Designation: D5475 – 93 (Reapproved 2002)

Standard Test Method for Nitrogen- and Phosphorus-Containing Pesticides in Water by Gas Chromatography with a Nitrogen-Phosphorus Detector[1]

This standard is issued under the fixed designation D5475; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This is a gas chromatographic (GC) test method applicable to the determination of certain nitrogen- and phosphorus-containing pesticides in ground water and finished drinking water.[2] The analytes listed in Table 1 have been validated using this test method.

1.2 This test method has been validated on reagent water and finished drinking water by 10 volunteer laboratories. Summary statistics were calculated for mean recovery, overall method precision and bias and single analyst precision using a computer program, Interlaboratory Method Validation Study (IMVS).[2]

1.3 Collaborative study showed the test method to be acceptable for all analytes tested except merphos, which decomposed in the GC injection port.

1.4 This test method is restricted to use by or under the supervision of analysts experienced in the use of GC and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this test method using the procedure described in 12.3.

1.5 Analytes that are not separated chromatographically, that is, analytes which have very similar retention times, cannot be individually identified and measured in the same calibration mixture or water sample unless an alternative technique for identification and quantitation exist (13.5).

1.6 When this test method is used to analyze unfamiliar samples for any or all of the analytes above, analyte identifications should be confirmed by at least one additional qualitative technique.

1.7 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D1129 Terminology Relating to Water

D1192 Guide for Equipment for Sampling Water and Steam in Closed Conduits[4]

D1193 Specification for Reagent Water

D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water

D3370 Practices for Sampling Water from Closed Conduits

D3694 Practices for Preparation of Sample Containers and for Preservation of Organic Constituents

2.2 *U.S. EPA Method:*

EPA Method 507, Revision 2.0 Determination of Nitrogen- and Phosphorus-Containing Pesticides in Water by Gas Chromatography with a Nitrogen-Phosphorus Detector[5]

3. Terminology

3.1 *Definitions*—For definitions of water terms used in this practice, refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *internal standard*—a pure analyte(s) added to a solution in known amount(s) and used to measure the relative responses of other test method analytes and surrogates that are components of the same solution.

3.2.1.1 *Discussion*—The internal standard must be an analyte that is not a sample component.

[1] This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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[2] Edgell, K. W., Jenkins, E. L., Lopez-Avila, V., and Longbottom, J., "Capillary Column Gas Chromatography with Nitrogen-Phosphorus Detection for Determination of Nitrogen- and Phosphorus-Containing Pesticides in Finished Drinking Waters: Collaborative Study," Journal of Association of Official Analytical Chemists, Vol 74, 1991, pp. 295–309.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[4] Withdrawn. The last approved version of this historical standard is referenced on www.astm.org.

[5] Available as part of publication PB91–231480 from National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

TABLE 1 Chemical Service Registry Numbers, Retention Times, and Estimated Method Detection Limits for Forty-Five Pesticides

