SUPPLEMENT TO THE
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6710 Tributyl Tin ..... François Rodigari

Carbamate Pesticides (6610) has been revised to use citrate instead of monochloroacetic acid (MCAA) as a preservative. MCAA is considered a hazardous substance that requires special labeling, whereas samples preserved with citrate do not require special labeling.

Tributyl Tin (6710) is a new section that contains two methods: gas chromatography/mass spectrometry and gas chromatography/flame photometry.

6610 CARBAMATE PESTICIDES*

6610 A. Introduction

1. Sources and Significance

Carbamates are used as insecticides, nematicides, and aracides to control pests on agricultural crops, as well as to control lawn and garden insects. Their toxicity comes from their ability to act as cholinesterase inhibitors. Residues of several carbamates have been found in groundwater in a number of states.[1,2] Two of the target compounds in this method, carbofuran and oxamyl, are regulated by the U.S. Environmental Protection Agency, with aldicarb and its metabolites, aldicarb sulfoxide and aldicarb sulfone, under consideration for regulation.[3]

2. Selection of Method

High-performance liquid chromatography (HPLC) is the method of choice for analysis for carbamates, many of which are thermally labile. This HPLC analysis method is an updated version of a previous method developed using direct injection, post-column derivatization, and fluorescence detection to yield sensitivity and selectivity while keeping sample preparation to a minimum.[4–6] This method is suitable for the analysis of the following carbamate compounds and metabolites in drinking waters: aldicarb sulfoxide, aldicarb sulfone, oxamyl, methomyl, 3-hydroxycarbofuran, aldicarb, propoxur, carbofuran, carbaryl, 1-naphthol, and methiocarb.

3. References

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3. U.S. Environmental Protection Agency. 2000. Drinking Water Standards and Health Advisories. EPA 822-B-00-001, U.S. Environmental Protection Agency, Off. Water, Washington, D.C.
4. Moye, H.A., S.J. Scherrer & P.A. St. John. 1977. Dynamic labeling of pesticides for high performance liquid chromatography: Detection of *N*-methylcarbamates and *o*-phthalaldehyde. *Anal. Lett.* 10:1049.
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6. U.S. Environmental Protection Agency. 2001. Method 531.2—Measurement of *N*-methylcarbamoyloximes and *N*-methylcarbamates in water by direct aqueous injection HPLC with postcolumn derivatization. EPA 815-B-01-002, U.S. Environmental Protection Agency, Off. Ground Water and Drinking Water, Cincinnati, Ohio.

* Approved by Standard Methods Committee, 2004.

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6610 B. High-Performance Liquid Chromatographic Method

1. General Discussion

a. Principle: After the addition of a surrogate compound and filtration, water samples are injected directly onto a HPLC and separated by use of a gradient and a C_{18} column. The carbamates analyzable by this method (Table 6610:I), which are generally classified as phenyl and oxime carbamates, all have an *N*-methyl group in common. After chromatographic separation, these compounds are hydrolyzed with 0.05*N* NaOH at 80 to 95°C, yielding a methyl amine which is then reacted with *o*-phthalaldehyde (OPA) and 2-mercaptoethanol (or *N,N*-dimethyl-2-mercaptoethylamine) to form a highly fluorescent isoindole that is detected instrumentally. The external standard technique is used for quantitation.

b. Interferences: Method interferences may be caused by contaminants, especially primary amines and ammonia, in solvents, reagents (including reagent water), sample bottles and caps, and other sample-processing hardware, that lead to discrete artifacts and/or elevated baselines in the chromatograms. The samples or analytical system may be contaminated from being

Table 6610:I. Detection Levels in Reagent Water

Analyte	Fortification Level µg/L	Detection Level* µg/L	Signal-to-Noise Ratio
Aldicarb sulfoxide	0.20	0.059	8:1
Aldicarb sulfone	0.10	0.057	3:1
Oxamyl	0.20	0.065	10:1
Methomyl	0.20	0.050	10:1
3-Hydroxycarbofuran	0.20	0.029	18:1
Aldicarb	0.20	0.026	9:1
Propoxur	0.20	0.037	6:1
Carbofuran	0.20	0.043	9:1
Carbaryl	0.20	0.045	13:1
1-Naphthol	0.20	0.063	10:1
Methiocarb	0.20	0.061	11:1
BDMC (SUR)	2.00	N/A	N/A

* Detection levels were determined by analyzing 7 replicates over 3 d under the conditions outlined in Table 6610:IV with a 1000-µL injection.
N/A = not applicable

TABLE 6610:II. SINGLE-ANALYST PRECISION AND ACCURACY OF COMPOUND DETECTION IN VARIOUS WATERS AT LOW (0.20 μG/L) AND HIGH (10 μG/L) FORTIFICATION LEVELS*

	Reagent Water				Drinking Water, Surface Water				Drinking Water, Groundwater			
	0.20 μg/L		10.0 μg/L		0.20 μg/L		10.0 μg/L		0.20 μg/L		10.0 μg/L	
Compound	MR%	%RSD	MR%	%RSD	MR%	%RSD	MR%	%RSD	MR%	%RSD	MR%	%RSD
Aldicarb sulfoxide	112	6.2	106	1.8	113	7.0	104	2.8	111	7.3	106	1.1
Aldicarb sulfone	92	9.5	106	2.6	104	5.5	106	1.4	98	9.2	106	0.9
Oxamyl	101	8.6	106	2.2	107	6.4	104	2.2	99	8.4	105	1.2
Methomyl	101	6.5	106	2.9	110	9.8	104	1.6	99	10.2	105	1.4
3-Hydroxycarbofuran	105	6.8	108	1.2	128	3.9	107	1.1	107	3.0	108	0.4
Aldicarb	95	7.4	106	1.3	123	2.7	105	1.5	100	6.3	105	0.6
Propoxur	109	5.9	109	2.0	128	6.0	106	2.1	112	6.1	107	0.8
Carbofuran	112	6.7	110	2.2	140	5.6	105	2.5	112	4.1	107	1.6
Carbaryl	112	7.0	107	2.1	112	9.7	106	0.9	119	5.1	108	1.3
1-Naphthol	113	12.6	108	3.1	113	12.1	101	1.3	109	8.2	109	1.2
Methiocarb	105	5.9	107	1.5	104	13.3	107	1.1	105	3.9	107	1.0
BDMC (SUR)†	108	4.3	101	2.3	108	2.1	96	3.9	109	2.0	97	4.3

* MR% = mean recovery expressed as % recovery; %RSD = percent relative standard deviation
† Surrogate concentration in all samples was 2.0 μg/L; all data from $n=7$ replicates using a 1000-μL injection volume.

handled with bare fingers. Routinely demonstrate that all items used in analysis are free from interferences under the conditions of the analysis by analyzing laboratory reagent blanks. Do not subtract blank values from sample results. Clean all glassware meticulously: wash glassware with detergent and tap water, rinse with tap water, and rinse again with reagent water. A final rinse with solvents may be needed. In place of a solvent rinse, nonvolumetric glassware can be heated in a muffle furnace at 400°C for 2 h. Do not heat volumetric glassware above 120°C.

