is present in aqueous samples, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine (Reference 7).
- 9.4 Begin sample extraction within 7 days of collection, and analyze all extracts within 28 days of extraction.

10. **SAMPLE EXTRACTION AND CONCENTRATION**

Samples containing 1% solids or less are extracted directly using continuous liquid/liquid extraction techniques (Section 10.2.1). Samples containing 1 to 30% solids are diluted to the 1% level with reagent water (Section 10.2.2) and extracted using continuous liquid-liquid extraction techniques. Samples containing greater than 30% solids are extracted by tumbling with water in a rotary agitation apparatus. The aqueous phase is then extracted using continuous liquid-liquid extraction techniques. Figure 1 outlines the extraction and concentration steps.

10.1 Determination of percent solids.

10.1.1 Weigh 5 to 10 g of sample into a tared beaker. Record the weight to three significant figures.

10.1.2 Dry overnight (12 hours minimum) at 110°C (±5°C), and cool in a desiccator.

10.1.3 Determine percent solids as follows:

Equation 2

$$\% \text{ solids} = \frac{\text{weight of dry sample}}{\text{weight of wet sample}} \times 100$$

10.2 Preparation of samples for extraction.**10.2.1 Samples containing 1% solids or less.**

10.2.1.1 Measure 1.00 L (± 0.01 L) of sample into a clean 1.5- to 2.0-L beaker.

10.2.1.2 Spike 0.5 mL of the surrogate spiking solution (Section 6.12) into the sample aliquot.

10.2.1.3 Proceed to preparation of the QC aliquots for low-solids samples (Section 10.2.3).

10.2.2 Samples containing 1 to 30% solids.

10.2.2.1 Mix sample thoroughly.

10.2.2.2 Using the percent solids found in Section 10.1.3, determine the weight of sample required to produce 1 L of solution containing 1% solids as follows:

Equation 3

$$\frac{1000}{\% \text{ solids}} = \text{grams}$$

10.2.2.3 Place the weight determined in Section 10.2.2.2 in a clean 1.5- to 2.0-L beaker. Discard all sticks, rocks, leaves, and other foreign material prior to weighing.

10.2.2.4 Bring the volume of the sample aliquot(s) to 400 to 500 mL with reagent water.

10.2.2.5 Spike 0.5 mL of the surrogate spiking solution (Section 6.12) into each sample aliquot.

10.2.2.6 Using a clean metal spatula, break any solid portions of the sample into small pieces.

10.2.2.7 Place the $\frac{3}{4}$ -inch horn on the ultrasonic probe approximately $\frac{1}{2}$ inch below the surface of each sample aliquot and pulse at 50% for 3 minutes at full power. If necessary, remove the probe from the solution and break any large pieces using the metal spatula or a stirring rod and repeat the sonication. Clean the probe with 5% aqueous sodium bicarbonate and then methylenechloride: acetone (1:1) between samples to prevent damage to the horn and preclude cross-contamination.

10.2.2.8 Bring the sample volume to 1.0 L (± 0.1 L) with reagent water.

10.2.3 Preparation of QC aliquots for samples containing low solids (less than 30%).

10.2.3.1 For each sample or sample batch (to a maximum of 20) to be extracted at the same time, place two 1.0 L (± 0.01 L) aliquots of reagent water in clean 1.5- to 2.0-L beakers. Acidify to pH to less than 2 with HCl.

10.2.3.2 Blank spike 0.5 mL of the surrogate spiking solution (Section 6.12) into one reagent water aliquot.

10.2.3.3 Spike the combined QC standard (Section 7.4) into a reagent water aliquot.

10.2.3.4 If a matrix spike is required, prepare an aliquot at the concentrations specified in Section 8.4.

10.2.4 Hydrolysis of acid esters and flocculation of particulates.

10.2.4.1 While on a stirring plate, raise the pH of the sample and QC aliquots to pH 12 to 13.

10.2.4.2 Stir and equilibrate all sample and QC solutions for 1 to 2 hours. Check the pH after approximately 0.5 hour and adjust if necessary.

10.2.4.3 Add sufficient NaCl to saturate the solution. Approximately 350 g are required. Stir to dissolve.

10.2.4.4 If the solution appears cloudy, add 2 g (± 0.2 g) of CaCl_2 and allow to stand for approximately 10 minutes to flocculate particulates.

10.2.4.5 Pre-extract the samples and QC aliquots to remove interferents per Section 10.3.

10.2.5 Samples containing 30% solids or greater.^a

10.2.5.1 Mix the sample thoroughly.

10.2.5.2 Weigh 30 g (± 0.3 g) of sample into a clean tumbler bottle. Discard all sticks, rocks, leaves, and other foreign material prior to weighing.

10.2.5.3 Add 1000 mL (± 100 mL) of reagent water and adjust the pH to 12 to 13 using NaOH.

10.2.5.4 QC aliquots: For each sample or sample batch (to a maximum of 20) to be extracted at the same time, place two 30 g (± 0.3 -g) aliquots of the high-solids reference matrix in tumbler bottles. One aliquot will serve as the blank.

10.2.5.5 Spike 0.5 mL of the surrogate spiking solution (Section 6.12) into each aliquot.

10.2.5.6 To serve as the ongoing precision and recovery standard, spike 1.0 mL of the combined QC standard (Section 7.4) into the remaining aliquot. Raise the pH of the QC aliquots to 12 to 13.

10.2.5.7 Tightly cap the tumbler bottles and tumble for 2 to 4 hours.

10.3 Pre-extraction to remove interferents: Place 100 to 150 mL methylene chloride in each continuous extractor and 200 to 300 mL in each distilling flask.

10.3.1 Pour the sample(s), blank, and standard aliquots into the extractors.

10.3.1.1 If a precipitate formed in the flocculation step (Section 10.2.4.4), or if the sample contains other solids, pour the sample through filter paper into the extractor.

10.3.1.2 Rinse the containers with 50 to 100 mL methylene chloride and add to the respective extractors. For samples that were filtered, pour the rinse over the residual sample in the filter funnel and drain into the respective extractor.

10.3.2 Verify that the pH of the water in the extractors is 12 to 13.

10.3.3 Begin the extraction by heating the flask until the methylene chloride is boiling. When properly adjusted, one to two drops of methylene chloride per second will fall from the condenser tip into the water. Test and adjust the pH of the waters during the first 1 to 2 hours of extraction. Extract for 2 to 4 hours.

10.3.4 After extraction, remove the distilling flask, discard the methylene chloride, and add a fresh charge of methylene chloride to the flask.

10.4 Extraction.

10.4.1 Adjust the pH of the water in the extractors to less than 2 with sulfuric acid.

10.4.2 Test and adjust the pH of the waters during the first 1 to 2 hours of the extraction. Extract for 18 to 24 hours.

10.4.3 Remove the distilling flask, estimate and record the volume of extract (to the nearest 100 mL), and pour the contents through a prerinsed drying column containing 7 to 10 cm of acidified anhydrous sodium sulfate. Rinse the distilling flask with 30 to 50 mL of methylene chloride and pour through the drying column. Collect the solution in a 500-mL K-D evaporator flask equipped with a 10-mL concentrator tube. Seal, label, and concentrate per Sections 10.5 and 10.6.

10.5 Macro concentration.

10.5.1 Concentrate the extracts in separate 500-mL K-D flasks equipped with 10-mL concentrator tubes. Add one or two clean, acid-rinsed boiling chips to the flask and attach a three-ball macro Snyder column. Prewet the column by adding approximately 1 mL of methylene chloride through the top. Place the K-D apparatus in a hot water bath so that the entire lower rounded surface of the flask is bathed with steam. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

10.5.2 When the liquid has reached an apparent volume of 1 mL, remove the K-D apparatus from the bath and allow the solvent to drain and cool for at least 10 minutes.

10.5.3 For extracts to be cleaned up using GPC, remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommended for this operation. Adjust the final volume to 10 mL and proceed to GPC cleanup in Section 11.

10.5.4 For extracts to be cleaned up using the SPE cartridge, adjust the final volume to 5.0 mL for those that have been cleaned up using GPC, and to 10 mL for those that have not. Proceed to SPE cleanup in Section 11.

10.6 Hexane exchange: Extracts containing acids to be derivatized, extracts to be subjected to Florisil cleanup, and extracts that have been cleaned up are exchanged into hexane.

- 10.6.1** Remove the Snyder column, add approximately 50 mL of hexane and a clean boiling chip, and reattach the Snyder column. Concentrate the extract as in Section 10.5, except use hexane to prewet the column. The elapsed time of the concentration should be 5 to 10 minutes.
- 10.6.2** Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane.
- 10.6.2.1** For extracts containing acids to be esterified, adjust the final volume to 10 mL for those that have not been cleaned up by GPC, and to 5 mL for those that have been cleaned up by GPC (the difference accounts for the 50% loss in the GPC cleanup). Proceed to Section 12 for esterification.
- 10.6.2.2** For extracts to be cleaned up using Florisil, adjust the final volume to 5 to 10 mL and proceed to Florisil cleanup in Section 11.
- 10.6.2.3** For extracts to be analyzed by GC (Section 13), adjust the final volume to 10 mL for those that have not been cleaned up by GPC, and to 5 mL for those that have been cleaned up by GPC.

11. CLEANUP

- 11.1** Cleanup procedures may not be necessary for relatively clean samples (treated effluents, ground water, drinking water). If particular circumstances require the use of a cleanup procedure, the analyst may use any or all of the procedures below or any other appropriate procedure. However, the analyst shall first repeat the tests in Section 8.2 to demonstrate that the requirements of Section 8.2 can be met using the cleanup procedure(s) as an integral part of the method. Figure 1 outlines the cleanup steps.
- 11.1.1** Gel permeation chromatography (Section 11.2) removes many high molecular weight interferents that cause GC column performance to degrade. It is used for all soil and sediment extracts and may be used for water extracts that are expected to contain high molecular weight organic compounds (e.g., polymeric materials, humic acids).
- 11.1.2** The solid-phase extraction cartridge (Section 11.3) removes polar organic compounds such as phenols.
- 11.1.3** The Florisil column (Section 11.4) allows for selected fractionation of the herbicides and will also eliminate polar interferences.
- 11.2** Gel permeation chromatography (GPC).
- 11.2.1** Column packing.
- 11.2.1.1** Place 70 to 75 g of SX-3 Bio-beads in a 400- to 500-mL beaker.
- 11.2.1.2** Cover the beads with methylene chloride and allow to swell overnight (12 hours minimum).
- 11.2.1.3** Transfer the swelled beads to the column and pump solvent through the column, from bottom to top, at 4.5 to 5.5 mL/min prior to connecting the column to the detector.
- 11.2.1.4** After purging the column with solvent for 1 to 2 hours, adjust the column head pressure to 7 to 10 psig, and purge for 4 to 5 hours to remove air.

Maintain a head pressure of 7 to 10 psig. Connect the column to the detector.

11.2.2 Column calibration.

11.2.2.1 Load 5 mL of the calibration solution (Section 6.5) into the sample loop.

11.2.2.2 Inject the calibration solution and record the signal from the detector. The elution pattern will be corn oil, bis (2-ethylhexyl) phthalate, pentachlorophenol, perylene, and sulfur.

11.2.2.3 Set the "dump time" to allow >85% removal of the corn oil and >85% collection of the phthalate.

11.2.2.4 Set the "collect time" to the peak minimum between perylene and sulfur.

11.2.2.5 Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the pentachlorophenol is greater than 85%. If calibration is not verified, the system shall be recalibrated using the calibration solution, and the previous 20 samples shall be re-extracted and cleaned up using the calibrated GPC system.

11.2.3 Extract cleanup: GPC requires that the column not be overloaded. The column specified in this method is designed to handle a maximum of 0.5 gram of high molecular weight material in a 5 mL extract. If the extract is known or expected to contain more than 0.5 g, the extract is split into fractions for GPC and the fractions are combined after elution from the column. The solids content of the extract may be obtained gravimetrically by evaporating the solvent from a 50- μ L aliquot.

11.2.3.1 Filter the extract or load through the filter holder to remove particulates. Load the 5.0-mL extract onto the column.

11.2.3.2 Elute the extract using the calibration data determined in Section 11.2.2. Collect the eluate in a clean 400- to 500-mL beaker.

11.2.3.3 Rinse the sample loading tube thoroughly with methylene chloride between extracts to prepare for the next sample.

11.2.3.4 If a particularly dirty extract is encountered, a 5.0-mL methylene chloride blank shall be run through the system to check for carry-over.

11.2.3.5 Concentrate the extract and exchange to hexane per Section 10.6.

11.3 Solid-phase extraction (SPE).

11.3.1 Setup.

11.3.1.1 Attach the Vac-elute manifold to a water aspirator or vacuum pump with the trap and gauge installed between the manifold and vacuum source.

11.3.1.2 Place the SPE cartridges in the manifold, turn on the vacuum source, and adjust the vacuum to 5 to 10 psia.

11.3.2 Cartridge washing: Pre-elute each cartridge prior to use sequentially with 10-mL portions each of hexane, methanol, and water using vacuum for 30 seconds after each eluant. Follow this pre-elution with 1 mL methylene chloride and three 10-mL portions of the elution solvent (Section 6.6.2.2) using vacuum for 5 minutes after each eluant. Tap the cartridge lightly while under vacuum to dry between eluants. The three portions

of elution solvent may be collected and used as a blank if desired. Finally, elute the cartridge with 10 mL each of methanol and water, using the vacuum for 30 seconds after each eluant.

11.3.3 Cartridge certification: Each cartridge lot must be certified to ensure recovery of the compounds of interest and removal of 2,4,6-trichlorophenol.

11.3.3.1 To make the test mixture, add the trichlorophenol solution (Section 6.6.2.1) to the combined calibration standard (Section 7.4). Elute the mixture using the procedure in Section 11.3.4.

11.3.3.2 Concentrate the eluant to 1.0 mL and inject 1.0 μ L of the concentrated eluant into the GC using the procedure in Section 13. The recovery of all analytes (including the unresolved GC peaks) shall be within the ranges for recovery specified in Table 4, and the peak for trichlorophenol shall not be detectable; otherwise the SPE cartridge is not performing properly and the cartridge lot shall be rejected.

11.3.4 Extract cleanup.

11.3.4.1 After cartridge washing (Section 11.3.2), release the vacuum and place the rack containing the 50-mL volumetric flasks (Section 5.6.2.4) in the vacuum manifold. Reestablish the vacuum at 5 to 10 psia.

11.3.4.2 Using a pipette or a 1-mL syringe, transfer 1.0 mL of extract to the SPE cartridge. Apply vacuum for 5 minutes to dry the cartridge. Tap gently to aid in drying.

11.3.4.3 Elute each cartridge into its volumetric flask sequentially with three 10-mL portions of the elution solvent (Section 6.6.2.2), using vacuum for 5 minutes after each portion. Collect the eluants in the 50-mL volumetric flasks.

11.3.4.4 Release the vacuum and remove the 50-mL volumetric flasks.

11.3.4.5 Using the nitrogen blow-down apparatus, concentrate the eluted extracts to 1.0 mL, and proceed to Section 13 for GC analysis.

11.4 Florisil column.

11.4.1 Place a weight of Florisil (nominally 20 g) predetermined by calibration (Section 7.5) in a chromatographic column. Tap the column to settle the Florisil and add 1 to 2 cm of anhydrous sodium sulfate to the top.

11.4.2 Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate layer to the air, stop the elution of the hexane by closing the stopcock on the chromatographic column. Discard the eluate.

11.4.3 Transfer the concentrated extract (Section 10.6.2) onto the column. Complete the transfer with two 1-mL hexane rinses.

- 11.4.4 Place a clean 500-mL K-D flask and concentrator tube under the column. Drain the column into the flask until the sodium sulfate layer is nearly exposed. Elute Fraction 1 with 200 mL of 6% ethyl ether in hexane (V/V) at a rate of approx 5 mL/min. Remove the K-D flask. Elute Fraction 2 with 200 mL of 15% ethyl ether in hexane (V/V) into a second K-D flask. Elute Fraction 3 with 200 mL of 50% ethyl ether in hexane (V/V).
- 11.4.5 Concentrate the fractions as in Section 10.6, except use hexane to prewet the column. Readjust the final volume to 5 or 10 mL as in Section 10.6, depending on whether the extract was subjected to GPC cleanup, and analyze by gas chromatography per the procedure in Section 13.

12. ESTERIFICATION

NOTE: Observe the safety precautions regarding diazomethane in Section 4.

- 12.1 Set up the diazomethane generation apparatus as given in the instructions in the Diazald kit.
- 12.2 Transfer 1 mL of the hexane solution containing the herbicides to a clean vial and add 0.5 mL of methanol and 3 mL of ether.
- 12.3 Add 2 mL of diazomethane solution and let the sample stand for 10 minutes with occasional swirling. The yellow color of diazomethane should persist throughout this period. If the yellow color disappears, add 2 mL of diazomethane solution and allow to stand, with occasional swirling, for another 10 minutes. Colored or complex samples will require at least 4 mL of diazomethane to ensure complete reaction of the herbicides. Continue adding diazomethane in 2-mL increments until the yellow color persists for the entire 10-minute period or until 10 mL of diazomethane solution has been added.
- 12.4.3 Rinse the inside wall of the container with 0.2 to 0.5 mL of diethyl ether and add 10 to 20 mg of silicic acid to react excess diazomethane. Filter through Whatman #41 paper into a clean sample vial. If the solution is colored or cloudy, evaporate to near dryness using the nitrogen blowdown apparatus, bring to 1.0 mL with hexane, and proceed to Section 11.3 for SPE cleanup. If the solution is clear and colorless, evaporate to near dryness, bring to 1.0 mL with hexane and proceed to Section 13 for GC analysis.