Analyte	CAS No.	Retention Time, min		Estimated MDL, µg/L
		Primary [A]	Confirmation [A]	
Alachlor	15972-60-8	35.96	34.1	0.38
Ametryn	834-12-8	36.0	34.52	2.0
Atraton	111-44-4	31.26	29.97	0.6
Atrazine	1912-24-9	31.77	31.23	0.13
Bromacil	314-40-9	37.22	40.0	2.5
Butachlor	23184-66-9	41.45	39.0	0.38
Butylate	2008-41-5	22.47	18.47	0.15
Carboxin	5234-68-5	42.77	42.05	0.6
Chlorpropham	101-21-3	29.09	... [B]	0.5
Cycloate	1134-23-2	28.58	29.67	0.25
Diazinon	333-41-5	33.23	... [B]	0.25
Dichlorvox	62-73-7	16.54	15.35	2.5
Diphenamid	957-51-7	38.87	37.97	0.6
Disulfoton	298-04-4	33.42	30.9	0.3
Disulfoton sulfone	2497-06-5	41.31	42.42	3.8
Disulfoton sulfoxide [C]	2497-07-6	19.08	... [B]	0.38
EPTC	563-12-2	20.07	16.57	0.25
Ethoprop	13194-48-4	28.58	26.42	0.19
Fenamiphos	22224-92-6	41.78	41.0	1.0
Fenarimol	60168-88-9	51.32	50.02	0.38
Fluridone	59756-60-4	56.68	59.07	3.8
Hexazinone	51235-04-2	46.58	47.8	0.76
Merphos [D]	150-50-5	42.35	39.28	0.25
Methyl paraoxon	950-35-6	35.58	34.1	2.5
Metolachlor	51218-45-2	37.74	35.7	0.75
Metribuzin	21087-64-9	35.2	34.73	0.15
Mevinphos	7786-34-7	22.51	21.92	5.0
MGK-264 [E]	113-48-4	38.73	36.73	0.5
Molinate	2212-67-1	25.66	22.47	0.15
Napropamide	15299-99-7	41.83	... [B]	0.25
Norflurazon	27314-13-2	45.92	47.58	0.5
Pebulate	1114-71-2	23.41	19.73	0.13
Prometon [C]	1610-18-0	31.58	30.0	0.3
Pronamide [C]	23950-58-5	32.76	32.63	0.76
Propazine	139-40-2	32.01	31.13	0.13
Simazine	122-34-9	31.49	31.32	0.075
Simetryn	1014-70-6	35.72	34.55	0.25
Stirofos	22248-79-9	41.27	39.65	0.76
Tebuthiuron	34014-18-1	25.15	42.77	1.3
Terbacil	5902-51-2	33.79	... [B]	4.5
Terbufos [C]	13071-79-9	32.57	... [B]	0.5
Terbutryn	886-50-0	36.80	34.8	0.25
Triademefon	43121-43-3	38.12	37.0	0.65
Tricyclazole	41814-78-2	42.25	44.33	1.0
Vernolate	1929-77-7	22.94	19.25	0.13

[A] See 7.13.2 and 7.13.3 for column description and operating conditions.
[B] Data not available.
[C] Compound shows instability in aqueous solutions.
[D] Merphos is converted to S,S,S-tributylphosphorotrithioate (DEF) in the hot GC injection port; DEF is actually detected using the mothod conditions.
[E] MGK-264 gives 2 peaks; peak identified in this table was used for quantification.

3.2.2 *surrogate analyte*—a pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction and is measured with the same procedures used to measure other sample components.

3.2.2.1 *Discussion*—The purpose of a surrogate analyte is to monitor test method performance with each sample.

3.2.3 *laboratory duplicates (LD1 and LD2)*—two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures.

3.2.3.1 *Discussion*—Analyses of LD1 and LD2 give a measure of the precision with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.2.4 *field duplicates (FD1 and FD2)*—two separate samples collected at the same time and placed under identical circumstances and treated exactly the same throughout field and laboratory procedures.

3.2.4.1 *Discussion*—Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

3.2.5 *laboratory reagent blank (LRB)*—an aliquot of water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples.

3.2.5.1 *Discussion*—The LRB is used to determine if test method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.2.6 *field reagent blank (FRB)*—water transferred in a bottle from the laboratory and poured at the field site into a

sample container in the field and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation, and all analytical procedures.

3.2.6.1 *Discussion*—The purpose of the FRB is to determine if test method analytes or other interferences are present in the field environment.

3.2.7 *laboratory performance check solution (LPC)*—a solution of method analytes, surrogate compounds, and internal standards used to evaluate the performance of the instrument system with respect to a defined set of test method criteria.

3.2.8 *laboratory fortified blank (LFB)*—an aliquot of water to which known quantities of the test method analytes are added in the laboratory.

3.2.8.1 *Discussion*—The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements at the required method detection limit.

3.2.9 *laboratory fortified sample matrix (LFM)*—an aliquot of an environmental sample to which known quantities of the test method analytes are added in the laboratory.