Samples that are not properly preserved (¶ 2 below) may yield poor target analyte recovery due to degradation caused by chlorine residual and base-catalyzed hydrolysis at neutral and high pH.

c. Detection levels: Detection levels are compound, instrument, and matrix dependent. The detection level is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero.[1] Experimentally determined detection levels for the target analytes are provided in Table 6610:I. The detection level differs from, and is lower than, the minimum reporting level (MRL). The concentration range for target analytes in this method was evaluated between 0.2 μg/L and 10 μg/L. Precision and bias data are presented in Table 6610:II.

d. Safety: The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; treat each chemical compound as a potential health hazard, and minimize exposure. Handle pure standard materials and stock standards of these compounds with suitable protection to skin and eyes. Take care not to breathe the vapors or ingest the materials. Maintain a current-awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. Make a reference file of MSDSs available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available.[2–4]

2. Sampling and Storage

a. Sample bottle preparation: Use amber glass bottles fitted with polytetrafluoroethylene (PTFE)-lined screw caps. Before shipping sample bottles to the field, add preservatives, as dry solids, to each bottle. To adjust sample pH to ~3.8 to prevent hydrolysis of oxamyl, 3-hydroxycarbofuran, carbaryl, and methiocarb and to guard against biodegradation, add a sufficient amount of potassium dihydrogen citrate ($C_6H_7KO_7$) to yield a concentration in the sample of 9.2 to 9.5 g/L. To eliminate the residual free chlorine in the samples, which rapidly degrades aldicarb and methiocarb, add sodium thiosulfate ($Na_2S_2O_3$) to yield a sample concentration in the range of 80 to 320 mg/L.

b. Sample collection: Collect grab samples in accordance with conventional sampling practices. Do not prerinse sample bottles with sample before collection, because prerinsing will wash out the preservatives added to the bottles before shipment.

When sampling from a cold water tap, remove aerator so that no air bubbles will be trapped in the sample. Open tap and let system flush until water temperature has stabilized (usually about 3 to 5 min). Collect samples from the flowing system.

Fill sample bottles, taking care not to flush out sample preservation reagents. Samples do not need to be collected headspace-free. After collecting sample, cap carefully to avoid spillage and agitate by hand for 1 min. Keep samples sealed from collection time until analysis.

c. Sample storage and holding time: Keep all samples iced during shipment and do not let temperature exceed 10°C during the first 48 h after collection. Assuming that samples are in transit for 48 h or less, confirm that they are at or below 10°C when they are received at the laboratory. In the laboratory, store samples at or below 6°C and protect from light until analysis. Do not freeze samples. Results of the sample storage stability study of all method analytes indicated that all compounds are stable for 28 d in water samples that are collected, dechlorinated, preserved, shipped, and stored as described above.[5] Analyze samples within 28 d.

3. Apparatus

a. High-performance liquid chromatograph (HPLC): A system capable of reproducibly injecting up to 1000-μL portions,

and performing ternary linear gradients at a constant flow rate of approximately 1.5 mL/min. A column heater is desirable. Data included in this method were obtained with a heater set at 30°C.

1) *Analytical column:* Any column that provides adequate resolution, peak shape, capacity, accuracy, and precision (¶ 6 below) may be used. For development of this method, an HPLC "carbamate" column, 3.9 × 150 mm, packed with 4-μm d_p C_{18} solid-phase particles* was used.

2) *Postcolumn reaction system* capable of mixing reagents into the mobile phase. Use a reactor constructed of polyether-etherketone (PEEK) or PTFE tubing and equipped with two pumps capable of delivering up to 0.5 mL/min of each reagent; mixing tees; and two reaction coils. Various postcolumn system manufacturers recommend different reaction-coil temperatures for the carbamate hydrolysis reaction; therefore, the first reaction coil temperature may range from 80 to 95°C. Analyte signal can increase with temperature over this temperature range; however, baseline noise can also increase with increasing temperatures. The second reaction takes place at ambient temperature.

3) *Detector:* Use a fluorescence detector capable of excitation at approximately 340 nm and detection of emission energy at approximately 465 nm. Specific optimum excitation and emission wavelengths may vary slightly for each system.

4) *Data system:* Preferably use a computerized data system for data acquisition and processing.†

b. Vials: Screw-cap or crimp-top glass autosampler vials with PTFE-faced septa, amber or clear.

c. Volumetric flasks, Class A, various sizes, used for preparation of standards and samples.

d. Microsyringes, various sizes.

e. Disposable syringes, 5- to 30-mL size, used to filter sample extracts before analysis.‡

f. Filters, disposable, used to filter samples before analysis.§

g. Analytical balance, capable of weighing accurately to 0.0001 g.

4. Reagents

Use only reagent-grade or better chemicals and HPLC-grade solvents. Unless otherwise indicated, ensure that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.

a. Reagent water: Use purified water that does not contain measurable quantities of any target analytes or interfering compounds greater than ⅓ the MRL for each compound of interest.

b. Buffered reagent water: To reagent water, add sample preservation reagents in the same concentrations present in the samples. To prepare 1 L buffered reagent water, add a sufficient amount of potassium dihydrogen citrate to yield a concentration of 9.2 to 9.5 g/L and sodium thiosulfate to yield a concentration in the range of 80 to 320 mg/L to a graduated bottle or volumetric flask. Fill to 1-L mark with reagent water.

c. Acetonitrile, CH_3CN, high-purity, demonstrated to be free of analytes and interferences (HPLC grade or better).

d. Methanol, CH_3OH, high-purity, demonstrated to be free of analytes and interferences (HPLC grade or better).