13. GAS CHROMATOGRAPHY

NOTE: Table 2 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are the retention times and estimated detection limits that can be achieved under these conditions. Examples of the separations achieved by the primary and confirmatory columns are shown in Figures 2 and 3.

- 13.1 Calibrate the system as described in Section 7.

- 13.2 Set the injection volume on the auto-sampler to inject 1.0 μL of all standards and extracts of blanks and samples.
- 13.3 Set the data system or GC control to start the temperature program upon sample injection, and begin data collection after the solvent peak elutes. Set the data system to stop data collection after the last analyte is expected to elute and to return the column to the initial temperature.

14. SYSTEM AND LABORATORY PERFORMANCE

- 14.1 At the beginning of each 8-hour shift during which analyses are performed, GC system performance and calibration are verified for all pollutants and surrogates on both column/detector systems. For these tests, analysis of the combined QC standard (Section 7.4) shall be used to verify all performance criteria. Adjustment and/or recalibration (per Section 7) shall be performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks, and precision and recovery standards be analyzed.
- 14.2 Retention times: The absolute retention times of the peak maxima shall be within ± 10 seconds of the retention times in the initial calibration (Section 7.4.1).
- 14.3 GC resolution: Resolution is acceptable if the valley height between two peaks (as measured from the baseline) is less than 10% of the taller of the two peaks.
 - 14.3.1 Primary column (DB-608): Dicamba and MCPA.
 - 14.3.2 Confirmatory column (DB-1701): MCPP and MCPA.
- 14.5 Calibration verification: Calibration is verified for the combined QC standard only.
 - 14.5.1 Inject the combined QC standard (Section 7.4)
 - 14.5.2 Compute the percent recovery of each compound or coeluting compounds, based on the calibration data (Section 7.4).
 - 14.5.3 For each compound or coeluted compounds, compare this calibration verification recovery with the corresponding limits for ongoing recovery in Table 4. For coeluting compounds, use the coeluted compound with the least restrictive specification (the widest range). If the recoveries for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any recovery falls outside the calibration verification range, system performance is unacceptable for that compound. In this case, correct the problem and repeat the test, or recalibrate (Section 7).
- 14.6 Ongoing precision and recovery.
 - 14.6.1 Analyze the extract of the precision and recovery standard extracted with each sample lot (Sections 10.2.3.3 and 10.2.5.7).
 - 14.6.2 Compute the percent recovery of each analyte and coeluting compounds.
 - 14.6.3 For each compound or coeluted compounds, compare the percent recovery with the limits for ongoing recovery in Table 4. For coeluted compounds, use the coeluted compound with the least restrictive specification (widest range). If all analytes pass, the extraction, concentration, and cleanup processes are in control and analysis of blanks and samples may proceed. If, however, any of the analytes fail, these processes are not in control.

In this event, correct the problem, re-extract the sample batch, and repeat the on-going precision and recovery test.

- 14.6.4** Add results which pass the specifications in Section 14.6.3 to initial and previous ongoing data. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory data quality for each analyte by calculating the average percent recovery (R) and the standard deviation of percent recovery s_r . Express the accuracy as a recovery interval from $R - 2s_r$ to $R + 2s_r$. For example, if $R = 95\%$ and $s_r = 5\%$, the accuracy is 85 to 105%.

15. QUALITATIVE DETERMINATION

- 15.1** Qualitative determination is accomplished by comparison of data from analysis of a sample or blank with data from analysis of the shift standard (Section 14.2), and with data stored in the retention-time and calibration libraries (Sections 7.3.2 and 7.3.3.2). Identification is confirmed when retention time and amounts agree per the criteria below.
- 15.2** For each compound on each column/detector system, establish a retention-time window ± 20 seconds on either side of the retention-time in the calibration data (Section 7.3.1). For compounds that have a retention time curve (Section 7.3.1.2), establish this window as the minimum -20 seconds and maximum +20 seconds.
- 15.2.1** Compounds not requiring a retention-time calibration curve: If a peak from the analysis of a sample or blank is within a window (as defined in Section 15.2) on the primary column/detector system, it is considered tentatively identified. A tentatively identified compound is confirmed when (1) the retention time for the compound on the confirmatory column/detector system is within the retention-time window on that system, and (2) the computed amounts (Section 16) on each system (primary and confirmatory) agree within a factor of 3.
- 15.2.2** Compounds requiring a retention-time calibration curve: If a peak from the analysis of a sample or blank is within a window (as defined in Section 15.2) on the primary column/detector system, it is considered tentatively identified. A tentatively identified compound is confirmed when (1) the retention-times on both systems (primary and confirmatory) are within ± 30 seconds of the retention times for the computed amounts (Section 16), as determined by the retention-time calibration curve (Section 7.3.1.2), and (2) the computed amounts (Section 16) on each system (primary and confirmatory) agree within a factor of 3.

16. QUANTITATIVE DETERMINATION

- 16.1** Using the GC data system, compute the concentration of the analyte detected in the extract (in micrograms per milliliter) using the calibration factor or calibration curve (Section 7.3.3.2).
- 16.2** Liquid samples: Compute the concentration in the sample using the following equation:

Equation 4

$$C_s = 10 \frac{(C_e)}{(V_e)}$$

where

C_s = The concentration in the sample, in $\mu\text{g/L}$

10 = The extract total, in mL

C_e = The concentration in the extract, in $\mu\text{g/mL}$

V_e = The sample extracted, in L

16.3 Solid samples: Compute the concentration in the solid phase of the sample using the following equation:

Equation 5

$$C_s = 10 \frac{(C_e)}{1000(W_s)(\text{solids})}$$

where

C_s = Concentration in the sample, in $\mu\text{g/kg}$

10 = Extract total, in mL

C_e = Concentration in the extract, in $\mu\text{g/mL}$

1000 = Conversion factor, g to kg

W_s = Sample weight, in g

solids = Percent solids in Section 10.1.3 divided by 100

16.4 If the concentration of any analyte exceeds the calibration range of the system, the extract is diluted by a factor of 10, and a 1- μL aliquot of the diluted extract is analyzed.

16.5 Report results for all pollutants found in all standards, blanks, and samples to three significant figures. Results for samples that have been diluted are reported at the least dilute level at which the concentration is in the calibration range.

17. ANALYSIS OF COMPLEX SAMPLES

17.1 Some samples may contain high levels ($> 1000 \text{ ng/L}$) of the compounds of interest, interfering compounds, and/or polymeric materials. Some samples may not concentrate to 10 mL (Section 10.6); others may overload the GC column and/or detector.

17.2 The analyst shall attempt to clean up all samples using GPC (Section 11.2), the SPE cartridge (Section 11.3), and Florisil (Section 11.4). If these techniques do not remove the interfering compounds, the extract is diluted by a factor of 10 and reanalyzed (Section 16.4).

- 17.3** Recovery of surrogates: in most samples, surrogate recoveries will be similar to those from reagent water or from the high-solids reference matrix. If the surrogate recovery is outside the range specified in Section 8.3, the sample shall be reextracted and reanalyzed. If the surrogate recovery is still outside this range, the sample is diluted by a factor of 10 and reanalyzed (Section 16.4).
- 17.4** Recovery of matrix spikes: In most samples, matrix spike recoveries will be similar to those from reagent water or from the high-solids reference matrix. If the matrix spike recovery is outside the range specified in Table 4, the sample shall be diluted by a factor of 10, respiked, and reanalyzed. If the matrix spike recovery is still outside the range, the method does not work on the sample being analyzed and the result may not be reported for regulatory compliance purposes.

18. METHOD PERFORMANCE

- 18.1** Development of this method is detailed in References 9 and 10.

References

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3. "Safety in Academic Chemistry Laboratories," American Chemical Society Committee on Chemical Safety: 1979.
4. Mills, P. A., "Variation of Florisil Activity: Simple Method for Measuring Adsorbent Capacity and Its Use in Standardizing Florisil Columns," *Journal of the Association of Official Analytical Chemists*, 51, 29: 1968.
5. "Handbook of Quality Control in Wastewater Laboratories," U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH,: EPA-600/4-79-019, March 1979.
6. "Standard Practice for Sampling Water" (ASTM Annual Book of Standards), American Society for Testing and Materials, Philadelphia, Pennsylvania: 76, 1980.
7. "Methods 330.4 and 330.5 for Total Residual Chlorine," U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH: EPA-600/4-70-020, March 1979.
8. Jackson, Cary B. and Workman, Stephen M., "Analysis of Chlorophenoxy-Acid Herbicides in Soil and Water," presented at the 14th Annual EPA Conference on the Analysis of Pollutants in the Environment, Norfolk, Virginia: May 1991.
9. "Consolidated GC Method for the Determination of ITD/RCRA Pesticides using Selective GC Detectors," S-CUBED, A Division of Maxwell Laboratories, Inc., La Jolla, CA: Ref. 32145-01, Document R70, September 1986.
10. "Method Development and Validation, EPA Method 1618, Cleanup Procedures," Colorado State University, Colorado Pesticide Center: November 1988 and January 1989.

Table 1. Phenoxyacid Herbicides Determined by Large-Bore, Fused-Silica
Capillary Column Gas Chromatography with Halide-Specific Detector

<i>EPA EGD</i>	<i>Compound</i>	<i>CAS Registry</i>
481	2,4-D	94-75-7
480	Dinoseb	88-85-7
482	2,4,5-T	93-76-5
483	2,4,5-TP	93-72-1

Other phenoxyacid herbicides that can be analyzed by this method:

<i>Compound</i>	<i>CAS Registry</i>
Dalapon	75-99-0
2,4-DB (Butoxon)	94-82-6
Dicamba	1918-00-9
Dichlorprop	120-36-5
MCPA	94-74-6
MCPP	7085-19-0

Table 2. Gas Chromatography of Phenoxy-Acid Herbicides

EPA EGD	Compound	Retention Time (min) ¹		Method Detection Limit ² (ng/L)
		DB-608	DB-1701	
481	2,4-D	16.57	16.39	100
480	Dinoseb	20.75	23.55	50 (est)(ECD)
482	2,4,5-T	20.42	20.25	50
483	2,4,5-TP (Silvex)	18.65	18.66	40
	Dalapon	3.52	3.63	100 est
	2,4-DB (Butoxon)	21.94	21.87	50
	Dicamba	13.51	12.97	110
	Dichlorprop	15.21	15.19	40
	MCPA	14.42	14.30	90
	MCPP	13.51	13.49	56
	2,4-DCPA (surrogate)	12.88	12.51	

Notes:

1. Columns: 30 m long by 0.53 mm ID, i.e., DB-608: 0.83 μ ; DB-1701: 1.0 μ . Conditions suggested to meet retention times shown: 175 to 270°C at 5°C/min., 175 to 270 @ 5°C/min. Carrier gas flow rates approximately 7 mL/min.

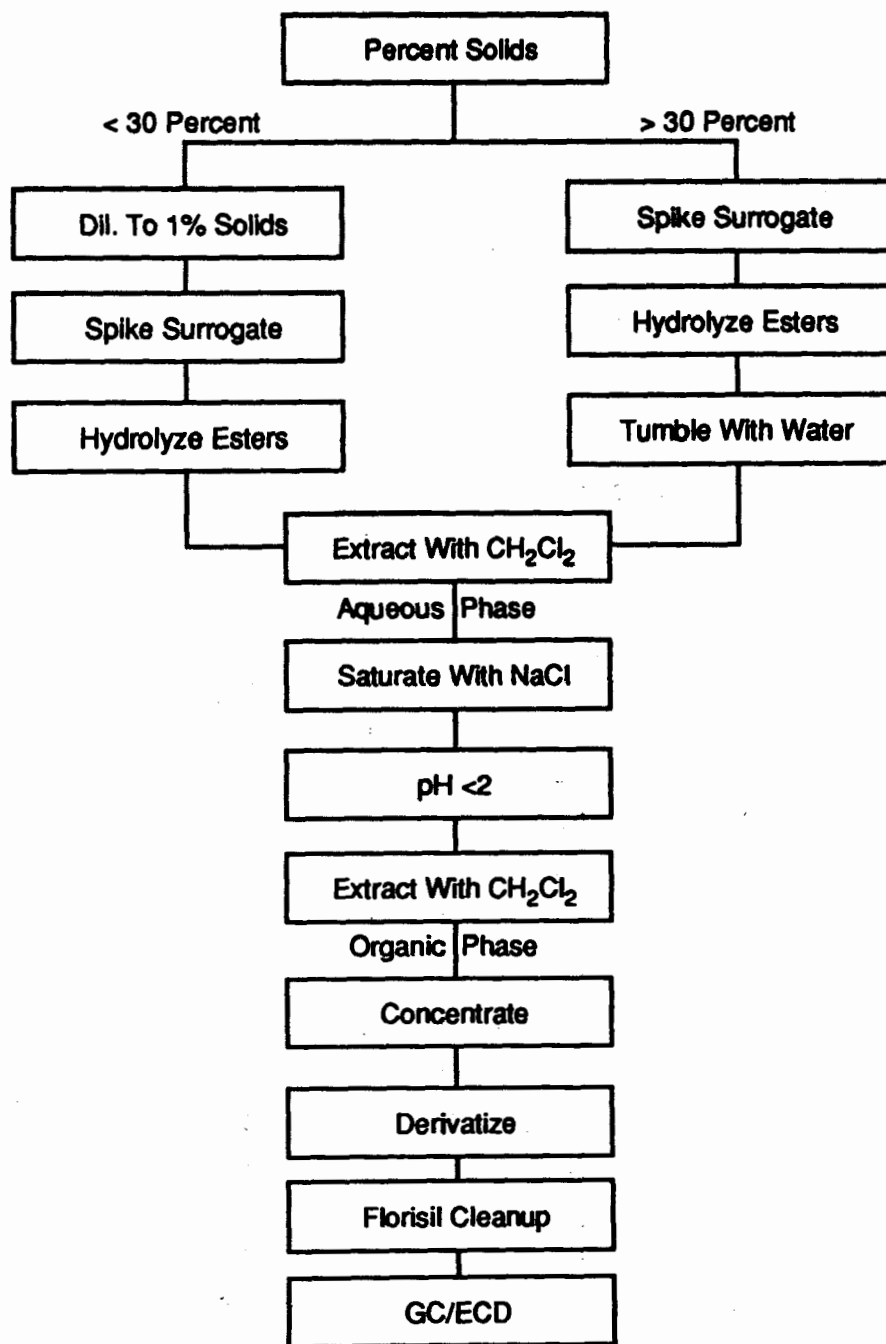
2. 40 CFR Part 136, Appendix B (49 FR 43234). Detection limits for soils (in ng/kg) are estimated to be 30 to 100 times this level.