3.2.9.1 *Discussion*—The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.2.10 *calibration standard (CAL)*—a solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes.

3.2.10.1 *Discussion*—The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.2.11 *quality control sample (QCS)*—a sample matrix containing test method analytes or a solution of test method analytes in a water-miscible solvent which is used to fortify water or environmental samples.

3.2.11.1 *Discussion*—The QCS is obtained from a source external to the laboratory, and is used to check laboratory performance with externally prepared test materials.

4. Summary of Test Method

4.1 A measured volume of sample of approximately 1 L is extracted with methylene chloride by shaking in a separatory funnel or mechanical tumbling in a bottle. The methylene chloride extract is isolated, water is removed, and concentrated to a volume of 5 mL during a solvent exchange to methyl tert-butyl ether (MTBE). Chromatographic conditions are described that permit the separation and measurement of the analytes in the extract by capillary column GC with a nitrogen-phosphorus detector (NPD).

4.2 This test method is based largely on USEPA Method 507.

5. Significance and Use

5.1 Nitrogen- and phosphorus-containing compounds are widely used in agriculture as pre-emergent agents to increase crop yields. Runoff from farmlands into lakes and streams as well as accidental discharge from irrigation systems into groundwater introduces these compounds into the environment. This discharge from agricultural areas along with possible health implications dictates a need to monitor the presence of these compounds.

6. Interferences

6.1 Test 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 12.2.

6.1.1 Glassware must be scrupulously cleaned as described in Practice D3694. 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 1 h. Do not heat volumetric ware. Thermally stable materials might not be eliminated by this treatment. 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.

6.1.2 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. (**Warning—** When a solvent is purified, stabilizers added by the manufacturer may be removed thus potentially making the solvent hazardous. Also, when a solvent is purified, preservatives added by the manufacturer are removed thus potentially reducing the shelf life.)

6.2 Interfering contamination may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. Between-sample rinsing of the sample syringe and associated equipment with MTBE can minimize sample cross contamination. After analysis of a sample containing high concentrations of analytes, one or more injections of MTBE should be made to ensure that accurate values are obtained for the next sample.

6.3 Matrix interferences may be caused by contaminants that are coextracted from the sample. Also, note that all the analytes listed in the scope and application section are not resolved from each other on any one column, that is, one analyte of interest may be an interferent for another analyte of interest. The extent of matrix interferences will vary considerably from source to source, depending upon the water sampled. Further processing of sample extracts may be necessary. Positive identifications should be confirmed (see 13.5).

6.4 It is important that samples and working standards be contained in the same solvent. The solvent for working standards must be the same as the final solvent used in sample preparation. If this is not the case, chromatographic comparability of standards to sample may be affected.

7. Apparatus and Equipment

7.1 *Sample Bottles*—Borosilicate, 1-L volume with graduations, fitted with screw caps lined with TFE-fluorocarbon. Protect samples from light. The container must be washed and dried as described in 6.1.1 before use to minimize contamination. Cap liners are cut to fit from sheets and extracted with methanol overnight prior to use.

7.2 *Separatory Funnel*, 2000-mL, with TFE-fluorocarbon stopcock, ground glass, or TFE-fluorocarbon stopper.

7.3 *Tumbler Bottle*, 1.7-L, with TFE-fluorocarbon-lined screw cap. Cap liners are cut to fit from sheets and extracted with methanol overnight prior to use.

7.4 *Concentrator Tube*, Kuderna-Danish (K-D), 10 or 25-mL, graduated. Calibration must be checked at the volumes employed in the test. Ground glass stoppers are used to prevent evaporation of extracts.

7.5 *Evaporation Flask*, K-D, 500-mL. Attach to concentrator tube with springs.

7.6 *Snyder Column*, K-D, three-ball macro.

7.7 *Snyder Column*, K-D, two-ball micro.

7.8 *Vials*, glass, 5 to 10-mL capacity with TFE-fluorocarbon-lined screw cap.