e. Hydrolysis solution (postcolumn reagent 1): Make sodium hydroxide, NaOH, 0.05*N*, by diluting 4 mL 50% (w/w) sodium hydroxide solution to 1 L with reagent water. Because hydrolysis solution concentration can dramatically affect analyte response, use extra care in preparing. Filter and degas (with helium or other appropriate gas) just before use.

f. Sodium borate solution, 0.05*N:* Dissolve 19.1 g sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$) in a 1-L volumetric flask. Bring volume up to 1.0 L with reagent water. The sodium borate will dissolve in less than 2 h if a stir bar is used. Filter and degas.

g. o-Phthalaldehyde (OPA): Dissolve 100 ± 10 mg of OPA in 5 to 10 mL methanol.

h. OPA derivatization solution (postcolumn reagent 2): Prepare by *either* 1) or 2) below. Both 2-mercaptoethanol and *N, N*-dimethyl-2-mercaptoethylamine hydrochloride react with OPA and the target methylamine to form the isoindole detected by the fluorescence detector. Both reagents have characteristic strong odors. Prepare solutions in a fume hood. This reagent, as prepared by either method, is stable for at least 36 h. However, individual laboratory conditions vary and daily preparation of this solution may be necessary.

1) Preparation with 2-mercaptoethanol—Add dissolved OPA (¶ *g* above) to 1 L filtered and degassed sodium borate solution (¶ *f*). Add 1.0 mL 2-mercaptoethanol and mix.

2) Preparation with *N,N*-dimethyl-2-mercaptoethylamine hydrochloride—Dissolve 2.0 ± 0.2 g *N,N*-dimethyl-2-mercaptoethylamine hydrochloride in approximately 10 mL sodium borate solution (¶ *f* above). Add dissolved OPA (¶ *g*) to 1 L filtered and degassed sodium borate solution. Add dissolved *N,N*-dimethyl-2-mercaptoethylamine-hydrochloride to the sodium borate solution and mix.

i. Calibration standards: Prepare standard solutions either from certified, commercially available solutions or from neat compounds. Compounds used to prepare solutions must be ≥ 96% pure; their weights may be used without correction for purity to calculate the concentration of the stock standard. Solution concentrations mentioned in this section were used to develop this method and are included as an example, not a requirement. Generally, prepare standards for sample fortification in the smallest volume that can be measured accurately, to minimize the addition of organic solvent to aqueous samples. NOTE: Although stability times for standard solutions are suggested below, use standard QC practices to determine when standard solutions need to be replaced.

1) *Surrogate analyte (SUR) standard solution, 4-bromo-3,5-dimethylphenyl* n*-methylcarbamate (BDMC):*

a) *SUR stock standard:* If preparing from neat material, accurately weigh approximately 25 to 35 mg neat SUR to nearest 0.1 mg into a tared, 5-mL volumetric flask. Dilute to the mark with methanol. Stock solutions have been shown to be stable for 12 months when stored at −10°C or less.

b) *SUR primary dilution standard (PDS):* Prepare by adding enough of the SUR stock standard to a volumetric flask partially filled with methanol to make a final concentration of 10 μg/mL

* Waters catalog No. WAT035577.
† A Waters Millennium software system was used to generate all data contained in the tables provided in this method.
‡ B-D catalog No. 309603, 309650 or equivalent.
§ Millipore 0.22-μm PVDF membrane, catalog No. SLGV 013 NL, or equivalent.

TABLE 6610:III. PREPARATION OF CALIBRATION (CAL) CURVE SOLUTIONS

CAL Level	Analyte PDS Conc. μg/mL	Volume of Analyte PDS μL	Volume of 10-μg/mL SUR PDS μL	Final Volume of CAL Solution mL	Final Conc. of CAL Solution μg/L
1	1.0	5.0	5.0	25	0.20
2	1.0	12.5	5.0	25	0.50
3	1.0	25.0	5.0	25	1.00
4	10.0	5.0	5.0	25	2.00
5	10.0	12.5	5.0	25	5.00
6	10.0	25.0	5.0	25	10.0

when filled to the mark with methanol. The PDS has been shown to be stable for 6 months when stored at −10°C or less.

2) *Target analyte standard solution:*

a) *Target analyte stock standard:* Obtain analytes listed in Table 6610:I as neat or solid standards or as commercially prepared ampulized solutions. If preparing from neat material, accurately weigh approximately 25 to 35 mg of pure material to the nearest 0.1 mg into a tared, 5-mL volumetric flask. Dilute to the mark with methanol. Repeat for each target analyte.

b) *Target analyte primary dilution standard (PDS):* Prepare by dilution of the target analyte stock standards. Add enough of each target stock standard to a volumetric flask partially filled with methanol to make a final concentration near 10 μg/mL when filled to the mark with methanol. A serial dilution of this PDS, to make a 1.0-μg/mL solution, is useful for low-level fortification. The PDSs have been shown to be stable for 6 months when stored at −10°C or less.

3) *Calibration solutions (CAL):* Prepare the initial calibration curve with at least 5 calibration concentrations. Prepare working calibration solutions over the concentration range of interest from dilutions of the analyte PDSs in buffered reagent water. Add SUR PDS to each CAL. Filter CAL solutions in same manner as samples (¶ 5*c*). The lowest concentration of calibration solution must be at or below the MRL, which may depend on system sensitivity. Prepare calibration standards using buffered reagent water (¶ 4*b*). An example of the dilutions used to prepare the CALs used to collect the data in this method is shown in Table 6610:III. These standards also may be used as continuing calibration checks (¶ 6*d*).

5. Procedure

a. Chromatographic conditions: Establish operating conditions as recommended in Table 6610:IV for the HPLC system. Other HPLC conditions may be used as long as all QC requirements (¶ 6 below) are met. Establish an appropriate retention time window for each target and surrogate to identify them in the QC and field samples. Base this retention time window on measurements of actual retention time variation for each compound in standard solutions analyzed on the HPLC over the course of time. Plus or minus three times the standard deviation of the retention time for each compound during initial calibration and completion of IDC can be used to calculate a suggested window size; however, consider the experience of the analyst in determination of the appropriate retention window size. Confirm that retention times, compound separation, and resolution are similar to those listed in Table 6610:V and Figure 6610:1. Check resolution for closely eluting compounds that are not baseline resolved according to ¶ 6*h*.

b. Calibration:

1) Initial calibration — Calibrate system by using the external standard technique. Prepare a set of at least five calibration standards as described in ¶ 4*i*3).