Table 3. Concentrations of Calibration Solutions

EPA EGD	Compound	Concentration (ng/mL)		
		Low	Medium	High
481	2,4-D	100	1,000	10,000
	Dalapon	50	500	5,000
	2,4-DB	200	2,000	20,000
	2,4-DCPA (Surrogate)	10	1,000	10,000
	Dicamba	20	200	2,000
	Dichlorprop	100	1,000	10,000
480	Dinoseb	50	500	5,000
	MCPA	5,000	50,000	500,000
	MCPP	5,000	50,000	500,000
	Picloram	50	500	5,000
482	2,4,5-T	20	200	2,000
483	2,4,5-TP (Silvex)	20	200	2,000
Electrolytic Conductivity Detector				
481	2,4-D	500	5,000	50,000
	Dalapon	500	5,000	50,000
	2,4-DB	1,000	10,000	100,000
	2,4-DCPA (surrogate)	500	5,000	50,000
	Dicamba	500	5,000	50,000
	Dichlorprop	500	5,000	50,000
480	Dinoseb		No Response	
	MCPA	500	5,000	50,000
	MCPP	500	5,000	50,000
482	2,4,5-T	500	5,000	50,000
483	2,4,5-TP (Silvex)	250	2,500	25,000

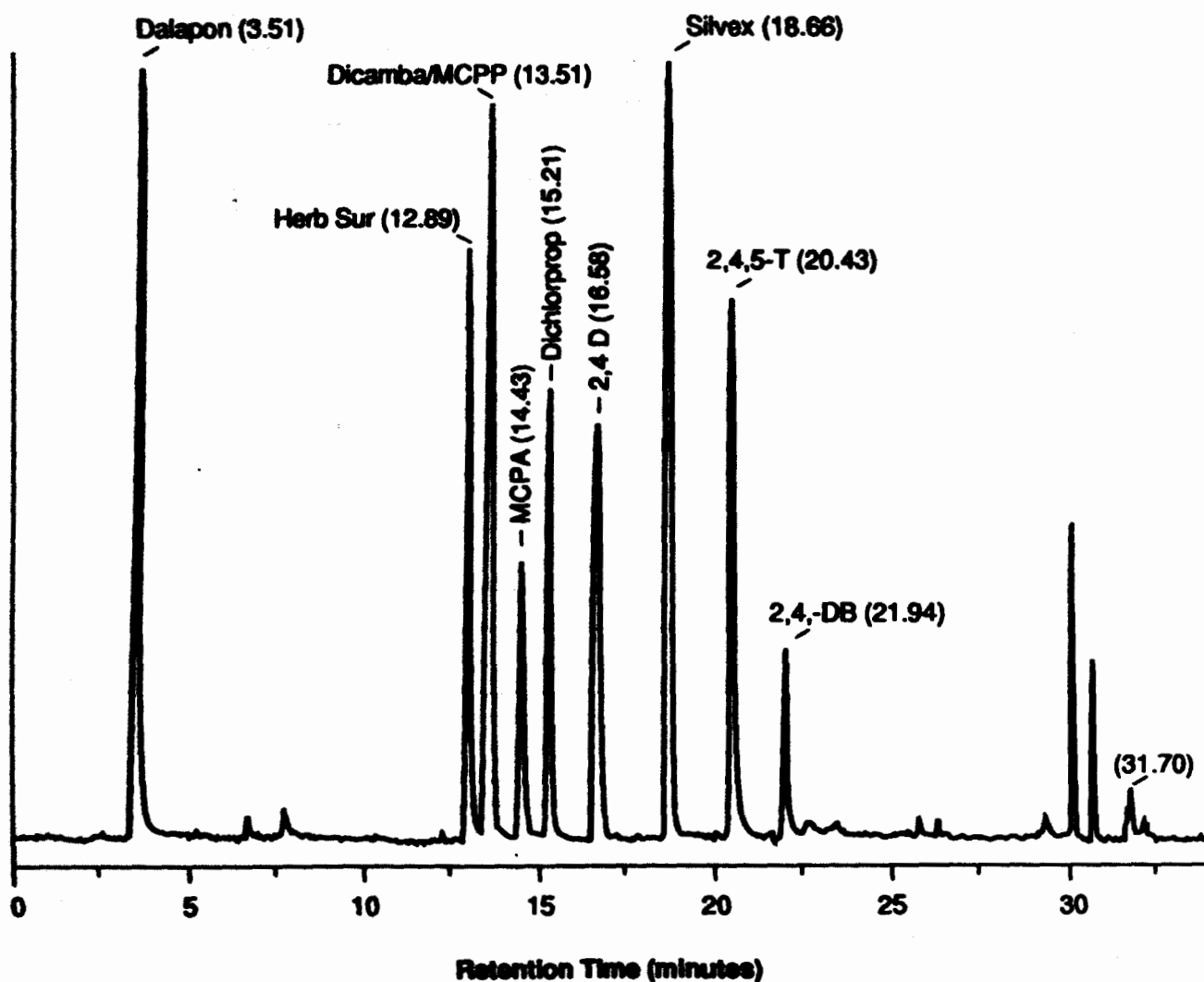
Table 4. Acceptance Criteria for Performance Tests for Phenoxy-Acid Compounds

EGD No.	Compound	Spike level (ng/L)	Acceptance criteria			
			Initial precision and accuracy (%)		Calibration verification (µg/mL)	Recovery Ongoing accuracy R (%)
			s	X		
481	2,4-D	200	16	41-107	78-121	23-131
480	Dinoseb	500	18	24-154	64-136	19-159
482	2,4,5-T	100	17	30-132	70-130	5-158
483	2,4,5-TP (Silvex)	100	14	36-120	75-126	15-141
	Dalapon	500	15	43-137	74-125	39-140
	2,4-DB (Butoxon)	100	22	22-118	42-157	0-142
	Dicamba	200	18	37-145	59-139	10-172
	Dichlorprop	100	14	49-133	71-128	28-154
	MCPA	200	14	46-130	67-132	25-151
	MCPP	400	14	65-149	71-129	42-170
	Picloram	500	13	46-140	73-126	42-144



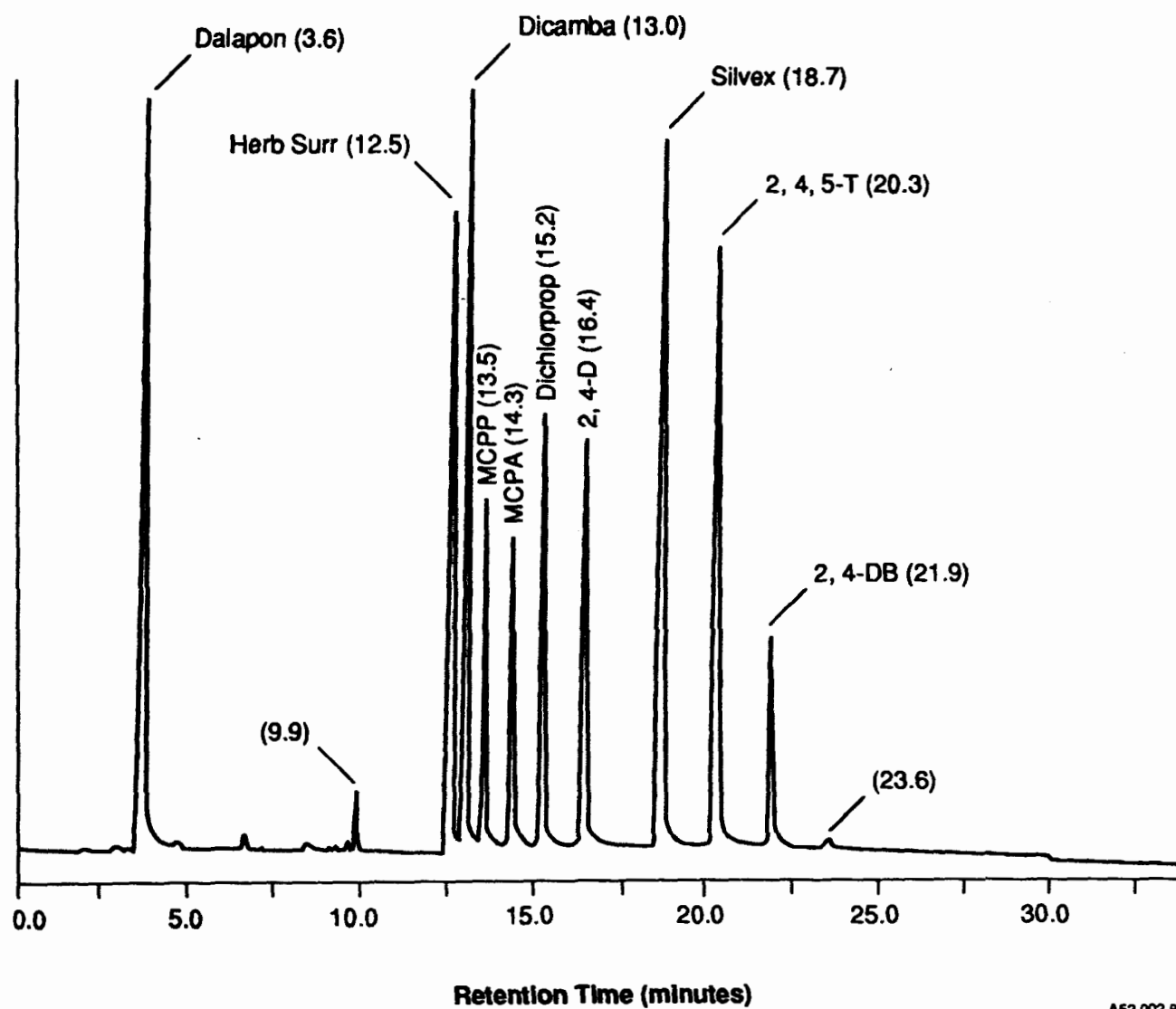
A52-002-82

Figure 1. Extraction, Cleanup, Derivatization, and Analysis



A62-002-79

Figure 2. Chromatogram of Herbicides DB-608 Column



A52-002-80

Figure 3. Chromatogram of Herbicides (DB-1701 Column)

Method 1659
The Determination of Dazomet
in Municipal and Industrial
Wastewater

Method 1659

The Determination of Dazomet in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of dazomet (CAS 533-74-4) by base hydrolysis to methyl isothiocyanate (MITC; CAS 556-61-6) and subsequent determination of MITC by wide bore, fused-silica column gas chromatography (GC) with a nitrogen-phosphorus detector (NPD).
- 1.2 This method is designed to meet the monitoring requirements of the U.S. Environmental Protection Agency under the Clean Water Act at 40 *CFR* Part 455. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3 When this method is applied to analysis of unfamiliar samples, compound identity must be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Gas chromatography mass spectrometry (GC/MS) can be used to confirm dazomet in extracts produced by this method when the level is sufficient.
- 1.4 The detection limit of this method is usually dependent on the level of interferences rather than instrumental limitations. The limit in Table 1 typifies the minimum quantity that can be detected with no interferences present.
- 1.5 This method is for use by or under the supervision of analysts experienced in the use of a gas chromatograph and in the interpretation of gas chromatographic data. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 8.2.

2. SUMMARY OF METHOD

- 2.1 A 50-mL sample is adjusted to pH 10 to 12 and allowed to stand for 3 hours to hydrolyze dazomet to MITC. After hydrolysis, the sample is saturated with salt and extracted with 2.5 mL of ethyl acetate. Gas chromatographic conditions are described that permit the separation and measurement of MITC in the extract by wide-bore, fused-silica capillary column with nitrogen-phosphorus detector (GC/NPD).
- 2.2 Identification of MITC (qualitative analysis) is performed by comparing the GC retention time of the MITC on two dissimilar columns with the respective retention times of an authentic standard. Compound identity is confirmed when the retention times agree within their respective windows.
- 2.3 Quantitative analysis is performed using an authentic standard of MITC to produce a calibration factor or calibration curve, and using the calibration data to determine the concentration of MITC in the extract. The concentration in the sample is calculated using the sample volume, the extract volume, and a factor to convert MITC to dazomet.
- 2.4 Quality is assured through reproducible calibration and testing of the extraction and GC systems.

3. CONTAMINATION AND INTERFERENCES

- 3.1** Solvents, reagents, glassware, and other sample-processing hardware may yield artifacts and/or elevated baselines causing misinterpretation of chromatograms. All materials used in the analysis shall be demonstrated to be free from interferences under the conditions of analysis by running method blanks as described in Section 8.4.
- 3.2** Glassware and, where possible, reagents are cleaned by rinsing with and baking at 450°C for 1 hour minimum in a muffle furnace or kiln. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment and thorough rinsing with acetone and pesticide-quality hexane may be required.
- 3.3** Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.
- 3.4** Interferences coextracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled.

4. SAFETY

- 4.1** The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 1 through 3.
- 4.2** Unknown samples may contain high concentrations of volatile toxic compounds. Sample containers should be opened in a hood and handled with gloves that will prevent exposure.

5. APPARATUS AND MATERIALS

NOTE: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance meeting the requirements of this method is the responsibility of the laboratory.

- 5.1** Sampling equipment for discrete or composite sampling.
 - 5.1.1** Sample bottle: Amber glass, 1-L, with screw-cap. If amber bottles are not available, samples shall be protected from light.
 - 5.1.2** Bottle caps: Threaded to fit sample bottles. Caps shall be lined with PTFE.
 - 5.1.3** Cleaning.
 - 5.1.3.1** Bottles are detergent water washed, then rinsed with solvent or baked at 450°C for 1 hour minimum before use.
 - 5.1.3.2** Liners are detergent water washed, then rinsed with reagent water and solvent, and baked at approximately 200°C for 1 hour minimum prior to use.

- 5.1.4** Compositing equipment: Automatic or manual compositing system incorporating glass containers cleaned per bottle cleaning procedure above. Sample containers are kept at 0 to 4°C during sampling. Glass or PTFE tubing only shall be used. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, the tubing shall be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.
- 5.2** Extraction bottle: 4 oz. with PTFE-lined screw-cap, cleaned by solvent rinse or baking at 450°C for 1 hour minimum.
- 5.3** pH meter, with combination glass electrode.
- 5.4** Sample vials: Amber glass, 1- to 5-mL with PTFE-lined screw- or crimp-cap, to fit GC autosampler.
- 5.5** Balance: Analytical, capable of weighing 0.1 mg.
- 5.6** Miscellaneous glassware.
- 5.6.1** Pipettes, glass, volumetric, 1.00-, 5.00-, and 10.0-mL.
- 5.6.2** Pipettes, glass, Pasteur.
- 5.6.3** Volumetric flasks, 10.0-, 25.0-, and 50.0-mL.
- 5.7** Gas chromatograph: Shall have splitless or on-column simultaneous automated injection into separate capillary columns with a nitrogen-phosphorus detector at the end of each column, temperature program with isothermal holds, data system capable of recording simultaneous signals from the two detectors, and shall meet all of the performance specifications in Section 12.
- 5.7.1** GC columns: Bonded-phase fused-silica capillary.
- 5.7.1.1** Primary: 30 m long (± 3 m) by 0.5 mm (± 0.05 mm) ID, DB-608 (or equivalent).
- 5.7.1.2** Confirmatory: DB-1701, or equivalent, with same dimensions as primary column.
- 5.7.2** Data system: Shall collect and record GC data, store GC runs on magnetic disk or tape, process GC data, compute peak areas, store calibration data including retention times and calibration factors, identify GC peaks through retention times, compute concentrations, and generate reports.
- 5.7.2.1** Data acquisition: GC data shall be collected continuously throughout the analysis and stored on a mass storage device.
- 5.7.2.2** Calibration factors and calibration curves: The data system shall be used to record and maintain lists of calibration factors, and multi-point calibration curves (Section 7). Computations of relative standard deviation (coefficient of variation) are used for testing calibration linearity. Statistics on initial (Section 8.2) and ongoing (Section 12.5) performance shall be computed and maintained.
- 5.7.2.3** Data processing: The data system shall be used to search, locate, identify, and quantify the compounds of interest in each GC analysis. Software routines shall be employed to compute and record retention times and peak

areas. Displays of chromatograms and library comparisons are required to verify results.

- 5.7.3** Nitrogen phosphorus detector: Thermionic bead or alkali flame detector, capable of detecting 600 pg of MITC under the analysis conditions given in Table 1.

6. REAGENTS AND STANDARDS

NOTE: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance meeting the requirements of this method is the responsibility of the laboratory.

- 6.1** Sample preservation: Sodium thiosulfate (ACS), granular.
- 6.2** pH adjustment.
- 6.2.1** Sodium hydroxide (10N): Dissolve 40 g NaOH in 100 mL reagent water.
- 6.2.2** Sulfuric acid (1 + 1): Reagent grade, 6N in reagent water. Slowly add 50 mL H₂SO₄ (specific gravity 1.84) to 50 mL reagent water.
- 6.2.3** Potassium hydroxide: 37% (W/V). Dissolve 37 g KOH in 100 mL reagent water.
- 6.3** Solvents: Methylene chloride, ethyl acetate, and acetone; pesticide-quality; lot-certified to be free of interferences.
- 6.4** Reagent water: Water in which the compounds of interest and interfering compounds are not detected by this method.
- 6.5** Salt: Sodium chloride, spread approximately 1 cm deep in a baking dish and baked at 450°C for 1 hour minimum.
- 6.6** Standard solutions: Purchased as solutions or mixtures with certification to their purity, concentration, and authenticity, or prepared from materials of known purity and composition. If compound purity is 96% or greater, the weight may be used without correction to compute the concentration of the standard. When not being used, standards are stored in the dark at -20 to -10°C in screw-capped vials with PTFE-lined lids. A mark is placed on the vial at the level of the solution so that solvent evaporation loss can be detected. The vials are brought to room temperature prior to use.
- 6.7** Preparation of stock solutions: Prepare in ethyl acetate per the steps below. Observe the safety precautions in Section 4.
- 6.7.1** Dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 10 mg MITC in a 10-mL ground-glass stoppered volumetric flask and fill to the mark with ethyl acetate. After the MITC is completely dissolved, transfer the solution to a 15-mL vial with PTFE-lined cap.
- 6.7.2** Stock solutions should be checked for signs of degradation prior to the preparation of calibration or performance test standards.
- 6.7.3** Stock solutions shall be replaced after 6 months, or sooner if comparison with quality control check standards indicates a change in concentration.

- 6.8** Secondary mixtures: Using stock solutions (Section 6.7), prepare mixtures for calibration and calibration verification (Sections 7.3 and 12.4), for initial and ongoing precision and recovery (Sections 8.2 and 12.5), and for spiking into the sample matrix (Section 8.3).
- 6.8.1** Calibration solutions: Prepare MITC in ethyl acetate at concentrations of 0.2, 1.0, and 5.0 $\mu\text{g/mL}$. The midpoint solution (1.0 $\mu\text{g/mL}$) is used for calibration verification (Section 12.4).
- 6.8.2** Precision and recovery standard: Prepare MITC in acetone at a concentration of 25 $\mu\text{g/mL}$.
- 6.8.3** Matrix spike solution: Prepare dazomet in acetone at a concentration of 25 $\mu\text{g/mL}$.
- 6.9** Stability of solutions: All standard solutions (Sections 6.7 and 6.8) shall be analyzed within 48 hours of preparation and on a monthly basis thereafter for signs of degradation. Standards will remain acceptable if the peak area remains within $\pm 15\%$ of the area obtained in the initial analysis of the standard.