7.9 *Separatory Funnel Shaker*, (optional), capable of holding 2-L separatory funnels and shaking them with rocking motion to achieve thorough mixing of separatory funnel contents.

7.10 *Tumbler*, capable of holding tumbler bottles and tumbling them end-over-end at 30 turns/min.

7.11 *Boiling Stones*, carborundum, No. 12 granules. Heat at 400°C for 30 min prior to use. Cool and store in desiccator.

7.12 *Water Bath*, heated, capable of temperature control (±2°C). The bath should be used in a well-ventilated hood.

7.13 *Gas Chromatograph*, analytical system complete with temperature programmable GC suitable for use with capillary columns and all required accessories including syringes, analytical columns, gases, detector, and stripchart recorder. A data system is recommended for measuring peak areas. Table 2 lists retention times observed for most of the method analytes using the columns and analytical conditions described below.

7.13.1 *Column 1 (Primary Column)*, 30 m long by 0.25-mm inside diameter (ID) by 0.25-µm film thickness, 95 % cross-bonded dimethyl-5 % diphenyl polysiloxane fused silica column. Helium carrier gas flow is established at 30- cm/s linear velocity and oven temperature is programmed from 60 to 300°C at 4°C/min. Data presented in this test method were obtained using this column. The injection volume was 2 µL in splitless mode with a 45-s delay. The injector temperature was 250°C and the detector temperature was 300°C. Alternative columns may be used in accordance with the provisions described in 12.4

7.13.2 *Column (Confirmation Column)*, 30 m long by 0.25-mm ID by 0.25-µm film thickness cross-bonded 14 % cyanopropylphenyl-86 % methyl polysiloxane fused silica column. Helium carrier gas flow is established at 30-cm/s linear velocity and oven temperature is programmed from 60 to 300°C at 4°C/min.

TABLE 2 Acceptance Limits (as Percent of Mean Recovery) for Analysis of Laboratory Quality Control Sample

Analyte	Concentration Level [A]	Mean Recovery [B]	Overall Std Dev	Acceptance Limits [C]
Alachlor	5.00	4.63	0.78	49.5–150
Ametryn	2.00	1.88	0.28	55.3–145
Atraton	5.00	4.72	0.87	44.9–155
Atrazine	2.00	1.86	0.27	55.9–144
Bromacil	10.0	9.55	1.60	49.7–151
Butachlor	10.0	9.41	1.46	53.2–147
Butylate	5.00	3.81	0.89	29.9–170
Carboxin	10.0	9.37	2.00	35.0–164
Chlorpropham	10.0	9.76	1.78	45.3–155
Cycloate	5.00	4.29	0.84	41.2–159
Diazinon	2.00	1.78	0.34	42.7–157
Dichlorvos	5.00	4.84	0.76	53.1–147
Diphenamid	5.00	4.72	0.75	52.3–148
Disulfoton	2.00	1.73	0.33	42.9–157
Disulfoton sulfone	10.0	10.4	2.26	34.8–165
Disulfoton sulfoxide	10.0	9.51	1.86	41.3–159
EPTC	2.00	1.72	0.32	44.2–156
Ethoprop	2.00	1.84	0.37	39.7–160
Fenamiphos	20.0	18.0	3.50	41.7–158
Fenarimol	5.00	4.86	1.09	32.7–167
Fluridone	10.0	10.2	2.47	27.4–172
Hexazinone	5.00	4.96	1.06	36.1–164
Merphos	... [D]	...	...	...
Methyl paraoxon	10.0	10.0	1.73	48.1–152
Metolachlor	10.0	9.26	1.22	60.5–139
Metribuzin	2.00	1.94	0.38	41.2–159
Mevinphos	10.0	9.70	1.28	60.4–139
MGK-264	10.0	9.23	1.66	46.0–154
Molinate	2.00	1.88	0.34	45.7–154
Napropamide	5.00	4.37	0.69	52.6–147
Norflurazon	5.00	4.80	1.19	25.4–174
Pebulate	2.00	1.78	0.39	34.3–166
Prometon	2.00	1.92	0.33	48.4–152
Pronamide	10.0	9.72	1.47	54.5–145
Propazine	2.00	1.86	0.32	48.4–152
Simazine	2.00	1.90	0.28	55.8–144
Simetryn	2.00	1.93	0.34	47.2–153
Stirofos	20.0	18.6	3.87	37.5–162
Tebuthiuron	10.0	9.72	1.79	44.6–155
Terbacil	50.0	49.8	9.35	43.8–156
Terbufos	10.0	8.30	1.50	45.8–154
Terbutryn	2.00	1.90	0.24	52.1–138
Triademefon	2.00	1.96	0.40	38.8–161
Tricyclazole	20.0	18.1	2.95	51.1–149
Vernolate	2.00	1.62	0.41	24.1–176