TABLE 6610:IV. INSTRUMENT GRADIENT AND CONDITIONS

Time min	% Water	% Methanol	% Acetonitrile
Initial	88.0	12.0	0.0
5.30	88.0	12.0	0.0
5.40	68.0	16.0	16.0
14.00	68.0	16.0	16.0
16.10	50.0	25.0	25.0
20.00	50.0	25.0	25.0
22.00	88.0	12.0	0.0
30.00	88.0	12.0	0.0

Instrument conditions:
HPLC: A ternary gradient of water, methanol, and acetonitrile with a flow of 1.5 mL/min as shown above.
Injection volume: System-dependent; for the development of this method: 250 to 1000 μL.
Column: See ¶ 3*a*1).
Postcolumn reactor: Reactor coil: 80°C; reagent 1 and 2 flow rates are instrument-dependent; 0.3 to 0.5 mL/min.
Fluorescence detector: 340-nm excitation, 465-nm emission with a 18-nm band width; gain=100, attenuation=16; response=standard; 16-μL flow cell.

TABLE 6610:V. RETENTION TIMES FOR ANALYTES*

Analyte	Retention Time min	Standard Deviation	Relative Standard Deviation %
Aldicarb sulfoxide	4.369	0.0092	0.21
Aldicarb sulfone	5.072	0.0089	0.17
Oxamyl	5.744	0.0095	0.17
Methomyl	6.526	0.0077	0.12
3-Hydroxycarbofuran	9.824	0.0128	0.13
Aldicarb	11.461	0.0129	0.11
Propoxur	14.321	0.0195	0.14
Carbofuran	14.834	0.0244	0.16
Carbaryl	16.993	0.0264	0.16
1-Naphthol	18.579	0.0187	0.10
Methiocarb	21.826	0.015	0.07
BDMC (SUR)	22.341	0.015	0.07

* Retention time data calculated from precision and accuracy data results presented in Table 6610:II reagent water injections and calibration curve used to quantitate these data.

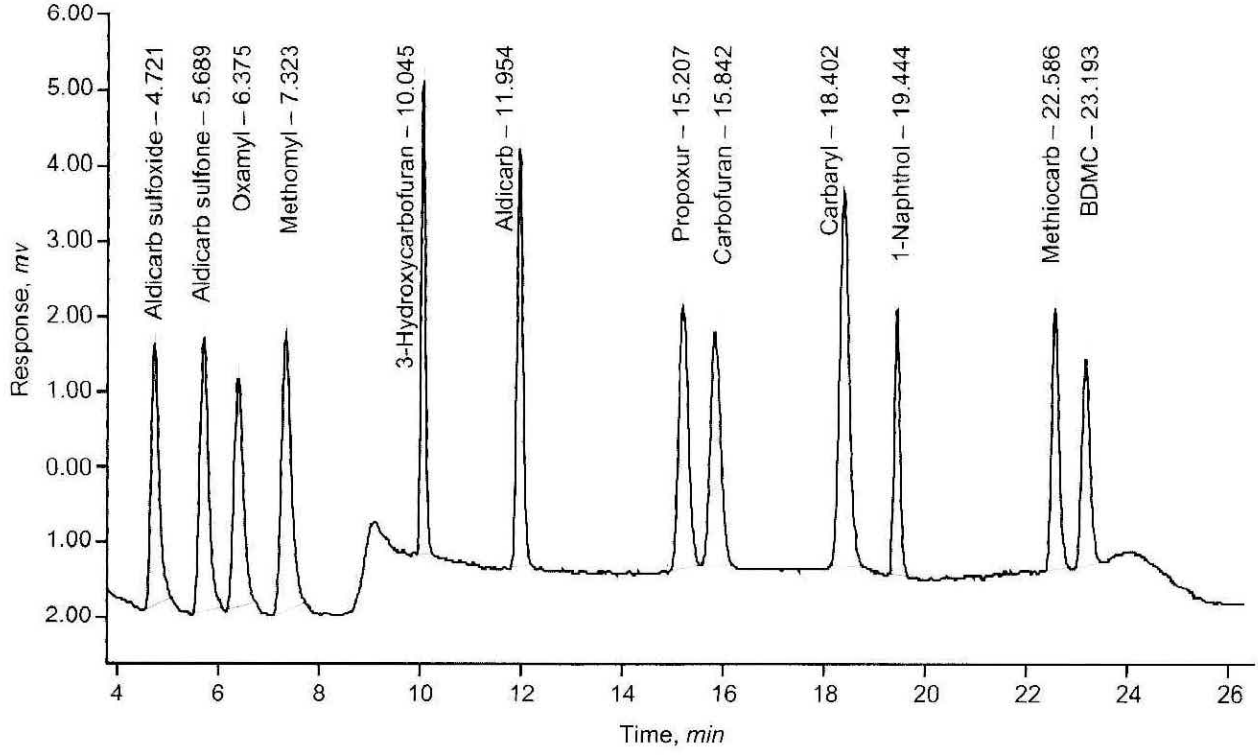

Figure 6610:1. Sample chromatogram of target analytes. Concentration 2.0 μg/L, 1000-μL injection volume, analyzed under conditions stated in Table 6610:IV.

Generate a calibration curve for each analyte by plotting peak response (preferably area) against analyte concentration. The instrument used during method development yielded linear curves for the target analytes over the concentration range of interest. However, data may be fitted with either a linear regression (response vs. concentration) or quadratic fit (response vs. concentration). Alternatively, if the ratio of the analyte peak area to concentration (or response factor) is relatively constant (RSD < 30%), an average response factor may be used to calculate analyte concentration.

When each calibration standard is calculated as an unknown using the calibration curve, the results must be 70 to 130% of the true value for all but the lowest standard, and the lowest standard must be 50 to 150% of the true value.

2) Daily calibration verification — Verify accuracy of initial calibration during each analysis batch (set of samples prepared and analyzed on the same instrument during a 24-h period). Begin an analysis batch with a continuing calibration check (CCC) at or below the MRL, analyze a CCC after every 10 field samples (alternate between medium and high concentrations), and end batch with a CCC. Limit analysis batch to 20 field samples. See ¶ 6 for the required QC samples for each analysis batch.

Inject a portion of the appropriate concentration calibration solution and analyze with the same conditions used during initial calibration.