7. SETUP AND CALIBRATION

- 7.1** Configure the GC system as given in Section 5.7 and establish the operating conditions in Table 1.
- 7.2** Attainment of minimum level: Determine that each column/detector system meets the minimum level for MITC (Table 1).
- 7.3** Calibration.
- 7.3.1** Inject 3 μL of each calibration solution (Section 6.8.1) into each GC column/detector pair, beginning with the lowest level mixture and proceeding to the highest. For each compound, compute and store, as a function of the concentration injected, the retention time and peak area on each column/detector system (primary and confirmatory).
- 7.3.2** Calibration factor (ratio of area to amount injected).
- 7.3.2.1** Compute the coefficient of variation (relative standard deviation) of the calibration factor over the calibration range for MITC on each column/detector system.
- 7.3.2.2** Linearity: If the calibration factor is constant ($C_v < 20\%$) over the calibration range, an average calibration factor may be used; otherwise, the complete calibration curve (area vs. amount) shall be used.

8. QUALITY CONTROL

- 8.1** Each laboratory that uses this method is required to operate a formal quality control program.⁴ The minimum requirements of this program consist of an initial demonstration of laboratory capability, an ongoing analysis of standards and blanks as tests of continued performance, and analysis of spiked samples to assess accuracy. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.
- 8.1.1** The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

- 8.1.2** The analyst is permitted to modify this method to improve separations or lower the costs of measurements, provided all performance requirements are met. Each time a modification is made to the method or a cleanup procedure is added, the analyst is required to repeat the procedure in Section 8.2 to demonstrate method performance. If detection the detection limit for dazomet will be affected by the modification, the analyst is required to repeat demonstration of the detection limit (Section 7.2).
- 8.1.3** The laboratory shall, on an ongoing basis, demonstrate through calibration verification and the analysis of the precision and recovery standard (Section 6.8.2) that the analysis system is in control. These procedures are described in Sections 12.1, 12.4, and 12.5.
- 8.1.4** The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Section 8.3.
- 8.1.5** Analyses of blanks are required to demonstrate freedom from contamination. The procedures and criteria for analysis of a blank are described in Section 8.4.
- 8.2 Initial precision and recovery:** To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations:
- 8.2.1** Extract, concentrate, and analyze one set of four 50-mL aliquots of reagent water spiked with 0.1 mL of the precision and recovery standard (Section 6.8.2) according to the procedure in Section 10.
- 8.2.2** Using results of the set of four analyses, compute the average percent recovery (\bar{X}) and the coefficient of variation (C_v) of percent recovery(s) for MITC.
- 8.2.3** Compare s and \bar{X} with the corresponding limit for initial precision and recovery in Table 1. If s and \bar{X} meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, s exceeds the precision limit or \bar{X} falls outside the range for accuracy, system performance is unacceptable. In this case, correct the problem and repeat the test.
- 8.3 Method accuracy:** The laboratory shall spike (matrix spike) at least 10% of the samples from a given site type (e.g., influent to treatment, treated effluent, produced water). If only one sample from a given site type is analyzed, a separate aliquot of that sample shall be spiked.
- 8.3.1 The concentration of the matrix spike shall be determined as follows:**
- 8.3.1.1** If, as in compliance monitoring, the concentration of dazomet in the sample is being checked against a regulatory concentration limit, the matrix spike shall be at that limit or at 1 to 5 times higher than the background concentration determined in Section 8.3.2, whichever concentration is larger.
- 8.3.1.2** If the concentration is not being checked against a regulatory limit, the matrix spike shall be at 50 $\mu\text{g/L}$ or at 1 to 5 times higher than the background concentration, whichever concentration is larger.
- 8.3.1.3** If it is impractical to determine the background concentration before spiking (e.g., maximum holding times will be exceeded), the matrix spike concentration shall be the regulatory concentration limit, if any; otherwise, the larger of either 5 times the expected background concentration, or 50 $\mu\text{g/L}$ (the concentration produced by 0.1 mL of the matrix spike solution spiked into a 50-mL sample).

- 8.3.2** Analyze one sample aliquot to determine the background concentration (B) of dazomet. If necessary, prepare a standard solution appropriate to produce a level in the sample 1 to 5 times the background concentration. Spike a second sample aliquot with the standard solution and analyze it to determine the concentration after spiking (A) with dazomet. Calculate the percent recovery (P):

Equation 1

$$P = \frac{100(A-B)}{T}$$

where

T = True value of the spike

- 8.3.3** Compare the percent recovery for dazomet with the corresponding QC acceptance criteria in Table 1. If dazomet fails the acceptance criteria for recovery, the sample is complex and must be diluted and reanalyzed per Section 15.
- 8.3.4** As part of the QC program for the laboratory, method accuracy for samples shall be assessed and records shall be maintained. After the analysis of five spiked samples of a given matrix type (treated effluent, influent to treatment, produced water) in which the recovery test (Section 8.3.3) is passed, compute the average percent recovery (P) and the standard deviation of the percent recovery (s_p). Express the accuracy assessment as a percent recovery interval from $P - 2s_p$ to $P + 2s_p$ for each matrix. For example, if $P = 90\%$ and $s_p = 10\%$ for five analyses of wastewater, the accuracy interval is expressed as 70 to 110%. Update the accuracy assessment in each matrix on a regular basis (e.g., after each five to ten new accuracy measurements).
- 8.4** Blanks: Reagent water blanks are analyzed to demonstrate freedom from contamination.
- 8.4.1** Extract and concentrate a 50-mL reagent water blank with each sample batch (samples started through the extraction process on the same 8 hour shift, to a maximum of 20 samples). Analyze the blank immediately after analysis of the precision and recovery standard (Section 12.5) to demonstrate freedom from contamination.
- 8.4.2** If MITC or any potentially interfering compound is found in an aqueous blank at greater than $2 \mu\text{g/L}$ (assuming the same calibration factor as MITC for interfering compounds), analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level.
- 8.5** The specifications contained in this method can be met if the apparatus used is calibrated properly, then maintained in a calibrated state. The standards used for calibration (Section 7), calibration verification (Section 12.4), and for initial (Section 8.2) and ongoing (Section 12.5) precision and recovery should be identical, so that the most precise results will be obtained. The GC instrument will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of the analytes given in this method.
- 8.6** Depending on specific program requirements, field replicates and field spikes may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

- 9.1** Collect samples in glass containers following conventional sampling practices,⁵ except that the bottle shall not be prerinsed with sample before collection. Aqueous samples which flow freely are collected in refrigerated bottles using automatic sampling equipment.
- 9.2** Maintain samples at 0 to 4°C from the time of collection until extraction. If the samples will not be extracted within 72 hours of collection, adjust the sample to a pH greater than 9.0 using sodium hydroxide solution. Record the volume used. If residual chlorine is present in aqueous samples, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine.⁶
- 9.3** Begin sample extraction within seven days of collection, and analyze all extracts within 40 days of extraction.

10. SAMPLE HYDROLYSIS AND EXTRACTION

- 10.1** Hydrolysis and preparation of QC aliquots.
 - 10.1.1** Pour 50 mL of sample into a clean 4-oz. bottle. If a matrix spike is to be prepared, pour 50 mL into a second clean bottle.
 - 10.1.2** For each sample or sample batch (to a maximum of 20) to be extracted at the same time, place two 50-mL aliquots of reagent water in clean 4-oz. bottles. One reagent water aliquot serves as the blank.
 - 10.1.3** Spike 0.1 mL of the precision and recovery standard (Section 6.8.2) into the remaining reagent water aliquot.
 - 10.1.4** Spike 0.1 mL of the matrix spike solution (Section 6.8.3) into the sample aliquot used for the matrix spike.
 - 10.1.5** Test the pH of the sample and QC aliquots with a pH meter and adjust to 10 to 12 with potassium hydroxide solution. Cap and shake the bottles vigorously to mix. Allow to stand.
 - 10.1.6** Test and adjust the pH after 0.5 to 1 hour. Allow to stand for an additional 2 to 3 hours.
 - 10.1.7** Extract the sample and QC aliquots per Section 10.2.
- 10.2** Extraction.
 - 10.2.1** Add 20 g of clean NaCl (Section 6.5) and 2.5 mL of ethyl acetate to each sample and QC aliquot and cap tightly.
 - 10.2.2** Shake vigorously for 2 to 5 minutes. Allow the bottle to stand for 10 minutes for the phases to separate.
 - 10.2.3** Using a Pasteur pipette, transfer the organic phase to a GC autosampler vial. Measure its volume.

11. GAS CHROMATOGRAPHY

Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table is the retention time for MITC achieved under these conditions. An example of the separation achieved by the primary column is shown in Figure 1.

- 11.1 Calibrate the system as described in Section 7.
- 11.2 Set the injection volume on the autosampler to inject 3.0 μL of all standards and extracts of blanks and samples.
- 11.3 Set the data system or GC control to start the temperature program upon sample injection, and begin data collection after the solvent peak elutes. Set the data system to stop data collection after the last analyte is expected to elute and to return the column to the initial temperature.

12. SYSTEM AND LABORATORY PERFORMANCE

- 12.1 At the beginning of each 8-hour shift during which analyses are performed, GC system performance and calibration are verified on both column/detector systems. For these tests, analysis of the calibration verification standard (Section 6.8.1) shall be used to verify all performance criteria. Adjustment and/or recalibration (per Section 7) shall be performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks, and precision and recovery standards be analyzed.
- 12.2 Retention times: The absolute retention time of the peak maxima shall be within ± 10 seconds of the retention times in the initial calibration (Section 7.3.1).
- 12.3 GC resolution: Resolution is acceptable if the peak width at half-height is less than 10 seconds.
- 12.4 Calibration verification.
 - 12.4.1 Inject the calibration verification standard (Section 6.8.1).
 - 12.4.2 Compute the concentration of MITC based on the calibration factor or calibration curve (Section 7.3).
 - 12.4.3 Compare this concentration with the limits for calibration verification in Table 1. If the recovery meets the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, the recovery falls outside the calibration verification range, system performance is unacceptable. In this case, correct the problem and repeat the test, or recalibrate (Section 7).
- 12.5 Ongoing precision and recovery.
 - 12.5.1 Analyze the extract of the precision and recovery standard extracted with each sample batch (Section 10.1.3).
 - 12.5.2 Compute the percent recovery of MITC.
 - 12.5.3 Compare the percent recovery with the limits for ongoing recovery in Table 1. If the recovery meets the acceptance criteria, the extraction and concentration processes are in control and analysis of blanks and samples may proceed. If, however, the recovery falls outside the acceptable range, these processes are not in control. In this event, correct the problem, re-extract the sample batch, and repeat the ongoing precision and recovery test.
 - 12.5.4 Add results which pass the specifications in Section 12.5.3 to initial and previous ongoing data. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory data quality for each analyte by calculating the average percent recovery (R) and the standard deviation of percent recovery s_r . Express the accuracy as a recovery interval from $R - 2s_r$ to $R + 2s_r$. For example, if $R = 95\%$ and $s_r = 5\%$, the accuracy is 85 to 105%.

13. QUALITATIVE DETERMINATION

- 13.1** Qualitative determination is accomplished by comparison of data from analysis of a sample or blank with data from analysis of the shift standard (Section 12.1), and with data stored in the retention time and calibration libraries (Section 7.3.1). Identification is confirmed when retention-time and amounts agree per the criteria below.
- 13.2** On each column/detector system, establish a retention-time window ± 20 seconds on either side of the retention-time in the calibration data (Section 7.3.1).
- 13.3** If the MITC peak from the analysis of a sample or blank is within a window (as defined in Section 13.2) on the primary column/detector system, it is considered tentatively identified. A tentatively identified compound is confirmed when (1) the retention time for the compound on the confirmatory column/detector system is within the retention-time window on that system, and (2) the computed amounts (Section 14) on each system (primary and confirmatory) agree within a factor of 3.

14. QUANTITATIVE DETERMINATION

- 14.1** Using the GC data system, compute the concentration of the analyte detected in the extract (in milligrams per milliliter) using the calibration factor or calibration curve (Section 7.3.2).
- 14.2** Compute the concentration in the sample using the following equation:

Equation 2

$$C_s = \frac{(2.22)(V_e)(C_e)}{V_s}$$

where

- C_s = The concentration in the sample, in $\mu\text{g/L}$
 2.22 \rightarrow Converts MITC (MW 73.12) to dazomet (MW 162.27)
 V_e = The extract total volume, in mL
 C_e = The concentration in the extract, in $\mu\text{g/mL}$
 V_s = The volume of sample extracted, in L
-

- 14.3** If the concentration of MITC exceeds the calibration range of the system, the extract is diluted by a factor of 10, and a 3- μL aliquot of the diluted extract is analyzed.
- 14.4** Report results for dazomet found in all standards, blanks, and samples to three significant figures. Results for samples that have been diluted are reported at the least dilute level at which the concentration is in the calibration range.

15. ANALYSIS OF COMPLEX SAMPLES

- 15.1** Some samples may contain high levels (> 1000 ng/L) of dazomet or of interfering compounds, and/or polymeric materials. Some samples may form emulsions when extracted (Section 10.2); others may overload the GC column and/or detector. In these instances, the extract is diluted by a factor of 10 and reanalyzed (Section 14.3).

- 15.2** Recovery of matrix spikes: In most samples, matrix spike recoveries will be similar to those from reagent water. If the matrix spike recovery is outside the range specified in Table 1, the sample is diluted by a factor of 10, respiked, and reanalyzed. If the matrix spike recovery is still outside the range, the method may not work on the sample being analyzed and the result may not be reported for regulatory compliance purposes.

16. METHOD PERFORMANCE

- 16.1** This method is based on industry Method 131.⁷
- 16.2** Development of this method is detailed in Reference 8.

References

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4. "Handbook of Quality Control in Wastewater Laboratories," U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH,: EPA-600/4-79-019, March 1979.
5. "Standard Practice for Sampling Water" (ASTM Annual Book of Standards), American Society for Testing and Materials, Philadelphia, Pennsylvania: 76, 1980.
6. "Methods 330.4 and 330.5 for Total Residual Chlorine," U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH: EPA-600/4-70-020, March 1979.
7. "Determination of Metham (Vapam) in Wastewater" (Method 131), *Methods for Nonconventional Pesticides Chemicals Analysis of Industrial and Municipal Wastewater*. U.S. Environmental Protection Agency Effluent Guidelines Division (WH-552), Washington, DC: EPA 440/1-83/079-C, January 31, 1983.
8. "Narrative for SAS 1019," Pacific Analytical, Inc.: September 1989. Available from the U.S. Environmental Protection Agency Sample Control Center, 300 N. Lee St., Alexandria, VA 22314 (703-557-5040).

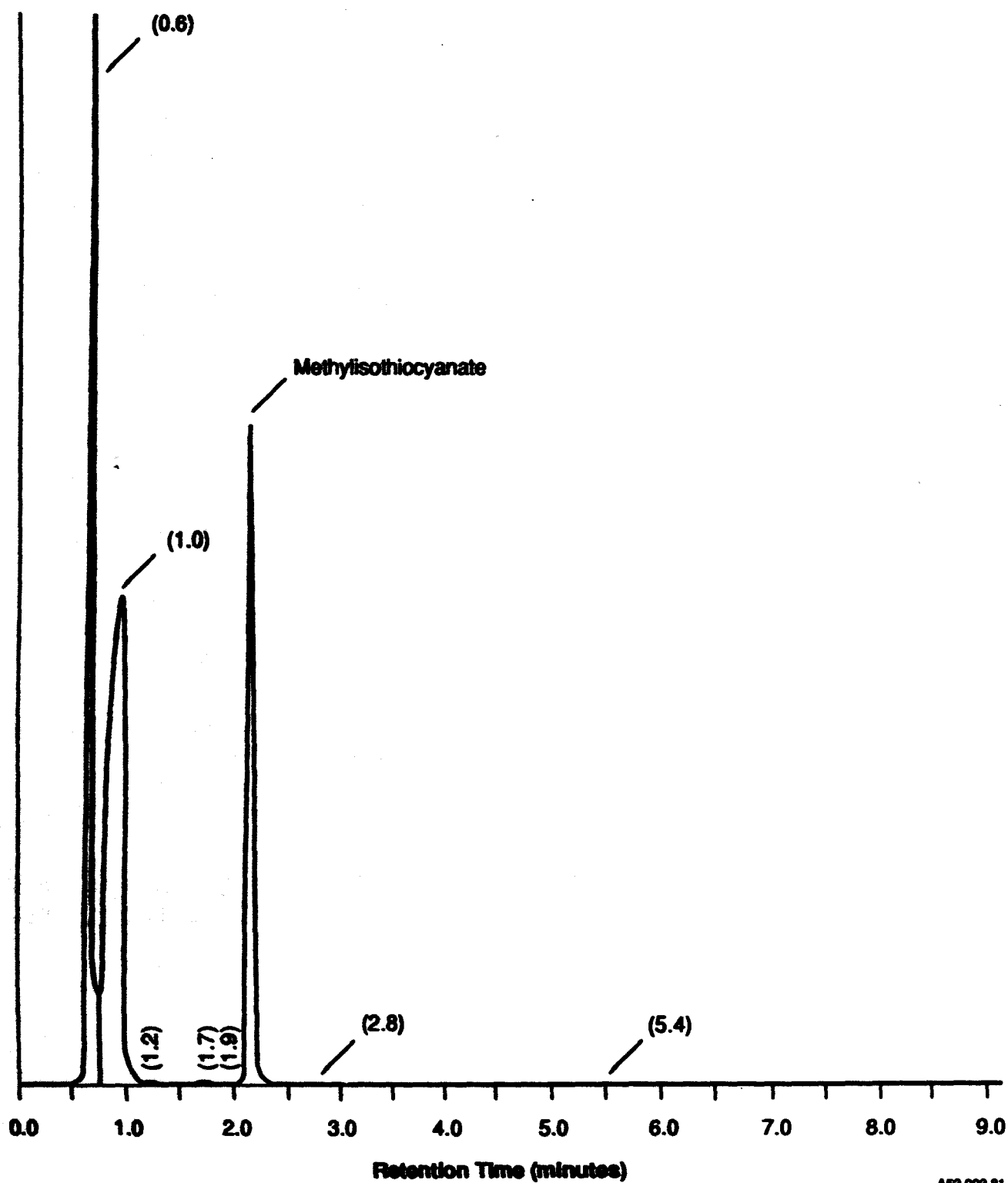
Table 1. GC Data and Method Acceptance Criteria for Dazomet*

Acceptance Criterion	Specification	Note
Minimum Level	10 µg/L	1
Method Detection Limit	3 µg/L	2
Calibration Verification (Section 12.4)	0.8 to 1.3 µg/mL	3
Initial Precision and Recovery (Section 8.2)		
Precision [standard deviation; s]	23 µg/L	
Recovery [mean; X]	18 to 75 µg/L	
Ongoing Precision and Recovery (Section 12.5)	15 to 78 µg/L	
Matrix Spike Recovery (Section 8.3.3)	16 to 123 %	
MITC Retention-time		5
DB-608	2.17 minutes	
DB-1701	3.80 minutes	

*(3,5-dimethyl-2H-tetrahydro-1,3,5-thiadiazine-2-thione) detected as Methyl isothiocyanate (MITC)

Notes:

1. This is a minimum level at which the analytical system shall give recognizable signals and acceptable calibration points.
2. Estimated; 40 CFR Part 136, Appendix B.
3. Test concentration 1.0 µg/mL.
4. Test concentration 50 µg/L.
5. Columns: 30 mm long by 0.53 mm ID; DB-608: 0.83 µ DB-1701: 1.0 µ. Conditions suggested to meet retention-times shown: 50°C for 1.0 minute, 50 to 200° at 10°C/min. Carrier gas flow rates approximately 7 mL/min.