[A] Concentration level is 10 to15 times the estimated MDL, µg/L.
[B] Calculated from the regression equations for mean recovery and overall standard deviation obtained in collaborative study of the method for reagent water matrix.
[C] Acceptance limits are defined as the mean recovery ±3 standard deviations.
[D] Merphos breakdown to DEF was incomplete and resulted in poor recovery and precision.

7.13.3 *Detector, Nitrogen-Phosphorus (NPD)*—An NPD was used to generate the validation data presented in this test method. Alternative detectors may be used in accordance with the provisions described in 12.4

8. Reagents

8.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of

sufficiently high purity to permit its use without lessening the accuracy of the determination.[6] For trace analysis using organic solvents for liquid-liquid extraction, solvents specified as distilled-in-glass, nano-grade, or pesticide-grade frequently have lower levels of interfering impurities. In all cases, sufficient reagent blanks must be processed with the samples to ensure that all compounds of interest are not present as blanks due to reagents or glassware.

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification D1193, Type II, and shown to contain no interfering contaminants at concentrations sufficient to interfere with the analysis.

8.3 *Acetone*, Distilled-in-glass quality or equivalent.

8.4 *Mercuric Chloride*, for use as a bactericide. If any other bactericide can be shown to work as well as mercuric chloride, it may be used.

8.5 *Methylene Chloride*, Distilled-in-glass quality or equivalent.

8.6 *Methyl Tert-Butyl Ether (MTBE)*, Distilled-in-glass quality or equivalent.

8.7 *Phosphate Buffer, pH 7*—Prepare by mixing 29.6 mL 0.1 N HCl and 50 mL 0.1 M dipotassium phosphate.

8.8 *Sodium Chloride (NaCl), crystal*—Heat in a shallow tray at 450°C for a minimum of 4 h to remove interfering organic substances.

8.9 *Sodium Sulfate*, granular, anhydrous. Heat in a shallow tray at 450°C for a minimum of 4 h to remove interfering organic substances.

8.10 *Sodium Thiosulfate*, granular, anhydrous.

8.11 *1,3-dimethyl-2-nitrobenzene*, 98 % purity, for use as surrogate standard.

8.12 *Standard Solution, Stock* (1.00 µg/µL). Stock standard solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedure:

8.12.1 Prepare stock standard solutions by accurately weighing 0.0100 g of pure material. Dissolve the material in MTBE and dilute to volume in a 10-mL volumetric flask. The stock solution for simazine should be prepared in methanol. Larger volumes may be prepared 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.

8.12.2 Transfer the stock standard solutions into TFE-fluorocarbon sealed screw-cap amber vials. Store at room temperature and protect from light.

8.12.3 Stock standard solutions should be replaced after 2 months or sooner if comparison with laboratory fortified blanks or QC samples indicate a problem.