Calculate concentration of each analyte and surrogate in the CCC standard. The calculated amount for each analyte for medium- and high-level CCCs must be ± 30% of the true value, and the calculated amount for the lowest calibration point for each analyte must be within ± 50% of the true value. If the values are outside these ranges, consider all data for the problem analyte invalid and take remedial action (possibly including recalibration). After adequate calibration has been restored, reanalyze any field or QC samples that have been analyzed since the last acceptable calibration verification.

c. Sample preparation: Preserve, collect, and store samples as directed in 6610B.2, above. Ensure that all field and QC samples contain the required preservatives. Measure a portion of sample into a volumetric flask (25-mL recommended). When the volumes of SUR and analyte PDS added to the field and QC samples are kept to a minimum as described below, no volume adjustment is necessary.

Add a portion of the SUR PDS [¶ 4*i*1)b)] to all samples and mix by capping and inverting sample. For the development of this method, the addition of 5.0 μL of a 10-μg/mL SUR PDS to a 25-mL sample resulted in a SUR concentration of 2.0 μg/L.

If sample is a CCC or other target-analyte-fortified QC sample (¶s 6*f* and *g*), add the necessary amount of analyte PDS [¶ 4*i*2)b)]. Cap and invert each sample to ensure that all components are properly mixed.

Filter samples before filling appropriate autosampler vials. Preferably use filters specified in ¶ 3*f*.

d. Analysis of samples: Inject and analyze samples, including CCCs and QC samples, using conditions identical with those used for initial calibration. Do not extrapolate beyond the established calibration range. If an analyte peak area exceeds the range of the initial calibration curve, the extract may be diluted

TABLE 6610:VI. SUMMARY OF REQUIREMENTS FOR INITIAL DEMONSTRATION OF CAPABILITY (IDC)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
¶ 6*a*1)	Initial demonstration of low system background	Analyze laboratory reagent blank before any other IDC steps.	Target analytes below 1/3 intended MRL; possible interferences from reagents and glassware do not prevent analyte identification and quantitation.
¶ 6*a*2)	Quality control sample (QCS)	Use second source standard to fortify buffered reagent water. Analyze as a CCC after initial calibration but before IDA sample analysis.	Verifies initial calibration accuracy; recovery within ± 30% of true value.
¶ 6*a*2)	Initial demonstration of accuracy (IDA)	Analyze 4–7 replicate LFBs/CCCs fortified at mid-range concentration. Calculate average recovery for replicates used in IDP.	Mean recovery within ± 20% of true value.
¶ 6*a*3)	Initial demonstration of precision (IDP)	Calculate average recovery for replicates used in IDA.	RSD ≤ 20%
¶ 6*a*4)	Detection level determination	Over 3-d period prepare at least 7 replicate LFBs fortified at concentration estimated to be near the detection level. Analyze replicates through all steps of analysis. Calculate detection level according to ¶ 6*a*4).	Data *not required* to meet method precision and accuracy criteria.[See note, ¶ 6*a*4)].

with buffered reagent water. Determine acceptable surrogate performance (¶ 6*i*) from the undiluted sample extract. Any dilutions will also affect analyte MRL. For final calculations, see ¶ 7 below.

6. Quality Control

Quality control (QC) requirements include the initial demonstration of capability (IDC), determination of the detection level, and subsequent analysis in each analysis batch of a laboratory reagent blank (LRB), continuing calibration check (CCC) standards, a laboratory-fortified blank (LFB), a laboratory-fortified sample matrix (LFSM), either a laboratory-fortified sample matrix duplicate (LFSMD) or a field duplicate sample, and the periodic analysis of a quality control sample (QCS). This section details the specific requirements for each QC parameter. The QC criteria discussed in the following sections are summarized in Tables 6610:VI and VII. These criteria are considered the minimum acceptable QC criteria; institute additional QC practices to meet specific needs.

a. Initial demonstration of capability: Requirements for the IDC are described in ¶s 1) through 4) below and are summarized in Table 6610:VI.

1) Initial demonstration of low system background — Before any field samples are analyzed, and any time a new set of reagents is used, demonstrate that a laboratory reagent blank is reasonably free of contamination and that the criteria in ¶ 6*c* are met.

2) Initial demonstration of accuracy — Before analysis of the IDC samples, verify calibration accuracy with the preparation and analysis of a mid-level QCS as defined in ¶ 6*j*. If the analyte recovery is not within ± 30% of the true value, the accuracy of the method is unacceptable; identify and correct the source of the problem. After calibration accuracy has been verified, prepare and analyze four to seven replicate LFBs (or CCCs in this method) fortified at 2 µg/L, or near the mid-range of the initial calibration curve, according to the procedure described in ¶ 4 *i*3). Also add sample preservatives (¶ 2*a*) to these samples. Average recovery of the replicate values must be within ± 20% of the true value.

3) Initial demonstration of precision — Using the same set of replicate data generated for ¶ 2) above, calculate the standard deviation and percent relative standard deviation of the replicate recoveries. The percent relative standard deviation (% RSD) of the results of the replicate analyses must be less than 20%.

4) Detection level determination — Prepare and analyze at least seven replicate LFBs at a concentration estimated to be near the detection level over at least 3 d by the procedure described in ¶ 4*i*3). This fortification level may be estimated by selecting a concentration with a signal of 2 to 5 times the noise level. The appropriate concentration will depend on the sensitivity of the HPLC system being used. Also add sample preservatives (¶ 2*a*) to these samples. Calculate the detection level using the equation

$$\text{Detection level} = St_{(n-1,\,1-\alpha\,=\,0.99)}$$

where:

S = standard deviation of replicate analyses,

$t_{(n-1,1-\alpha\,=\,0.99)}$ = Student's t value for the 99% confidence level with $n-1$ degrees of freedom, and

n = number of replicates.