A82-002-81

Figure 1. Chromatogram of Methyl isothiocyanate

Method 1660

***The Determination of Pyrethrins
and Pyrethroids in Municipal
and Industrial Wastewater***

Method 1660

The Determination of Pyrethrins and Pyrethroids in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of pyrethrins and pyrethroids in wastewater by extraction and high performance liquid chromatography (HPLC) with an ultra-violet detector (UV). The compounds in Table 1 may be determined by this method.
- 1.2 This method is designed to meet the monitoring requirements of the U.S. Environmental Protection Agency under the Clean Water Act at 40 *CFR* Part 455. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3 When this method is applied to analysis of unfamiliar samples, compound identity must be supported by at least one additional qualitative technique. This method lists a second UV wavelength that can be used to confirm measurements made with the primary wavelength.
- 1.4 This method is specific to the determination of two pyrethrins and seven pyrethroids, but should be applicable to other pyrethroids as well. The quality control requirements in this method give the steps necessary to determine this applicability.
- 1.5 The detection limit of this method is usually dependent on the level of interferences rather than instrumental limitations. The limits in Table 2 typify the minimum quantity that can be detected with no interferences present.
- 1.6 This method is for use by or under the supervision of analysts experienced in the use of a high performance liquid chromatograph and interpretation of liquid chromatographic data. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 8.2.

2. SUMMARY OF METHOD

- 2.1 A 750-mL sample is saturated with salt and extracted by stirring with acetonitrile in a 1-L volumetric flask. A small portion of the acetonitrile rises into the neck of the flask.¹ The extract is evaporated to a volume of 7.5 mL.
- 2.2 A 40- μ L aliquot of the extract is injected into the HPLC. Chromatographic conditions are described that permit the separation and measurement of the pyrethrins and pyrethroids by reverse-phase C18 column HPLC with a multiple-wavelength UV detector.
- 2.3 Identification of compound is performed by comparing the retention time of the compound with that of an authentic standard. Compound identity is confirmed when the retention times agree, and when the response at a second wavelength agrees with the response at the primary wavelength.
- 2.4 Quantitative analysis is performed using an authentic standard of each compound to produce a calibration factor or calibration curve, and using the calibration data to determine the

concentration of that compound in the extract. The concentration in the sample is calculated using the sample and extract volumes.

- 2.5 Quality is assured through reproducible calibration and testing of the extraction and HPLC systems.

3. CONTAMINATION AND INTERFERENCES

- 3.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or elevated baselines causing misinterpretation of chromatograms. All materials used in the analysis shall be demonstrated to be free from interferences under the conditions of analysis by running method blanks as described in Section 8.4.
- 3.2 Glassware and, where possible, reagents are cleaned by rinsing with solvent and baking at 450°C for 1 hour minimum in a muffle furnace or kiln. Some thermally stable materials may not be eliminated by this treatment and thorough rinsing with acetone and pesticide-quality acetonitrile may be required.
- 3.3 Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.
- 3.4 Interferences coextracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 2 through 4.
- 4.2 Unknown samples may contain high concentrations of volatile toxic compounds. Sample containers should be opened in a hood and handled with gloves that will prevent exposure.

5. APPARATUS AND MATERIALS

NOTE: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance meeting requirements of this method is the responsibility of the laboratory.

- 5.1 Sampling equipment for discrete or composite sampling.
- 5.1.1 Sample bottle: Amber glass, 1-L, with screw-cap. If amber bottles are not available, samples shall be protected from light.
- 5.1.2 Bottle caps: Threaded to fit sample bottles. Caps shall be lined with PTFE.

5.1.3 Cleaning.

5.1.3.1 Bottles are detergent water washed, then rinsed with solvent or baked at 450°C for 1 hour minimum before use.

5.1.3.2 Liners are detergent-water washed, then reagent water and solvent rinsed, and baked at approximately 200°C for 1 hour minimum prior to use.

5.1.4 Compositing equipment: Automatic or manual compositing system incorporating glass containers cleaned per bottle cleaning procedure above. Sample containers are kept at 0 to 4°C during sampling. Glass or PTFE tubing only shall be used. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, the tubing shall be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.

5.2 Equipment for glassware cleaning.

5.2.1 Laboratory sink with overhead fume hood.

5.2.2 Kiln: Capable of reaching 450°C within 2 hours and holding 450°C within $\pm 10^\circ\text{C}$, with temperature controller and safety switch (Cress Manufacturing Co, Sante Fe Springs, CA, B31H or X31TS, or equivalent).

5.3 Equipment for sample extraction.

5.3.1 Laboratory fume hood.

5.3.2 Stirring plate: Thermolyne Cimarec 2 (Model 546725), or equivalent.

5.3.3 Stirring bar: PTFE coated, approximately 1 by 4 cm.

5.3.4 Extraction flask: 1000-mL volumetric flask cleaned by rinsing with solvent or baking at 450°C for 1 hour minimum.

5.3.5 pH meter, with combination glass electrode.

5.4 Equipment for sample concentration.

5.4.1 Nitrogen evaporation device: Equipped with heated bath that can be maintained at 35 to 40°C (N-Evap, Organomation Associates, Inc., or equivalent).

5.4.2 Concentrator tube: 10- to 15-mL, graduated (Kontes K-570050-1025, or equivalent) with calibration verified.

5.5 Sample vials: Amber glass, 10- to 15-mL with PTFE-lined screw- or crimp-cap, to fit HPLC autosampler.

5.6 Balance: Analytical, capable of weighing 0.1 mg.

5.7 Miscellaneous glassware.

5.7.1 Pipettes, glass, volumetric, 1.00-, 5.00-, and 10.0-mL.

5.7.2 Pipettes, glass, Pasteur, 150 mm long by 5 mm ID (Fisher Scientific 13-678-6A, or equivalent).

5.7.3 Volumetric flasks, 10.0-, 25.0-, and 50.0-mL.

- 5.8 High performance liquid chromatograph (HPLC):** Analytical system complete with pumps, sample injector, column oven, and multiple-wavelength ultra-violet (UV) detector.
- 5.8.1 Pumping system:** Capable of isocratic operation and producing a linear gradient from 70% water/30% acetonitrile to 100% acetonitrile in 25 minutes (Waters 600E, or equivalent).
- 5.8.2 Sample injector:** Capable of automated injection of up to 30 samples (Waters 700, or equivalent).
- 5.8.3 Column oven:** Capable of operation at room ambient to 50°C (Waters TCM, or equivalent).
- 5.8.4 Column:** Two 150 mm long by 4.6 mm ID 300 Angstrom C18 columns (Vydac 201 TP5415, or equivalent) connected in series and preceded by a 30 mm long by 4.6 mm ID 300 Angstrom C18 guard column (Vydac 201 GCC54T, or equivalent), operated at the conditions shown in Table 2.
- 5.8.5 Detector:** UV operated at 235 and 245 nm (Waters 490E, or equivalent).
- 5.9 Data system.**
- 5.9.1 Data acquisition:** The data system shall collect and record LC peak areas and retention times on magnetic media.
- 5.9.2 Calibration:** The data system shall be used to calculate and maintain lists of calibration factors (response divided by concentration) and multi-point calibration curves. Computations of relative standard deviation (coefficient of variation) are used to test calibration linearity.
- 5.9.3 Data processing:** The data system shall be used to search, locate, identify, and quantify the compounds of interest in each analysis. Displays of chromatograms are required to verify results.
- 5.9.4 Statistics on initial (Section 8.2) and ongoing (Section 12.5) performance shall be computed and maintained.**

6. REAGENTS AND STANDARDS

- 6.1 Sample preservation:** Sodium thiosulfate (ACS), granular.
- 6.2 pH adjustment.**
- 6.2.1 Sodium hydroxide (10N):** Dissolve 40 g NaOH in 100 mL reagent water.
- 6.2.2 Sulfuric acid (1 + 1):** Reagent grade, 6N in reagent water. Slowly add 50 mL H₂SO₄ (specific gravity 1.84) to 50 mL reagent water.
- 6.3 Solvents:** Acetonitrile and acetone; pesticide-quality; lot-certified to be free of interferences.
- 6.4 Reagent water:** HPLC grade water in which the compounds of interest and interfering compounds are not detected by this method.
- 6.5 Salt:** Sodium chloride, spread approximately 1 cm deep in a baking dish and baked at 450°C for 1 hour minimum, cooled and stored in a precleaned glass bottle with PTFE-lined cap.
- 6.6 Standard solutions:** Purchased as solutions or mixtures with certification to their purity, concentration, and authenticity, or prepared from materials of known purity and composition. If

compound purity is 96% or greater, the weight may be used without correction to compute the concentration of the standard.

NOTE: The pyrethrins are normally available in a mixed standard consisting of the six naturally occurring compounds (pyrethrin I and II, cinerin I and II, and jasmolin I and II). The concentrations in this standard will be on the order of 10% each of pyrethrin I and II. The concentration in the stock solution prepared from this mixed standard is to be corrected for the exact concentration.

When not being used, standards are stored in the dark at -20 to -10°C in screw-capped vials with PTFE-lined lids. A mark is placed on the vial at the level of the solution so that solvent evaporation loss can be detected. The vials are brought to room temperature prior to use.

- 6.7** Preparation of stock solutions: Prepare in acetonitrile per the steps below. Observe the safety precautions in Section 4.
- 6.7.1** Dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 10-mg allethrin in a 10-mL ground-glass stoppered volumetric flask and fill to the mark with acetonitrile. After the allethrin is completely dissolved, transfer the solution to a 15-mL vial with PTFE-lined cap.
- 6.7.2** Stock solutions should be checked for signs of degradation prior to the preparation of calibration or performance test standards.
- 6.7.3** Stock solutions shall be replaced after 6 months, or sooner if comparison with quality control check standards indicates a change in concentration.
- 6.8** Secondary mixtures: Using stock solutions (Section 6.7), prepare mixtures for calibration and calibration verification (Sections 7.3 and 12.4), for initial and ongoing precision and recovery (Sections 8.2 and 12.5), and for spiking into the sample matrix (Section 8.3).
- 6.8.1** Calibration solutions: Prepare two solutions in acetonitrile at the concentrations given in Table 3. The midpoint solution is used for calibration verification (Section 12.4)
- 6.8.2** Precision and recovery standard and matrix spike solution: Prepare two solutions in acetone at the concentration of the midpoint standard (Table 3).
- 6.9** Stability of solutions: All standard solutions (Sections 6.7 through 6.8) shall be analyzed within 48 hours of preparation and on a monthly basis thereafter for signs of degradation. Standards will remain acceptable if the peak area remains within $\pm 15\%$ of the area obtained in the initial analysis of the standard.

7. SETUP AND CALIBRATION

- 7.1** Configure the HPLC system as given in Sections 5.8 through 5.9 and establish the operating conditions in Table 2.
- 7.2** Attainment of minimum level: Determine that the minimum levels in Table 2 are met at each wavelength.
- 7.3** Calibration.

- 7.3.1 Inject 40 μ L of each calibration solution (Table 3) into the HPLC system, beginning with the lowest level mixture and proceeding to the highest. For each compound, compute and store, as a function of the concentration injected, the retention time and the peak area at each wavelength (primary and confirmatory).
- 7.3.2 Calibration factor (ratio of area to amount injected).
 - 7.3.2.1 Compute the coefficient of variation (relative standard deviation) of the calibration factor over the calibration range for each at each wavelength.
 - 7.3.2.2 Linearity: If the calibration factor is constant ($C_v < 20\%$) over the calibration range, an average calibration factor may be used; otherwise, the complete calibration curve (area vs. amount) shall be used.

8. QUALITY CONTROL

- 8.1 Each laboratory that uses this method is required to operate a formal quality control program.⁵ The minimum requirements of this program consist of an initial demonstration of laboratory capability, an ongoing analysis of standards and blanks as tests of continued performance, and analysis of spiked samples to assess accuracy. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.
 - 8.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
 - 8.1.2 The analyst is permitted to modify this method to improve separations or lower the costs of measurements, provided all performance requirements are met. Each time a modification is made to the method or a cleanup procedure is added, the analyst is required to repeat the procedure in Section 8.2 to demonstrate method performance. If detection limits will be affected by the modification, the analyst is required to repeat demonstration of the detection limit (Section 7.2).
 - 8.1.3 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and the analysis of the precision and recovery standard (Section 6.8.2) that the analysis system is in control. These procedures are described in Sections 12.1, 12.4, and 12.5.
 - 8.1.4 The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Section 8.3.
 - 8.1.5 Analyses of blanks are required to demonstrate freedom from contamination. The procedures and criteria for analysis of a blank are described in Section 8.4.
- 8.2 Initial precision and recovery: To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations:
 - 8.2.1 Extract, concentrate, and analyze two sets of four 750-mL aliquots of reagent water spiked with 1.0 mL of each solution of the precision and recovery standard (Section 6.8.2) according to the procedure in Section 10.
 - 8.2.2 Using results of each set of four analyses, compute the average recovery (\bar{X}) and the standard deviation of recovery (s), in milligrams per liter, for the each compound.

- 8.2.3** Compare *s* and *X* with the corresponding limit for initial precision and recovery in Table 4. If *s* and *X* meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, *s* exceeds the precision limit or *X* falls outside the range for accuracy, system performance is unacceptable. In this case, correct the problem and repeat the test.
- 8.3** Method accuracy: The laboratory shall spike (matrix spike) at least 10% of the samples from a given site type (e.g., influent to treatment, treated effluent, produced water). If only one sample from a given site type is analyzed, a separate aliquot of that sample shall be spiked.
- 8.3.1** The concentration of the matrix spike shall be determined as follows.
- 8.3.1.1** If, as in compliance monitoring, the concentration of allethrin in the sample is being checked against a regulatory concentration limit, the matrix spike shall be at that limit or at 1 to 5 times higher than the background concentration determined in Section 8.3.2, whichever concentration is larger.
 - 8.3.1.2** If the concentration is not being checked against a regulatory limit, the matrix spike shall be at the level of the precision and recovery standard (Section 6.8.2) or at 1 to 5 times higher than the background concentration, whichever concentration is larger.
 - 8.3.1.3** If it is impractical to determine the background concentration before spiking (e.g., maximum holding times will be exceeded), the matrix spike concentration shall be the regulatory concentration limit, if any; otherwise, at the level of the precision and recovery standard (Section 6.8.2) or at 1 to 5 times the expected background concentration, whichever is larger.
- 8.3.2** Analyze one sample aliquot to determine the background concentration (*B*) of the pyrethrins and pyrethroids. If necessary, prepare a standard solution appropriate to produce a level in the sample 1 to 5 times the background concentration. Spike a second sample aliquot with the standard solution and analyze it to determine the concentration after spiking (*A*) of each analyte. Calculate the percent recovery (*P*):

Equation 1

$$P = \frac{100(A-B)}{T}$$

where

T = True value of the spike

- 8.3.3** Compare the percent recovery of each compound with the corresponding QC acceptance criteria in Table 4. If any analyte fails the acceptance criteria for recovery, the sample is complex and must be diluted and reanalyzed per Section 15.
- 8.3.4** As part of the QC program for the laboratory, method accuracy for samples shall be assessed and records shall be maintained. After the analysis of five spiked samples of a given matrix type (water, sludge) in which the recovery test (Section 8.3.3) is passed, compute the average percent recovery (*P*) and the standard deviation of the percent

recovery (s_p). Express the accuracy assessment as a percent recovery interval from $P - 2s_p$ to $P + 2s_p$ for each matrix. For example, if $P = 90\%$ and $s_p = 10\%$ for five analyses of wastewater, the accuracy interval is expressed as 70 to 110%. Update the accuracy assessment in each matrix on a regular basis (e.g., after each five to ten new accuracy measurements).