8.13 *Internal Standard Solution*—Prepare an internal standard solution by accurately weighing 0.0500 g of pure triphenylphosphate (TPP). Dissolve the TPP in MTBE and dilute to volume in a 100-mL volumetric flask. Transfer the internal standard solution to a TFE-fluorocarbon sealed screw-cap bottle and store at room temperature. Addition of 50 µL of the internal standard solution to 5 mL of sample extract results in a final TPP concentration of 5.0µ g/mL. Solution should be replaced when ongoing quality control (QC) (Section 12) indicates a problem. Note that TPP has been shown to be an effective internal standard for the test method analytes, [2] but other compounds may be used if the QC requirements in Section 12 are met.

8.14 *Surrogate Standard Solution*—Prepare a surrogate standard solution by accurately weighing 0.0250 g of pure 1,3-dimethyl-2-nitrobenzene. Dissolve the 1,3-dimethyl-2-nitrobenzene in MTBE and dilute to volume in a 100-mL volumetric flask. Transfer the surrogate standard solution to a TFE-fluorocarbon sealed screw-cap bottle and store at room temperature. Addition of 50 µL of the surrogate standard solution to a 1-L sample prior to extraction results in a surrogate standard concentration in the sample of 5 µg/L and, assuming quantitative recovery of 1,3-dimethyl-2-nitrobenzene, a surrogate standard concentration in the final extract of 12.5 µg/mL. Solution should be replaced when ongoing QC (Section 12) indicates a problem. The 1,3-dimethyl-2-nitrobenzene has been shown to be an effective surrogate standard for the test method analytes, [2] but other compounds may be used if the QC requirements in Section 12 are met.

8.15 *Laboratory Performance Check Solution*—Prepare the laboratory performance check solution by adding 5 µL of the vernolate stock solution, 0.5 mL of the bromacil stock solution, 30 µL of the prometon stock solution, 15 µL of the atrazine stock solution, 1.0 mL of the surrogate solution, and 500 µL of the internal standard solution to a 100-mL volumetric flask. Dilute to volume with MTBE and thoroughly mix the solution. Transfer to a TFE-fluorocarbon-sealed screw-cap bottle and store at room temperature. Solution should be replaced when ongoing QC (Section 12) indicates a problem.

9. Sample Collection, Preservation, and Handling

9.1 Collect the samples in accordance with Practices D3370, D3694, and Specification D1192.

9.2 *Instructions Specific to This Test Method*—Grab samples must be collected in glass containers. Conventional sampling Practices D3370 should be followed; however, the bottle must not be prerinsed with sample before collection.

10. Sample Preservation and Storage

10.1 Add mercuric chloride to the sample bottle in amounts to produce a concentration of 10 mg/L. Add 1 mL of a solution containing 10 mg/mL of mercuric chloride in water to the sample bottle at the sampling site or in the laboratory before shipping to the sampling site. (**Warning—**A major disadvantage of mercuric chloride is that it is a highly toxic chemical; mercuric chloride must be handled with caution, and samples containing mercuric chloride must be disposed of properly.)

[6] *Reagent Chemicals, American Chemical Society Specifications,* American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals,* BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary,* U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

10.2 If residual chlorine is present, add 80 mg of sodium thiosulfate per litre of sample to the sample bottle prior to collecting the sample.

10.3 After the sample is collected in a bottle containing preservative(s), seal the bottle and shake vigorously for 1 min.

10.4 Ice or refrigerate the samples at 4°C away from light from the time of collection until extraction. Preservation study results indicated that most test method analytes present in samples were stable for 14 days when stored under these conditions. [2] The analytes disulfoton sulfoxide, diazinon, pronamide, and terbufos exhibited significant aqueous instability, and samples to be analyzed for these compounds must be extracted immediately. The analytes carboxin, EPTC, fluridone, metolachlor, napropamide, tebuthiuron, and terbacil exhibited recoveries of less than 60 % after 14 days. Analyte stability may be affected by the matrix; therefore, the analyst should verify that the preservation technique is applicable to the samples under study.

10.5 *Extract Storage*—Store extracts at 4°C away from the light. Preservation study results indicate that most analytes are stable for 28 days; however, a 14 day maximum extract storage time is recommended. The analyst should verify appropriate extract holding times applicable to the samples under study.