TABLE 6610:VII. SUMMARY OF QUALITY CONTROL REQUIREMENTS

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
¶ 2*c*	Sample holding times	Ship preserved samples at or below 10°C; hold in lab at ≤ 6°C for up to 28 d. Do not freeze.	Do not report data for samples that have not been properly preserved or stored, or that have exceeded specified holding time.
¶ 6*c*	Laboratory reagent blank (LRB)	Include with each analysis batch (up to 20 samples). Analyze before samples and determine to be free from interferences.	All target analytes below 1/3 intended MRL; possible interferences from reagents and glassware do not prevent analyte identification and quantitation. If targets exceed 1/3 MRL, analytical batch results for that analyte are invalid.
¶ 5*b*2)	Continuing calibration check (CCC)	Verify initial calibration by analyzing CCC (at or below MRL) before analyzing samples. Inject CCCs after every 10 samples and after last sample, rotating concentrations to cover calibration range.	Recovery for each analyte within 70–130% of true value for all but lowest calibration level. Lowest calibration level CCC within 50–150% of true value. Results not bracketed by acceptable CCCs are invalid.
¶ 6*i*	Surrogate standards	Add surrogate, ¶ 4*i*1), to all field and QC samples.	Surrogate recovery within 70–130% of true value. Report samples that fail criteria as suspect.
¶s 6*f* & *g*	Laboratory-fortified sample matrix (LFSM) and laboratory-fortified sample matrix duplicate (LFSMD)	With each analysis batch, extract and analyze at least one LFSM. Extract LFSMD when occurrence of target analytes is low. Field duplicate analysis is not required for extraction batches containing a LFSMD.	Recoveries not within 70–130% (50–150% at MRL) of fortified amount may indicate matrix effect. If LFSMD is analyzed instead of a laboratory duplicate, accept if target RPDs within ± 30%. If all CCCs meet acceptance criteria and LFSM or LFSMD do not, designate sample "suspect/matrix."
¶ 6*g*	Field duplicates (FD)	Analyze at least one duplicate with each extraction batch (20 samples or less). LFSMD may be substituted when analyte occurrence is low.	RPDs within ± 30%. If all CCCs meet acceptance criteria and FDs do not, designate sample "suspect/matrix."
¶ 6*h*	Resolution check	Monitor once/ 24-h analysis period.	Resolution of ≥ 1.0 for closely eluting peaks that are not baseline resolved (see ¶ 6*h*).
¶ 6*j*	Quality control sample	Analyze at least quarterly or when preparing new standards, as well as during IDC.	Same acceptance criteria as CCC.
¶ 5*b*1)	Initial calibration	Use external standard calibration technique to generate calibration curve with at least 5 standards.	For each calibration standard, calculated as an unknown using calibration curve, results within 70–130% of true value for all but lowest standard; lowest standard (concentration ≤ MRL) results within 50–150% of true value.

NOTE: Calculated detection levels need only be less than ⅓ of the laboratory's MRL to be considered acceptable. Do not subtract blank values when performing detection-level calculations. The detection level is a statistical determination of precision only.[1] If the detection level replicates are fortified at a low enough concentration, it is likely that they will not meet precision and accuracy criteria, and may result in a calculated detection level higher than the fortified concentration.

b. Minimum reporting level (MRL): The MRL is the threshold concentration of an analyte that a laboratory can expect to quantitate accurately in an unknown sample. Do not establish the MRL at an analyte concentration lower than that of the lowest calibration standards. The MRL also should not be less than either three times the detection level or a concentration yielding a response less than a signal-to-noise ratio of five. Depending upon the study's data quality objectives, it may be set at a higher concentration.

c. Laboratory reagent blank (LRB): This is a direct injection method without a conventional extraction. A LRB, prepared using buffered reagent water and filtering in the same manner as the samples, is required with each analysis batch of samples to determine any background system contamination. If, within the retention time window of any analyte, the LRB produces a peak that would prevent determination of that analyte, determine source of contamination and eliminate the interference before processing samples. Reduce background contamination to an

acceptable level before proceeding. Keep background from method analytes or contaminants that interfere with the measurement of method analytes below ⅓ the MRL. If target analytes are detected in the LRB at concentrations equal to or greater than this level, consider all data for the problem analyte(s) invalid for all samples in the analysis batch.

d. Continuing calibration check (CCC): Prepare a CCC in the same manner as the initial calibration solutions, using buffered reagent water and filtering in the same manner as the samples. Analyze during an analysis batch at a required frequency to confirm that the instrument meets initial calibration criteria. See ¶ 5*b*2) for concentration requirements, frequency requirements, and acceptance criteria.

e. Laboratory-fortified blank (LFB): For this direct injection method, a CCC is the same as an LFB. Consequently, the analysis of an LFB is not required.

f. Laboratory-fortified sample matrix (LFSM): Analyze an LFSM in each analysis batch to determine that the sample matrix does not adversely affect method accuracy. If the occurrence of target analytes in the samples is infrequent, or if historical trends are unavailable, prepare a second LFSM, or LFMSD, from a duplicate of the field sample used to prepare the LFSM, and analyze to assess method precision. Extraction batches that contain LFSMDs do not require the analysis of a field duplicate. If a variety of different sample matrices are analyzed regularly, for example, drinking water from groundwater and surface water sources, establish method performance for each. Over time, document LFSM data for all routine sample sources for the laboratory.

Within each analysis batch, fortify a minimum of one field sample as an LFSM for every 20 samples processed. Prepare LFSM by adding an appropriate amount of the analyte PDS [¶ 4*i*2)b)] to a sample. Select a fortifying concentration at least twice the matrix background concentration, if known. Use historical data or rotate through the designated concentrations when selecting a fortifying concentration. Selecting a duplicate bottle of a sample that has already been analyzed aids in the selection of appropriate fortifying levels.

Calculate the percent recovery (R) for each analyte with the equation

$$R = \frac{(A - B)}{C} \times 100$$

where:

A = measured concentration in the fortified sample,
B = measured concentration in the unfortified sample, and
C = fortification concentration.

Analyte recoveries may exhibit a matrix bias. For samples fortified at or above their original concentration, recoveries should be between 70 and 130%. For LFSM fortification at the MRL, 50 to 150% recoveries are acceptable. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the CCCs, the recovery is matrix-biased. Label the result for that analyte in the unfortified sample "suspect/matrix".

g. Field duplicate or laboratory-fortified sample matrix duplicate (FD or LFSMD): Within each analysis batch, analyze a minimum of one FD or LFSMD. Duplicates check the precision associated with sample collection, preservation, storage, and laboratory procedures. If target analytes are not routinely observed in field samples, analyze a LFSMD rather than a FD.