8.4 Blanks: Reagent water blanks are analyzed to demonstrate freedom from contamination.

8.4.1 Extract and concentrate a 750-mL reagent water blank with each sample batch (samples started through the extraction process on the same 8 hour shift, to a maximum of 20 samples). Analyze the blank immediately after analysis of the precision and recovery standard (Section 12.5) to demonstrate freedom from contamination.

8.4.2 If any compound or any potentially interfering compound is found in an aqueous blank at greater than 20 $\mu\text{g/L}$ (assuming the same calibration factor as allethrin for interfering compounds), analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level.

8.5 Other pyrethroids may be determined by this method. To establish a quality control limit for another analyte, determine the precision and accuracy by analyzing four replicates of the analyte along with the precision and recovery standard per the procedure in Section 8.2. If the analyte coelutes with an analyte in the QC standard, prepare a new QC standard without the coeluting component(s). Compute the average percent recovery (A) and the standard deviation of percent recovery (s_p) for the analyte, and measure the recovery and standard deviation of recovery for the other analytes. The data for the new analyte is assumed to be valid if the precision and recovery specifications for the other analytes are met; otherwise, the analytical problem is corrected and the test is repeated. Establish a preliminary quality control limit of $A \pm 2s_p$ for the new analyte and add the limit to Table 4.

8.6 The specifications contained in this method can be met if the apparatus used is calibrated properly, then maintained in a calibrated state. The standards used for calibration (Section 7), calibration verification (Section 12.4), and for initial (Section 8.2) and ongoing (Section 12.5) precision and recovery should be identical, so that the most precise results will be obtained. The HPLC instrument will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of the analytes given in this method.

8.7 Depending on specific program requirements, field replicates and field spikes may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

9.1 Collect samples in glass containers following conventional sampling practices,⁶ except that the bottle shall not be prerinsed with sample before collection. Aqueous samples which flow freely are collected in refrigerated bottles using automatic sampling equipment.

9.2 Maintain samples at 0 to 4°C from the time of collection until extraction. If the samples will not be extracted within 72 hours of collection, adjust the sample to a pH of 5.0 to 7.0 using sodium hydroxide or hydrochloric acid solution. Record the volume used. If residual chlorine is present in aqueous samples, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine.⁷

- 9.3** Begin sample extraction within 7 days of collection, and analyze all extracts within 40 days of extraction.

10. SAMPLE EXTRACTION

10.1 Preparation of sample and QC aliquots.

10.1.1 Mix sample thoroughly.

10.1.2 Pour 750 mL of sample into a clean 1000-mL volumetric flask. If a matrix spike is to be prepared, pour two 750-mL aliquots into clean flasks.

10.1.3 For each sample or sample batch (to a maximum of 20) to be extracted at the same time, place three 750-mL aliquots of reagent water (Section 6.4) in clean 1000-mL volumetric flasks. One reagent water aliquot serves as the blank.

10.1.4 Spike 1.0 mL of each precision and recovery standard (Section 6.8.2) into the remaining reagent water aliquots.

10.1.5 Spike the samples designated as the matrix spike at the level directed in Section 8.3.

10.1.6 Extract the sample and QC aliquots per Section 10.2.

10.2 Extraction.

10.2.1 Place each sample or QC aliquot on a stirring plate and add a clean PTFE-coated stirring bar.

10.2.2 Add 230 g of clean NaCl (Section 6.5) to each sample and QC aliquot and stir 5 to 10 minutes to dissolve.

10.2.3 Add 160 mL of acetonitrile to each sample and QC aliquot and begin stirring. Increase the rate of stirring until the vortex is drawn approximately one-half the depth of the water. Stir for approximately 5 minutes.

10.2.4 Allow the solutions to stand for approximately 5 minutes for the phases to separate. If an acetonitrile layer does not appear, add acetonitrile in 5-mL increments, stirring and settling between increments, until a 2- to 5-mL layer appears. If the acetonitrile layer is more than 5 mL, add reagent water, stir, and settle until the acetonitrile volume is reduced to 2 to 5 mL.

10.2.5 Using a Pasteur pipette, transfer the organic phase to a clean K-D concentrator tube (Section 5.4.2).

10.2.6 Add 5 mL of acetonitrile to the extraction flasks, stir, and allow to settle. Transfer the organic phase to the respective concentrator tubes. Repeat the extraction a third time. If all of the extract will not fit into the concentrator tube, evaporate some of the acetonitrile (Section 10.3), then add the remaining extract.

10.3 Concentration of extracts.

10.3.1 Place the concentrator tubes in the evaporation device (Section 5.4.1). Adjust the height of the blow-down tubes to 1 to 3 cm above the surface of the liquid and gently evaporate the acetonitrile until a volume of approximately 5 mL is reached.

10.3.2 Adjust the final extract volume to 7.5 mL and transfer to an HPLC autosampler vial.

11. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Table 2 summarizes the recommended operating conditions for the HPLC system. Included in this table are the retention times for the pyrethrins and pyrethroids achieved under these conditions. An example of the separation achieved by the column system is shown in Figure 1. Pyrethrin I and II are the major peaks in the naturally occurring pyrethrin standard. Pyrethrin II elutes prior to pyrethrin I. Jasmolin II and I will normally coelute with pyrethrin II and I, respectively. Most HPLC columns will resolve cinerin II and I, which are small peaks that elute after the respective pyrethrins. Some HPLC columns may resolve all six of the naturally occurring pyrethrins.

- 11.1 Calibrate the system as described in Section 7.
- 11.2 Set the injection volume on the autosampler to inject 40 μ L of all standards and extracts of blanks and samples.
- 11.3 Set the data system or HPLC control to start the gradient upon sample injection, and begin data collection after 10 minutes. Set the data system or HPLC control to stop data collection after the last analyte is expected to elute and to return the gradient to the initial setting.

12. SYSTEM AND LABORATORY PERFORMANCE

- 12.1 At the beginning of each 8 hour shift during which analyses are performed, HPLC system performance and calibration are verified at both wavelengths. For these tests, analysis of the calibration verification standard (Section 6.8.1) shall be used to verify all performance criteria. Adjustment and/or recalibration (per Section 7) shall be performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks, and precision and recovery standards be analyzed.
- 12.2 Retention times.
 - 12.2.1 The absolute retention time of sumithrin shall be no earlier than 23 minutes.
 - 12.2.2 The absolute retention time of the peak maxima shall be within ± 15 seconds of the average retention times in the initial calibration (Section 7.3.1).
- 12.3 GC resolution: Resolution is acceptable if the height of the valley between tetramethrin and allethrin is less than 20% of the taller of the two peaks when chromatograms of the two calibration verification solutions (Section 6.8.1) are superimposed.
- 12.4 Calibration verification.
 - 12.4.1 Inject the two calibration verification standards (Section 6.8.1).
 - 12.4.2 Compute the concentration of the pyrethrins and pyrethroids based on the calibration factor or calibration curve (Section 7.3).
 - 12.4.3 Compare this concentration with the limits for calibration verification in Table 4. If calibration is verified, system performance is acceptable and analysis of blanks and samples may begin. If, however, the recovery falls outside the calibration verification range, system performance is unacceptable. In this case, correct the problem and repeat the test, or recalibrate (Section 7).
- 12.5 Ongoing precision and recovery.
 - 12.5.1 Analyze the extract of the two precision and recovery standards extracted with each sample batch (Section 10.1.3).

- 12.5.2 Compute the recovery of the compounds of interest in milligrams per liter.
- 12.5.3 Compare the recovery with the limits for ongoing recovery in Table 4. If the recovery meets the acceptance criteria, the extraction and concentration processes are in control and analysis of blanks and samples may proceed. If, however, the recovery falls outside the acceptable range, these processes are not in control. In this event, correct the problem, re-extract the sample batch, and repeat the ongoing precision and recovery test.
- 12.5.4 Add results which pass the specifications in 12.5.3 to initial and previous ongoing data. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory data quality for each analyte by calculating the average percent recovery (R) and the standard deviation of percent recovery s_r . Express the accuracy as a recovery interval from $R - 2s_r$ to $R + 2s_r$. For example, if $R = 95\%$ and $s_r = 5\%$, the accuracy is 85 to 105%.

13. QUALITATIVE DETERMINATION

- 13.1 Qualitative determination is accomplished by comparison of data from analysis of a sample or blank with data from analysis of the shift standard (Section 12.1), and with data stored in the retention-time and calibration libraries (Section 7.3.1). Identification is confirmed when retention time and amounts agree per the criteria below.
- 13.2 Establish a retention-time window of ± 20 seconds on either side of the mean retention -time in the calibration data (Section 7.3.1).
- 13.3 If a peak from the analysis of a sample or blank is within a window (as defined in Section 13.2) at the primary wavelength (235 nm), it is considered tentatively identified. A tentatively identified compound is confirmed when (1) the retention time of the peak maximum at the confirmatory wavelength (245 nm) is within ± 2 seconds of the retention-time of the peak maximum at the primary wavelength, and (2) the computed amounts (Section 14) on each system (primary and confirmatory) agree within a factor of 2.

14. QUANTITATIVE DETERMINATION

- 14.1 Using the HPLC data system, compute the concentration of the analyte detected in the extract (in micrograms per milliliter) using the calibration factor or calibration curve (Section 7.3.2).
- 14.2 Compute the concentration in the sample using the following equation:

Equation 2

$$C_s = \frac{(V_e)(C_{ex})}{V_s}$$

where

- C_s = The concentration in the sample, in $\mu\text{g/L}$
 V_e = The extract total volume, in mL (nominally, 7.5)
 C_{ex} = The concentration in the extract, in $\mu\text{g/L}$
 V_s = The volume of sample extracted, in L (nominally, 0.75)
-

- 14.3 If the concentration of any analyte exceeds the calibration range of the system, the extract is diluted by a factor of 10, and a 40- μ L aliquot of the diluted extract is analyzed.
- 14.4 Report results for pyrethrins and pyrethroids found in all standards, blanks, and samples to three significant figures. Results for samples that have been diluted are reported at the least dilute level at which the concentration is in the calibration range.

15. ANALYSIS OF COMPLEX SAMPLES

- 15.1 Some samples may contain high levels (> 1000 ng/L) of the pyrethrins and pyrethroids or of interfering compounds and/or polymeric materials. Some samples may form emulsions when extracted (Section 10.2); others may overload the HPLC column and/or detector. In these instances, the sample is diluted by a factor of 10 and re-extracted (Section 10), or the extract is diluted by a factor of 10 and reanalyzed (Section 14.3).
- 15.2 Recovery of matrix spikes: In most samples, matrix spike recoveries will be similar to those from reagent water. If the matrix spike recovery is outside the range specified in Table 4, the sample is diluted by a factor of 10, respiked, and reanalyzed. If the matrix spike recovery is still outside the range, the method does not work on the sample being analyzed and the result may not be reported for regulatory compliance purposes.

16. METHOD PERFORMANCE

- 16.1 Development of this method is detailed in Reference 8.

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Table 1. Pyrethrins and Pyrethroids Determined by High Performance Liquid Chromatography with Ultra-Violet Absorption Detector

<i>Compound</i>	<i>CAS Registry</i>
Allethrin (Pynamin)	583-79-2
Cyfluthrin (Baythroid)	68359-37-5
Fenvalerate (Pydrin)	51630-58-1
Cis-permethrin	61949-76-6
Trans-permethrin	61949-77-7
Pyrethrin I	121-21-1
Pyrethrin II	121-29-9
Resmethrin	10453-86-8
Sumithrin (phenothrin)	26002-80-2
Tetramethrin	7696-12-0

Table 2. High Performance Liquid Chromatography of Pyrethrins and Pyrethroids

<i>Compound</i>	<i>Retention Time (min)</i>	<i>Minimum Level¹ (µg/L)</i>	<i>MDL² (µg/L)</i>
Pyrethrin II	17.48	33	19
Tetramethrin	18.98	50	16
Allethrin	19.27	50	16
Pyrethrin I	20.89	31	22
Cyfluthrin	21.84	50	22
Resmethrin	22.07	50	22
Fenvalerate	22.68	25	10 est.
C/T-permethrin ³	22.98	50	20 est.
Sumithrin	23.47	50	25
C/T-permethrin ³	23.56	50	20 est.

1. This is a minimum level at which the analytical system shall give recognizable signals and acceptable calibration points.
2. 40 CFR Part 136, Appendix B. Column system and conditions: two 150 mm long by 4.6 mm ID 300 Angstrom C18 columns connected in series preceded by a 30 mm long by 4.6 mm ID 300 Angstrom C18 guard column. Column temperature 30°C. Solvent flow rate 1.5 mL/min. Gradient: linear from 70% water/30% acetonitrile at injection to 100% acetonitrile in 25 minutes.
3. Elution order of cis/trans isomers not known.

Table 3. Concentration of Calibration Solutions

<i>Compound</i>	<i>Solution Concentration (µg/mL)</i>		
	<i>Low</i>	<i>Median</i>	<i>High</i>
Calibration Solution 1			
Cyfluthrin	0.50	4.00	40.0
Fenvalerate	0.25	2.00	20.0
Pyrethrin I	0.31	2.50	25.0
Pyrethrin II	0.33	2.65	26.5
Sumithrin	0.50	4.00	40.0
Tetramethrin	0.50	4.00	40.0
Calibration Solution 2			
Allethrin	0.50	4.00	40.0
Resmethrin	0.50	4.00	40.0
C/T-permethrin*	0.50	4.00	40.0
C/T-permethrin*	0.50	4.00	40.0

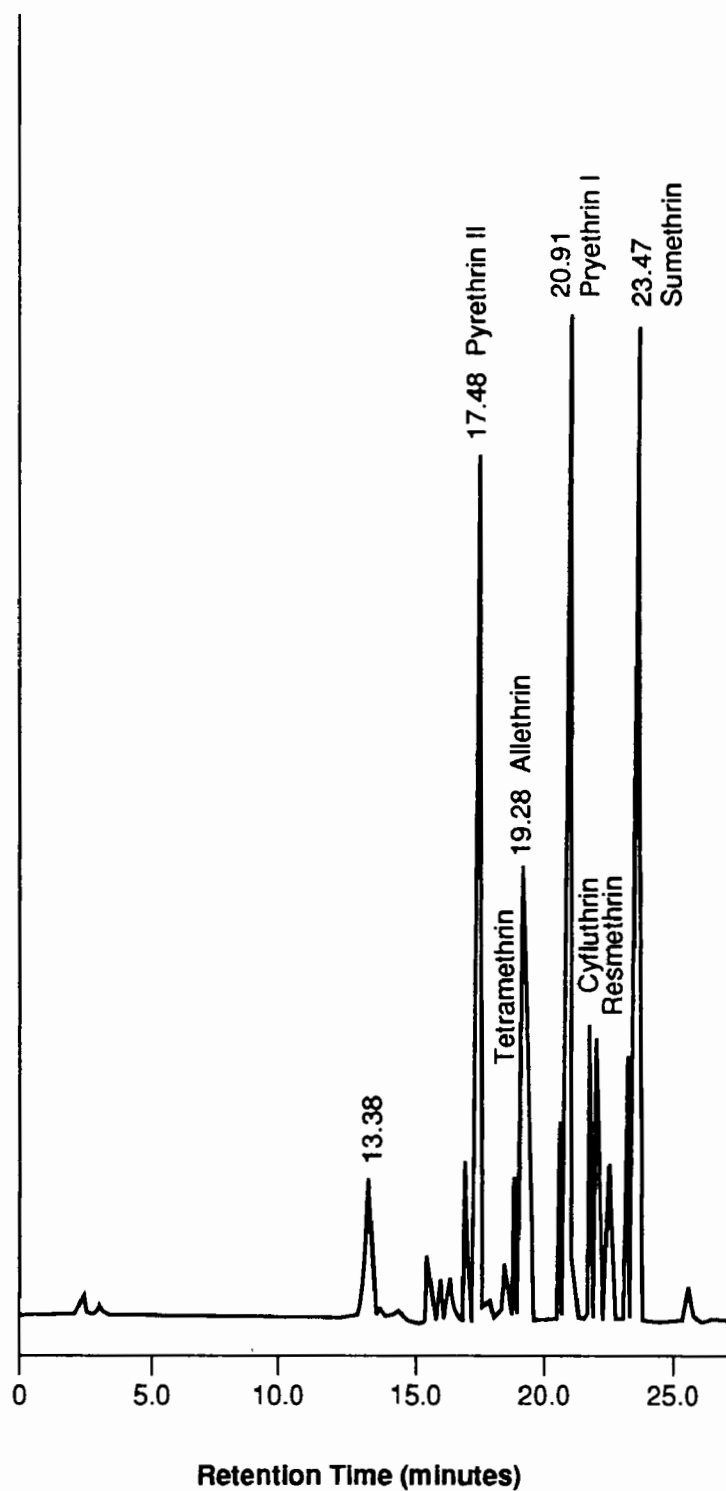
Table 4. Acceptance Criteria for Performance Tests for Pyrethrins and Pyrethroids

<i>Compound</i>	<i>Spike Level (µg/L)</i>	<i>Acceptance Criteria</i>			
		<i>Initial Precision and Accuracy (µg/L)</i>		<i>Calibration Verification¹ (µg/L)</i>	<i>Recovery Ongoing Accuracy R (µg/L)</i>
		<i>s</i>	<i>X</i>		
Allethrin	400	90	160 - 520	3.5 - 4.6	150 - 530
Cyfluthrin	400	125	110 - 610	3.0 - 5.2	94 - 630
Fenvalerate	200	35	120 - 260	1.6 - 2.4	62 - 314
C/T-permethrin ²	400	75	230 - 530	3.0 - 4.6	220 - 540
C/T-permethrin ³	400	75	230 - 530	3.0 - 4.6	210 - 540
Pyrethrin I	265	70	860 - 320	2.2 - 2.8	77 - 330
Pyrethrin II	250	60	110 - 330	2.0 - 3.5	100 - 340
Resmethrin	400	125	43 - 510	2.3 - 5.2	25 - 520
Sumithrin	400	140	46 - 570	3.5 - 4.7	25 - 590
Tetramethrin	400	90	170 - 530	1.5 - 6.1	150 - 550

1. Verified at the level of the median standard in Table 3.

2. First of two permethrin peaks

3. Second of two permethrin peaks



52-002-85

Figure 1. Chromatogram of Pyrethrins and Pyrethroids

Method 1661
***The Determination of
Bromoxynil in Municipal and
Industrial Wastewater***

Method 1661

The Determination of Bromoxynil in Municipal and Industrial Wastewater

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of bromoxynil in waste by direct aqueous injection high performance liquid chromatography (HPLC) with an ultraviolet detector (UV).
- 1.2 This method is designed to meet the monitoring requirements of the U.S. Environmental Protection Agency under the Clean Water Act at 40 *CFR* Part 455. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3 When this method is applied to the analysis of unfamiliar samples, compound identity must be supported by at least one additional qualitative technique. This method lists a second UV wavelength that can be used to confirm measurements made with the primary wavelength.
- 1.4 The detection limit of this method is usually dependent on the level of interferences rather than instrumental limitations. The limit in Table 1 typifies the minimum quantity that can be detected with no interferences present.
- 1.5 This method is for use by or under the supervision of analysts experienced in the use of a high performance liquid chromatograph and interpretation of liquid chromatographic data. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 8.2.