11. Calibration

11.1 Establish GC operating parameters equivalent to those indicated in 7.13. Consult the manufacturer's operation manual for the GC system, if necessary. Calibrate the GC system using either the internal standard technique (11.2) or the external standard technique (11.3). Be aware that NPDs may exhibit instability (that is, fail to hold calibration curves over time). The analyst may, when analyzing samples for target analytes that are rarely found, prefer to analyze on a daily basis a low level (for example, 5 to 10 times detection limit), sample (containing all analytes of interest) and require some minimum sensitivity (for example, ½ full-scale deflection) to show that if the analyte were present it would be detected. The analyst may then quantitate using single-point calibration (11.2.5 or 11.3.4).

11.1.1 Calibration standard solutions must be prepared such that no unresolved analysts are mixed together.

11.2 *Internal Standard Calibration Procedure*—To use this approach, the analyst must select one or more internal standards compatible 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. Triphenylphosphate has been identified as a suitable internal standard.

11.2.1 Prepare calibration standards at a minimum of three (recommend five) concentration levels for each analyte of interest by adding volumes of one or more stock standards to a volumetric flask. If Merphos is to be determined, calibrate with DEF (S,S,S-tributylphosphorus-trithioate). To each calibration standard, add a known constant amount of one or more of the internal standards and dilute to volume with MTBE. The standards should bracket the analyte concentrations expected in the sample extracts, or should define the working range of the detector.

11.2.2 Analyze each calibration standard according to the procedure (see 13.4). Tabulate response (peak height or area) against concentration for each compound and internal standard. Calculate the response factor (RF) for each analyte and surrogate using Eq 1.

$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)} \quad (1)$$

where:

A_s = response for the analyte to be measured,
A_{is} = response for the internal standard,
C_{is} = concentration of the internal standard, µg/L, and
C_s = concentration of the analyte to be measured, µg/L.

11.2.3 If the RF value over the working range is constant (20 % RSD or less) use the average RF for calculations. Alternatively, use the results to plot a calibration curve of response ratios (A_s/A_{is}) versus C_s.

11.2.4 Verify the working calibration curve or RF on each working shift by the measurement of one or more calibration standards. If the response for any analyte of interest varies from the predicted response by more than ± 20 %, repeat the test using a fresh calibration standard. If the repetition also fails, generate a new calibration curve for that analyte using freshly prepared standards.

11.2.5 Single-point calibration is a viable alternative to a calibration curve. Prepare single point standards from the secondary dilution standards in MTBE. Prepare the single point standards at a concentration that produces a response that deviates from the sample extract response by no more than 20 %.

11.2.6 Verify calibration standards periodically, at least quarterly, by analyzing a standard prepared from reference material obtained from an independent source. Results from these analyses must be within the limits used to routinely check calibration.

11.3 *External Standard Calibration Procedure*:

11.3.1 Prepare calibration standards at a minimum of three (recommend five) concentration levels for each analyte of interest and surrogate compound by adding volumes of one or more stock standards and 250 µL methanol to a volumetric flask. If Merphos is to be determined, calibrate with DEF (S,S,S-tributylphosphorus-trithioate). Dilute to volume with MTBE. The standards should bracket the analyte concentrations expected in the sample extracts, or should define the working range of the detector.

11.3.2 Starting with the standard of lowest concentration, analyze each calibration standard in accordance with 13.4 and tabulate response (peak height or area) versus the concentration curve for each compound. Alternatively, if the ratio of response to concentration (calibration factor) is a constant over the working range (20 % RSD or less), assume linearity through the origin and use the average ratio or calibration factor in place of a calibration curve.

11.3.3 Verify the working calibration curve or calibration factor on each working day by the measurement of a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day. These check standards should be at two different concentration levels to verify the calibration curve. For extended periods of analysis (greater than 8 h), it is strongly recommended that check standards be interspersed with samples at regular intervals during the course