Calculate relative percent difference (RPD) for duplicate measurements (FD1 and FD2) using the equation

$$RPD = \frac{(FD1 - FD2)}{(FD1 + FD2)/2} \times 100$$

If a LFSMD is analyzed instead of a FD, calculate relative percent RPD for duplicate LFSMs (LFSM and LFSMD) using the equation

$$RPD = \frac{(LFSM - LFSMD)}{(LFSM + LFSMD)/2} \times 100$$

RPDs for FDs and duplicate LFSMs should fall in the range of ± 30% for samples fortified at or above their original concentration. Greater variability may be observed when LFSMs are fortified near the MRL. At the MRL, RPDs should fall in the range of ± 50% for samples fortified at or above their original concentration.

h. Resolution check: In each analytical batch, monitor resolution of peaks in a calibration standard or CCC near the mid-level of calibration. During the development of this method, the 2-µg/L level was monitored. Check that closely eluting peaks that are not baseline-resolved have a resolution (R_s) of 1.0 or greater, calculated by the equation[6]

$$R_s = \frac{1.18(t_2 - t_1)}{W_{0.5,1} + W_{0.5,2}}$$

where:

t_1 and t_2 = retention times of the first and second adjacent peaks, and
$W_{0.5,1}$ and $W_{0.5,2}$ = widths of the adjacent peaks at half height.

Monitor resolution once for every 24-h analytical batch at any time during the 24-h period. Preferably check resolution before sample analysis, especially if the system in use has a history of resolution problems. If a resolution check fails, reanalyze all samples in the analytical batch, including the QC samples, after the problem is corrected.

i. Surrogate recovery: Fortify all samples, blanks, LRBs, and LFSMs and LFSMDs with surrogate standard before filtration and analysis. Also add it to the calibration curve and calibration check standards. The surrogate is a means of assessing method performance from preparation and filtration to final chromatographic measurement.

When surrogate recovery from a sample, blank, or CCC is less than 70% or greater than 130%, check the following: calculations to locate possible errors, standard solutions for degradation, possible contamination, and instrument performance. If those checks do not reveal the cause of the problem, reanalyze the sample.

If the reanalysis meets the surrogate recovery criterion, report only data for the reanalyzed sample.

If the sample reanalysis fails the 70–130% recovery criterion, check calibration by reinjecting the most recently acceptable

calibration standard. If the calibration standard fails the criteria of ¶ 5*b*2), recalibrate. If the calibration standard is acceptable, repeat preparation (including fortifying with surrogate and filtration) and analysis of the sample if the sample is still within the holding time. If this sample reanalysis also fails the recovery criterion, report all data for that sample as suspect because of unsatisfactory surrogate recovery.

j. Quality control sample (QCS): During the analysis of the IDC (¶ 6*a*), each time that new analyte standard solutions are prepared, or at least quarterly, analyze a QCS from a source different from the source of the calibration standards. Fortify QCS at the mid-level of calibration in buffered reagent water and analyze in same manner as a CCC. The acceptance criteria are the same as the CCC criteria at mid-level: the calculated amount for each analyte must be ± 30% of the true value. If measured analyte concentrations are not of acceptable accuracy, check entire analytical procedure to locate and correct the problem source.

7. Data Analysis and Calculation

Identify method analytes in the sample chromatogram by comparing the retention time of the suspect peak to the retention time of an analyte peak in a calibration standard. Confirm that surrogate retention times are within acceptance limits (¶ 6*i*), even if no target compounds are detected.

Calculate analyte concentrations using the initial calibration curve generated as described in ¶ 5*b*1). Quantitate only those values that fall between the MRL and the highest calibration standard. Samples with target analyte responses that exceed the highest standard require dilution and reanalysis (¶ 5*d*).

Adjust calculated concentrations of detected analytes to reflect initial sample volume and any dilutions performed.

Before reporting the data, review the chromatogram for any incorrect peak identification or poor integration.

Report analyte concentrations in micrograms per liter, usually to two significant figures.

8. References

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6710 TRIBUTYL TIN

6710 A. Introduction

1. Sources and Significance

Tributyl tin (TBT) is both a potent biocide and an endocrine disruptor.[1] Its primary uses have been as an antifouling agent in marine paint, household cleaning solutions, and cooling systems, and as a stabilizer in polyvinyl chloride (PVC) plastic. TBT has been detected in a variety of matrices, including wastewater, marine waters, shellfish tissues, and sediments.

2. Selection of Method

TBT in water or wastewater can be measured with a variety of analytical techniques, most of which involve the derivatization of TBT followed by gas chromatography as a separation technique. Detection of the derivatized TBT can be accomplished with mass spectrometry (GC/MS), flame photometric detector (GC/FPD), atomic absorption spectrometry (GC/AAS), or inductively coupled plasma (GC/ICP). Other methods include high-performance liquid chromatography (HPLC) coupled with a fluorescence detector or an ICP/MS.

This section covers the liquid/liquid extraction of TBT followed by derivatization with a Grignard reagent, hexylmagnesium bromide, and analysis by GC/MS (6710B) or GC/FPD (6710C). The detection levels for the two methods are comparable.

TBT can break down into dibutyl tin (DBT) and monobutyl tin (MBT). The extraction method presented in this section does not yield good recoveries for DBT and MBT. It is suspected that either the ionic properties of DBT and MBT lead to poor extraction recoveries or that losses of these two species occur during the extraction process. Recoveries for DBT can be improved by adding tropolone to the extraction solvent. Further study is needed to improve the recoveries of low-level MBT in an aqueous matrix.

3. Sampling and Storage

If possible, collect all samples in duplicate and one sample per sample batch in triplicate to allow for laboratory QA/QC. Suitable containers are 1-L amber glass or polycarbonate. Preserve samples with 1:1 HCl to a pH of less than 2 and store at 4°C in the dark. TBT in water, when properly preserved and stored, is stable for at least 13 weeks.[2]

4. References

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* Approved by Standard Methods Committee, 2004.
Joint Task Group: François Rodigari (chair), Philip D. Carpenter, Eric A. Crecelius, Lisa M. Ramirez, Michael A. Unger.

6710 B. Gas Chromatographic/Mass Spectrometric Method

This method is applicable to the determination of tributyl tin (TBT) in drinking water, marine water, and wastewater.

1. General Discussion

a. Principle: A measured volume of sample is extracted with methylene chloride. After a drying step followed by a solvent exchange to hexane, the sample is derivatized to tributyl hexyl tin with a Grignard reagent, hexylmagnesium bromide (HMB). Organic interferences are removed by passing the sample extract through a magnesium silica gel column. After concentration, the sample is injected into a gas chromatograph equipped with a mass spectrometer for separation and analysis. Identification and detection of TBT is performed with the mass spectrometer in selected ion monitoring mode.

b. Interferences: Most organic interferences in the samples can be removed by performing a cleanup step. Laboratory preparation of HMB is recommended because some commercially prepared solutions of HMB have been found to contain TBT as a contaminant. Preparing several blanks containing increasing amounts of HMB can assess HMB contamination.

c. Minimum detectable concentration: The method detection level (MDL)[1] for TBT in wastewater is 2 ng/L. Table 6710:I contains a single-laboratory detection level study performed in a wastewater matrix.