2. SUMMARY OF METHOD

- 2.1 A 40- μ L aliquot of sample is injected into the HPLC. Chromatographic conditions are described that permit the separation and measurement of bromoxynil by reverse-phase C18 column HPLC with a multiple-wavelength UV detector.
- 2.2 Identification of bromoxynil is performed by comparing the retention time of the chromatograph peak with that of an authentic standard. Compound identity is confirmed when the retention times agree, and when the response at a second wavelength agrees with the response at the primary wavelength.
- 2.3 Quantitative analysis is performed using an authentic standard of bromoxynil to produce a calibration factor or calibration curve, and using the calibration data to determine the concentration of bromoxynil in the sample.
- 2.4 Quality is assured through reproducible calibration and testing of the HPLC system.

3. CONTAMINATION AND INTERFERENCES

- 3.1 Solvents, reagents, glassware, and other sample-processing hardware may yield artifacts and/or elevated baselines causing misinterpretation of chromatograms. All materials used in the analysis

shall be demonstrated to be free from interferences under the conditions of analysis by running method blanks as described in Section 8.4.

- 3.2 Glassware and, where possible, reagents are cleaned by rinsing with solvent and baking at 450°C for 1 hour minimum in a muffle furnace or kiln. Some thermally stable materials may not be eliminated by this treatment and thorough rinsing with acetone and pesticide-quality acetonitrile may be required.
- 3.3 Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.
- 3.4 Interferences coextracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 1 to 3.
- 4.2 Unknown samples may contain high concentrations of volatile toxic compounds. Sample containers should be opened in a hood and handled with gloves that will prevent exposure.

5. APPARATUS AND MATERIALS

NOTE: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance meeting requirements of this method is the responsibility of the laboratory.

- 5.1 Sampling equipment for discrete or composite sampling.
 - 5.1.1 Sample bottle, amber glass, 40-mL minimum, with screw-cap. If amber bottles are not available, samples shall be protected from light.
 - 5.1.2 Bottle caps: Threaded to fit sample bottles. Caps shall be lined with PTFE.
 - 5.1.3 Cleaning.
 - 5.1.3.1 Bottles are detergent water washed, then rinsed with solvent rinsed or baked at 450°C for 1 hour minimum before use.
 - 5.1.3.2 Liners are detergent-water washed, then rinsed with reagent water and solvent, and baked at approximately 200°C for 1 hour minimum prior to use.
 - 5.1.4 Compositing equipment: Automatic or manual compositing system incorporating glass containers cleaned per bottle cleaning procedure above. Sample containers are kept at 0 to 4°C during sampling. Glass or PTFE tubing only shall be used. If the sampler uses

a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, the tubing shall be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.

5.2 Equipment for glassware cleaning.

5.2.1 Laboratory sink with overhead fume hood.

5.2.2 Kiln: Capable of reaching 450°C within 2 hours and holding 450°C within $\pm 10^\circ\text{C}$, with temperature controller and safety switch (Cress Manufacturing Co, Sante Fe Springs, CA, B31H or X31TS, or equivalent).

5.3 pH meter, with combination glass electrode.

5.4 Sample vials: Amber glass, 10- to 15-mL with PTFE-lined screw- or crimp-cap, to fit HPLC autosampler.

5.5 Balance: analytical, capable of weighing 0.1 mg.

5.6 Miscellaneous glassware.

5.6.1 Pipettes, glass, volumetric, 1.00-, 5.00-, and 10.0-mL.

5.6.2 Pipettes, glass, Pasteur, 150 mm long by 5 mm ID (Fisher Scientific 13-678-6A, or equivalent).

5.6.3 Volumetric flasks, 10.0-, 25.0-, and 50.0-mL.

5.7 High performance liquid chromatograph (HPLC): Analytical system complete with pumps, sample injector, column oven, and multiple-wavelength ultra-violet (UV) detector.

5.7.1 Pumping system: Capable of isocratic operation (Waters 600E, or equivalent).

5.7.2 Sample injector: Capable of automated injection of up to 30 samples (Waters 700, or equivalent).

5.7.3 Column oven: Capable of operation at room ambient to 50°C (Waters TCM, or equivalent).

5.7.4 Column: 150 mm long by 4.6 mm ID 300 Angstrom C18 column (Vydac 201 TP5415, or equivalent), operated at the conditions shown in Table 1.

5.7.5 Detector: UV operated at 255 and 280 nm (Waters 490E, or equivalent).

5.8 Data system.

5.8.1 Data acquisition: The data system shall collect and record LC peak areas and retention times on magnetic media.

5.8.2 Calibration: The data system shall be used to calculate and maintain lists of calibration factors (response divided by concentration) and multi-point calibration curves. Computations of relative standard deviation (coefficient of variation) are used to test calibration linearity.

5.8.3 Data processing: The data system shall be used to search, locate, identify, and quantify the compounds of interest in each analysis. Displays of chromatograms are required to verify results.

5.8.4 Statistics on initial (Section 8.2) and ongoing (Section 12.5) performance shall be computed and maintained.

6. REAGENTS AND STANDARDS

- 6.1 Sample preservation: Sodium thiosulfate (ACS), granular.
- 6.2 pH Adjustment.
 - 6.2.1 Sodium hydroxide (10N): Dissolve 40 g NaOH in 100 mL reagent water.
 - 6.2.2 Sulfuric acid (1 + 1): Reagent grade, 6N in reagent water. Slowly add 50 mL H₂SO₄ (specific gravity 1.84) to 50 mL reagent water.
- 6.3 Solvents: Methanol; pesticide-quality, lot-certified to be free of interferences.
- 6.4 Reagent water: HPLC grade water in which the compounds of interest and interfering compounds are not detected by this method.
- 6.5 Standard: Purchased as a solution with certification as to purity, concentration, and authenticity, or prepared from materials of known purity and composition. If compound purity is 96% or greater, the weight may be used without correction to compute the concentration of the standard. When not being used, standards are stored in the dark at -20°C to -10°C in screw-capped vials with PTFE-lined lids. A mark is placed on the vial at the level of the solution so that solvent evaporation loss can be detected. The vials are brought to room temperature prior to use.
- 6.6 Preparation of stock solutions: Prepare in water per the steps below. Observe the safety precautions in Section 4.
 - 6.6.1 Dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 10 mg bromoxynil in a 100-mL ground-glass stoppered volumetric flask and fill to the mark with water. After the bromoxynil is completely dissolved, transfer the solution to a 150-mL vial with PTFE-lined cap.
 - 6.6.2 Stock solutions should be checked for signs of degradation prior to the preparation of calibration or performance test standards.
 - 6.6.3 Stock solutions shall be replaced after 6 months, or sooner if comparison with quality control check standards indicates a change in concentration.
- 6.7 Secondary mixtures: Using stock solutions (Section 6.6), prepare mixtures for calibration and calibration verification (Sections 7.3 and 12.4), for initial and ongoing precision and recovery (Sections 8.2 and 12.5), and for spiking into the sample matrix (Section 8.3).
 - 6.7.1 Calibration solutions: Prepare in water at the concentrations given in Table 2. The low level solution is used for calibration verification (Section 12.4)
 - 6.7.2 Precision and recovery standard and matrix spike solution: Prepare in water at a concentration of 10 µg/mL.
- 6.8 Stability of solutions: All standard solutions (Sections 6.6 through 6.7) shall be analyzed within 48 hours of preparation and on a monthly basis thereafter for signs of degradation. Standards will remain acceptable if the peak area remains within ±15% of the area obtained in the initial analysis of the standard.

7. SETUP AND CALIBRATION

- 7.1 Configure the HPLC system described in Sections 5.7 through 5.8 and establish the operating conditions in Table 1.

- 7.2 Attainment of minimum level:** Determine that the minimum level in Table 1 is met at each wavelength.
- 7.3 Calibration.**
- 7.3.1** Inject 40 μL of each calibration solution (Table 2) into the HPLC system, beginning with the lowest concentration and proceeding to the highest. Compute and store, as a function of the concentration injected, the retention time and the peak area of Bromoxynil each wavelength (primary and confirmatory).
- 7.3.2 Calibration factor (ratio of area to amount injected).**
- 7.3.2.1** Compute the coefficient of variation (relative standard deviation) of the calibration factor over the calibration range at each wavelength.
- 7.3.2.2 Linearity:** If the calibration factor is constant ($C_v < 15\%$) over the calibration range, an average calibration factor may be used; otherwise, the complete calibration curve (area vs. amount) shall be used.

8. QUALITY CONTROL

- 8.1** Each laboratory that uses this method is required to operate a formal quality control program.⁴ The minimum requirements of this program consist of an initial demonstration of laboratory capability, an ongoing analysis of standards and blanks as tests of continued performance, and analysis of spiked samples to assess accuracy. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.
- 8.1.1** The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2** The analyst is permitted to modify this method to improve separations or lower the costs of measurements, provided all performance requirements are met. Each time a modification is made to the method or a cleanup procedure is added, the analyst is required to repeat the procedure in Section 8.2 to demonstrate method performance. If minimum level will be affected by the modification, the analyst is required to repeat demonstration of the minimum level (Section 7.2).
- 8.1.3** The laboratory shall, on an ongoing basis, demonstrate through calibration verification and the analysis of the precision and recovery standard (Section 6.7.2) that the analysis system is in control. These procedures are described in Sections 12.1, 12.4, and 12.5.
- 8.1.4** The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Section 8.3.
- 8.1.5** Analyses of blanks are required to demonstrate freedom from contamination. The procedures and criteria for analysis of a blank are described in Section 8.4.
- 8.2** Initial precision and recovery: To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations.

- 8.2.1** Analyze one set of four 10-mL aliquots of reagent water spiked with 100 μ L of the precision and recovery standard (Section 6.7.2) according to the procedure in Sections 10 and 11.
- 8.2.2** Using the results of the set of four analyses, compute the average recovery (X) and the standard deviation of recovery (s) for bromoxynil.
- 8.2.3** Compare s and X with the corresponding limit for initial precision and recovery in Table 1. If s and X meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, s exceeds the precision limit or X falls outside the range for accuracy, system performance is unacceptable. In this case, correct the problem and repeat the test.
- 8.3 Method accuracy:** The laboratory shall spike (matrix spike) at least 10% of the samples from a given site type (e.g., influent to treatment, treated effluent, produced water). If only one sample from a given site type is analyzed, a separate aliquot of that sample shall be spiked.
- 8.3.1** The concentration of the matrix spike shall be determined as follows.
- 8.3.1.1** If, as in compliance monitoring, the concentration of bromoxynil in the sample is being checked against a regulatory concentration limit, the matrix spike shall be at that limit or at 1 to 5 times higher than the background concentration determined in Section 8.3.2, whichever concentration is larger.
- 8.3.1.2** If the concentration is not being checked against a regulatory limit, the matrix spike shall be at the level of the precision and recovery standard (Section 6.7.2) or at 1 to 5 times higher than the background concentration, whichever concentration is larger.
- 8.3.1.3** If it is impractical to determine the background concentration before spiking (e.g., maximum holding times will be exceeded), the matrix spike concentration shall be the regulatory concentration limit, if any; otherwise, at the the level of the precision and recovery standard (Section 6.7.2) or at 1 to 5 times the expected background concentration concentration, whichever is larger.
- 8.3.2** Analyze one sample aliquot to determine the background concentration (B) of bromoxynil. If necessary, prepare a standard solution appropriate to produce a level in the sample 1 to 5 times the background concentration. Spike a second sample aliquot with the standard solution and analyze it to determine the concentration after spiking (A) of each analyte. Calculate the percent recovery (P):

Equation 1

$$P = \frac{100(A-B)}{T}$$

where

T = True value of the spike

- 8.3.3** Compare the percent recovery of bromoxynil with the corresponding QC acceptance criteria in Table 1. If it fails the acceptance criteria for recovery, the sample may be complex and must be diluted and reanalyzed per Section 15.
- 8.3.4** As part of the QC program for the laboratory, method accuracy for samples shall be assessed and records shall be maintained. After the analysis of five spiked samples of a given matrix type (e.g., influent to treatment, treated effluent, produced water) in which the the recovery test (Section 8.3.3) is passed, compute the average percent recovery (P) and the standard deviation of the percent recovery (s_p). Express the accuracy assessment as a percent recovery interval from $P - 2s_p$ to $P + 2s_p$ for each matrix. For example, if $P = 90\%$ and $s_p = 10\%$ for five analyses of wastewater, the accuracy interval is expressed as 70 to 110%. Update the accuracy assessment in each matrix on a regular basis (e.g., after each 5 to 10 new accuracy measurements).
- 8.4** Blanks: reagent water blanks are analyzed to demonstrate freedom from contamination.
- 8.4.1** Analyze a reagent water blank with each sample batch (samples started through the extraction process on the same 8-hour shift, to a maximum of 20 samples). Analyze the blank immediately after analysis of the precision and recovery standard (Section 12.5) to demonstrate freedom from contamination.
- 8.4.2** If any compound or any potentially interfering compound is found in an aqueous blank at greater than $100 \mu\text{g/L}$ (assuming the same calibration factor as bromoxynil for interfering compounds), analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level.
- 8.5** The specifications contained in this method can be met if the apparatus used is calibrated properly, then maintained in a calibrated state. The standards used for calibration (Section 7), calibration verification (Section 12.4), and for initial (Section 8.2) and ongoing (Section 12.5) precision and recovery should be identical, so that the most precise results will be obtained. The HPLC instrument will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of the analytes given in this method.
- 8.6** Depending on specific program requirements, field replicates and field spikes may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

- 9.1** Collect samples in glass containers following conventional sampling practices,⁵ except that the bottle shall not be prerinsed with sample before collection. Aqueous samples which flow freely are collected in refrigerated bottles using automatic sampling equipment.
- 9.2** Maintain samples at 0 to 4°C from the time of collection until extraction. If the samples will not be extracted within 72 hours of collection, adjust the sample to a pH of 3.0 to 7.0 using sodium hydroxide or hydrochloric acid solution. Record the volume used. If residual chlorine is present in aqueous samples, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine.⁶
- 9.3** Begin sample extraction within 7 days of collection, and analyze all extracts within 40 days of extraction.

10. PREPARATION OF SAMPLE AND QC ALIQUOTS

- 10.1** Mix sample thoroughly.
- 10.2** Pour approximately 10 mL of sample into a clean HPLC autosampler vial. If a matrix spike is to be prepared, pour 10.0 mL into a second clean vial.
- 10.3** For each sample or sample batch (to a maximum of 20) to be analyzed in the same 8-hour shift, place two 10.0-mL aliquots of reagent water (Section 6.4) in clean auto-sampler vials. One reagent water aliquot serves as the blank.
- 10.4** Spike 100 μ L of the precision and recovery standard (Section 6.7.2) into the remaining reagent water aliquot.
- 10.5** Spike the sample designated as the matrix spike at the level directed in Section 8.3.
- 10.6** Analyze the sample and QC aliquots per Section 11.

11. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Table 1 summarizes the recommended operating conditions for the HPLC system. Included in this Table is the retention times for bromoxynil achieved under these conditions. An example of the separation achieved by the column system is shown in Figure 1.

- 11.1** Calibrate the system as described in Section 7.
- 11.2** Set the injection volume on the auto-sampler to inject 40 μ L of all standards, blanks, and samples.
- 11.3** Set the data system or HPLC control to begin data collection upon injection and to stop data collection after bromoxynil is expected to elute.

12. SYSTEM AND LABORATORY PERFORMANCE

- 12.1** At the beginning of each 8-hour shift during which analyses are performed, HPLC system performance and calibration are verified at both wavelengths. For these tests, analysis of the calibration verification standard (Section 6.7.1) shall be used to verify all performance criteria. Adjustment and/or recalibration (per Section 7) shall be performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks, and precision and recovery standards be analyzed.
- 12.2** Retention times.
 - 12.2.1** The absolute retention time of bromoxynil shall be no less than 2.5 minutes.
 - 12.2.2** The absolute retention time of the bromoxynil peak maximum shall be within ± 15 seconds of the average of the retention times in the initial calibration (Section 7.3.1).
- 12.3** GC resolution: Resolution is acceptable if the peak width at half-height of bromoxynil is less than 15 seconds.
- 12.4** Calibration verification.
 - 12.4.1** Inject the calibration verification standard (Section 6.7.1).
 - 12.4.2** Compute the concentration of bromoxynil based on the calibration factor or calibration curve (Section 7.3).

12.4.3 Compare this concentration with the limits for calibration verification in Table 1. If calibration is verified, system performance is acceptable and analysis of blanks and samples may begin. If, however, the recovery falls outside the calibration verification range, system performance is unacceptable. In this case, correct the problem and repeat the test, or recalibrate (Section 7).

12.5 Ongoing precision and recovery.

12.5.1 Analyze the precision and recovery standard prepared with each sample batch (Section 10.3).

12.5.2 Compute the recovery of bromoxynil.

12.5.3 Compare the recovery with the limit for ongoing recovery in Table 1. If the recovery meets the acceptance criteria, the analytical process is in control and analysis of blanks and samples may proceed. If, however, the recovery falls outside the acceptable range, these processes are not in control. In this event, correct the problem and repeat the ongoing precision and recovery test.

12.5.4 Add results which pass the specifications in Section 12.5.3 to initial and previous ongoing data. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory data quality for each analyte by calculating the average percent recovery (R) and the standard deviation of percent recovery s_r . Express the accuracy as a recovery interval from $R - 2s_r$ to $R + 2s_r$. For example, if $R = 95\%$ and $s_r = 5\%$, the accuracy is 85 to 105%.

13. QUALITATIVE DETERMINATION

13.1 Qualitative determination is accomplished by comparison of data from analysis of a sample or blank with data from analysis of the shift standard (Section 12.1), and with data stored in the retention-time and calibration libraries (Section 7.3.1). Identification is confirmed when retention-time and amounts agree per the criteria below.

13.2 Establish a retention-time window ± 15 seconds on either side of the mean retention-time in the calibration data (Section 7.3.1).

13.3 If a peak from the analysis of a sample or blank is within this window (as defined in Section 13.2) at the primary wavelength (280 nm), it is considered tentatively identified. A tentatively identified compound is confirmed when (1) the retention time of the peak maximum at the confirmatory wavelength (255 nm) is within ± 2 seconds of the retention-time of the peak maximum at the primary wavelength, and (2) the computed amounts (Section 14) on each system (primary and confirmatory) agree within a factor of 2.

14. QUANTITATIVE DETERMINATION

14.1 Using the HPLC data system, compute the concentration of the analyte detected in the sample (in milligram per liter) using the calibration factor or calibration curve (Section 7.3.2).

14.2 If the concentration of any analyte exceeds the calibration range of the system, the sample is diluted by a factor of 10, and a 40- μ L aliquot of the diluted extract is analyzed.

- 14.3** Report results for bromoxynil found in all standards, blanks, and samples to three significant figures. Results for samples that have been diluted are reported at the least dilute level at which the concentration is in the calibration range.

15. ANALYSIS OF COMPLEX SAMPLES

- 15.1** Some samples may contain high levels ($> 1000 \mu\text{g/L}$) of bromoxynil or of interfering compounds and/or polymeric materials. Some samples may overload the HPLC column and/or detector. In these instances, the sample is diluted by a factor of 10 and reanalyzed (Section 14.2).
- 15.2** Recovery of matrix spikes: in most samples, matrix spike recoveries will be similar to those from reagent water. If the matrix spike recovery is outside the range specified in Table 1, the sample is diluted by a factor of 10, respiked, and reanalyzed. If the matrix spike recovery is still outside the range, the method does not work on the sample being analyzed and the result may not be reported for regulatory compliance purposes.

16. METHOD PERFORMANCE

- 16.1** Development of this method is detailed in Reference 7.

References

1. "Carcinogens: Working with Carcinogens." Department of Health, Education, and Welfare; Public Health Service; Center for Disease Control; National Institute for Occupational Health and Safety: Publication 77-206, Aug 1977.
2. "OSHA Safety and Health Standards, General Industry" (29 *CFR* 1910). Occupational Safety and Health Administration: Jan 1976.
3. "Safety in Academic Chemistry Laboratories," American Chemical Society Committee on Chemical Safety: 1979.
4. "Handbook of Quality Control in Wastewater Laboratories," U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH,: EPA-600/4-79-019, March 1979.
5. "Standard Practice for Sampling Water" (ASTM Annual Book of Standards), American Society for Testing and Materials, Philadelphia, Pennsylvania: 76, 1980.
6. "Methods 330.4 and 330.5 for Total Residual Chlorine," U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH: EPA-600/4-70-020, March 1979.
7. "Narrative for SAS 1019," Pacific Analytical, Inc.: September 1989. Available from the U.S. Environmental Protection Agency Sample Control Center, 300 N. Lee St., Alexandria, VA 22314 (703-557-5040).

Table 1. HPLC Data and Method Acceptance Criteria for Bromoxynil*

<i>Acceptance Criteria</i>	<i>Specification</i>	<i>Note</i>
Minimum Level	100 µg/L	1
Method Detection Limit	20 µg/L	2
Calibration Verification (Section 12.4)	86 to 128 µg/L	3
Initial Precision and Recovery (Section 8.2)		3
Precision [standard deviation; s]	28 µg/L	
Recovery [mean; X]	74 to 130 µg/L	
Ongoing Precision and Recovery (Section 12.5)	72 to 132 µg/L	
Matrix Spike Recovery (Section 8.3.3)	68 to 129%	
Bromoxynil Retention-time	2.62 minutes	4

*(3,5-dibromo-4-hydroxybenzonitrile; CAS 1689-84-5)

Notes:

1. This is a minimum level at which the analytical system shall give recognizable signals and acceptable calibration points.
2. Estimated; 40 CFR Part 136, Appendix B.
3. Test concentration 100 µg/L.
4. Column and conditions: 150 mm long by 4.6 mm ID 300 Angstrom C18 column Column temperature 30°C. Solvent flow rate 0.5 mL/min. Isocratic at 50% methanol in water.

Table 2. Concentration of Bromoxynil Calibration Solutions

<i>Level</i>	<i>Concentration (µg/L)</i>
Low	100
Median	600
High	3,000

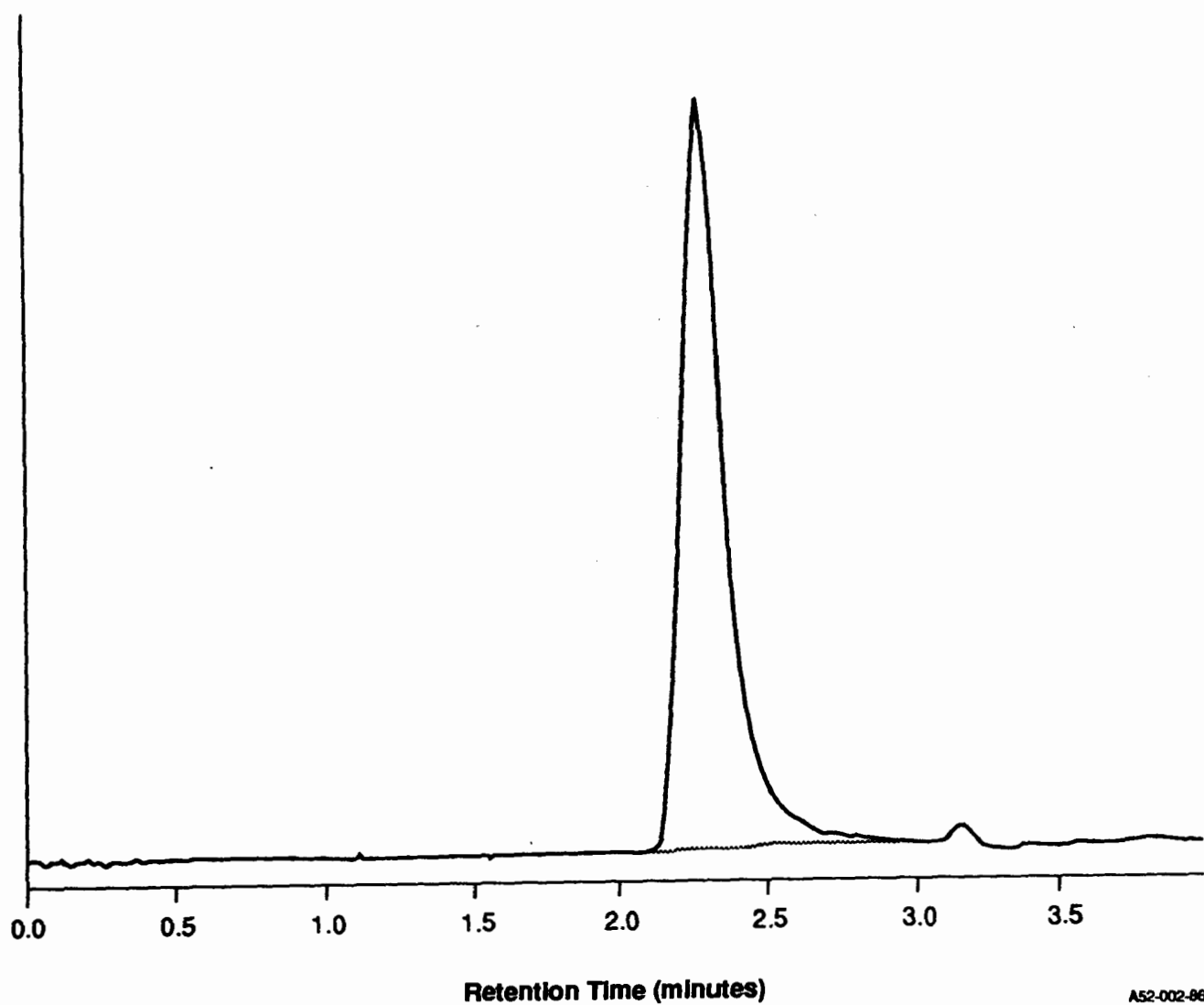


Figure 1. Chromatogram of Bromoxynil

APPENDIX

Methods EV-024 and EV-025

Analytical Procedures for Determining Total Tin and Triorganotin in Wastewater

**Provided by
ATOCHEM North America**

Methods EV-024 and EV-025

Analytical Procedures for Determining Total Tin and Triorganotin in Wastewater

EV-024

1. SAFETY

Wear rubber gloves and glasses with side shields. Follow standard laboratory safety procedures. Any special safety notes are included in the procedure body.

2. PROCEDURE

2.1 Prepare the AA & HGA 500 with the appropriate instrumental operating conditions and keyboard entries:

2.1.1 AA 2380

- 2.1.1.1** Turn power on.
- 2.1.1.2** Open H₂O drain exit valve and H₂O inlet valve (¼ open).
- 2.1.1.3** Install Sn hollow cathode element and regular, uncoated tube.
- 2.1.1.4** Check to see that all control knobs are in extreme counter-clockwise position.
- 2.1.1.5** Using lamp control knob, set lamp/energy to 30 mA.
- 2.1.1.6** Set slit nm to 0.7 ALT and wavelength to 286.3.
- 2.1.1.7** Adjust wavelength and lamp alignment (by turning signal to set up position, turning gain control knob clockwise until about 35 registers on lamp/energy display, then adjusting beat and lamp for maximum gain).
- 2.1.1.8** Position control knobs as follows:

Signal - conc
Mode - PkHT
Recorder - TCI
BG Correction - AA KN

- 2.1.1.9** Set integration time for 7 seconds.
- 2.1.1.10** Turn on inert gas supply (argon).

2.1.2 HGA 500

- 2.1.2.1** Turn power on.
- 2.1.2.2** Program keyboard with following entries.

	Step			
	1	2	3	4
Temp °C	100	700	2500	50
Ramp Time (s)	15	15	0	0
Hold Time (s)	15	15	7	5
Rec.	x	x	x	x
Read			x	
Mini Flow, Argon	x	x		
mL/min	50	50		
Stop flow			x	

- 2.2** Secure appropriate amount (usually 25 to 150 mL depending on source of sample) directly into previously cleaned (see comments) 500-mL screw-top bottle.
- 2.3** Add 10 mL hydrobromic acid to each sample. Shake well then let stand for 10 minutes.
- 2.4** Add 50 mL (or more, also dependent upon source of sample) 0.05% tropolone in toluene (T-T) solution. Shake for 10 minutes. Allow to separate into two layers.
- 2.5** If separation is sufficient (i.e., little emulsion), run 5 μ L on HGA-500 AA 2380 after standardization. (See Step 6); if separation unsatisfactory, add 10 mL more of T-T solution, shake 10 minutes and let stand to separate into two layers.
- 2.6** To standardize:
- 2.6.1** Inject 5 μ L of 0.05 ppm standard inorganic Sn into the graphite furnace with an Eppendorf pipette, equipped with a clean, disposable tip.
- 2.6.1** Engage program cycle and record peak height.
- 2.7** Repeat standard until numbers are reproducible and close in range and average.
- 2.8** Check cleanliness of furnace by engaging program cycle without sample until baseline returns.
- 2.9** Obtain 5 μ L directly from upper phase of each sample from Step 5 using the Eppendorf pipette technique with a clean tip for each sample and run through cycle at least three times and average results.
- 2.10** Run 5 μ L of blank (T-T solution) three times and average.

3. CALCULATIONS

$$\text{Total Tin ppm} = \frac{\text{Real conc.} \times \text{ml T-T solution used}}{\text{sample volume}}$$

$$\text{Conc} - \text{Average ABS normalized to 5 } \mu\text{L} \times \text{factor}$$

Example:

1. Normalize all average to 5 μ L;
e.g., 25 μ L sample average = $30.4 \div 5 = 6.08$
2. Determine factor of standard.

$$\frac{\text{Concentration of standard in ppm}}{\text{Average ABS (normalize to 5 } \mu\text{L)}}$$

Example:

Avg ABS of 0.5 ppm was .115

et) $0.5/.115 = 4.348 = \text{Factor}$

Example:

Avg ABS of sample is 0.146. Dilution factor is 10x. Sample volume is 150 mL.

T-T solution volume is 50 mL. (Use previous example as factor for standard).

$$\frac{(4.38)(50)(0.146)(10x)}{150 \text{ mL}} = 2.116 \text{ ppm Total Tin}$$

4. COMMENTS

- 4.1** Mark original level of sample in sample bottle so that volume may be determined.
- 4.2** Amount of sample extract introduced into the graphite furnace may vary depending upon actual concentration.
- 4.3** Suitable signals for analysis are obtained in the 10X expansion mode.
- 4.4** All sample bottles and glassware used must be scrupulously cleaned with concentrated HCl, by soaking for several hours or overnight, and then rinsed several times with distilled water.
- 4.5** Label bottles with magic marker for sample name, date sample discharged, sample volume, and T-T volume.

EV-025**1. SAFETY**

- 1.1 Wear rubber gloves and glasses with side shields. Follow standard laboratory safety procedures. Any special safety notes are included in the procedure body.

2. PROCEDURE

- 2.1 Prepare AA and total tin samples as specified in EV-024.
- 2.2 Using clean dram vials, extract 2 mL from top phase of total tin sample.
- 2.3 Add equal amount of prepared 3% NaOH solution to sample in vial. Label vial with sample name using indelible marker.
- 2.4 Shake for 10 minutes.
- 2.5 Inject 5 mL on HGA 500, AA 2380 after standardization into graphite furnace with an Eppendorf pipette, equipped with a clean disposable tip.
- 2.6 Engage program cycle and record peak height.
- 2.7 Repeat until numbers are close in range and reproducible, and average.

3. CALCULATIONS

Calculations are the same as in ppm total tin.

$$\frac{(\text{Factor})(T-T \text{ Volume})(\text{Avg ABS})(\text{Dilution})}{\text{Sample Volume}}$$

Example:

0.5 ppm standard with Avg. 0.115 ABS

$$\frac{(0.5)}{0.115} = 4.348 = \text{Factor}$$

ppm Tri Tin: Avg ABS of sample is 0.123. Dilution factor is none, but 25 μL was used. Sample volume is 150 mL and T-T solution volume is 50 mL.

$$\frac{(4.348)(50)(0.123) + 5}{150} = 0.036 \text{ ppm Tri Tin}$$