2. Apparatus

a. Gas chromatograph capable of temperature programming and equipped for splitless injection.

TABLE 6710:I. SINGLE-LABORATORY METHOD DETECTION LEVEL IN WASTEWATER*

Replicate No.	Concentration *ng/L*	Recovery %
1	7.8	65.2
2	8.5	70.6
3	9.6	80.3
4	9.1	75.9
5	8.0	67.0
6	9.6	80.2
7	10.0	83.4
8	9.4	78.2
Average	9.0	
Standard deviation	0.8	
Calculated MDL	2.3	

* Known addition: 5 ng TBT/L; nominal background: 7 ng/L.

b. Mass spectrometer capable of scanning from 45 to 450 amu every 1 s or less, utilizing 70 eV (nominal) in the electron impact ionization mode, producing a mass spectrum that meets all criteria in Table 6710:II when 5 ng or less of DFTPP is introduced into the chromatograph, and operating in the selected ion monitoring (SIM) mode.

c. Data system: A computer interfaced with the GC/MS with adequate software to allow continuous acquisition, storage, and processing of all mass spectra data.

d. Column: Fused silica capillary column, 30 m long with 0.25-mm ID and 0.25-μm film thickness.*

e. Balance, analytical, capable of accurately weighing to the nearest 0.1 mg.

f. Syringes, 10-, 25-, 100-, and 1000-μL volume.

g. Volumetric flasks, Class A, appropriate sizes with ground-glass stoppers.

h. Hexylmagnesium bromide generation apparatus (not necessary if commercially prepared reagent is used):

1) *Boiling flask,* 50-mL.

* DB5-MS, J&W Scientific, or equivalent.

TABLE 6710:II. ION ABUNDANCE CRITERIA FOR DECAFLUOROTRIPHENYLPHOSPHINE (DFTPP)[2]

Mass *m/z*	Relative Abundance Criteria
51	10–80% of base peak
68	<2% of mass 69
70	<2% of mass 69
127	10–80% of base peak
197	<2% of mass 198
198	Base peak or >50% of 442
199	5–9% of mass 198
275	10–60% of base peak
365	<1% of base peak
441	Present and < mass 443
442	Base peak or >50% of 198
443	15–24% of mass 442

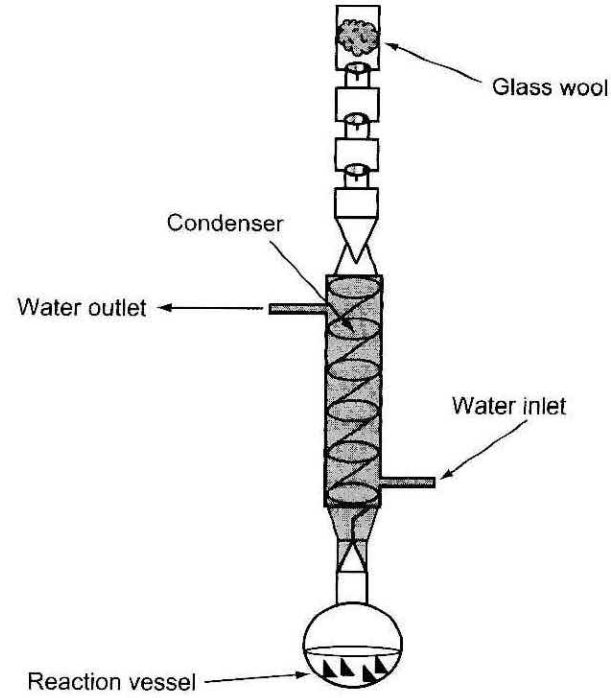

Figure 6710:1. Apparatus setup for HMB generation.

2) *Condenser topped with a macro Snyder column* (see Figure 6710:1).

3) *Glass wool.*

4) *Ice bath,* large enough for boiling flask.

5) *Hot plate.*

i. Extraction apparatus:

1) *Continuous liquid/liquid extractors.*† Alternatively, separatory funnels may be used.

2) *Boiling flasks,* heat-resistant glass, short neck, 500-mL.‡

3) *Heating mantles,* hemispherical, for flasks with 500-mL capacity, 270 W.§

4) *Variable transformers,* 0 – 120 V, 12 amp.‖

5) *Drying columns/cleanup columns,* 250-mL reservoir, 19 cm × 300 mm.#

6) *Vials,* 40-mL, precleaned.**

7) *Cooling container* (plastic or metal) to hold ice and 40-mL extract vials.

j. Sample concentrator.†† Kuderna-Danish concentration apparatus also can be used.

3. Reagents

a. Extraction reagents:

1) *Reagent water,* free of observable interferences at the method detection level. Water can be obtained by using a water

† Kontes No. 584191-0000 extractor body, or equivalent.
‡ VWR No. 29113-122 (Pyrex No. 7740), or equivalent.
§ Series O, VWR No. 33787-141, or equivalent.
‖ Powerstat® VWR No. 62546-364 Model No. 3PW117C, Superior Electric, or equivalent.
Kontes Custom Glass Shop No. 34-10070 or equivalent.
** Wheaton Clean-Pak 217857, or equivalent.
†† Labconco RapidVap N_2 or equivalent.

purification system‡‡ equipped with a UV lamp and an organic polishing cartridge. Do not use plastic pipes, especially PVC, in the plumbing of reagent water.

2) *Boiling chips,* TFE.§§

3) *Hydrochloric acid,* HCl, 1:1.||||

4) *Methylene chloride.*##

5) *Hexane.*##

6) *Tropolone.****

7) *Sodium sulfate,* Na_2SO_4, granular††† muffled at 400°C for 4 h.

b. Reagents for derivatization and sample cleanup: Either use reagents 1) through 3) for laboratory generation of HMB, or use reagent 4).

1) *Magnesium turnings,* >99.8%.‡‡‡

2) *Diethyl ether,* anhydrous§§§, preserved with BHT.

3) *1-bromo-hexane,* > 98% GC.‡‡‡

4) n-*Hexylmagnesium bromide (HMB),* 2*M*.||||||

5) *Activated magnesium silica gel,* ### 60–100 mesh, baked overnight at 130°C.

6) *Sodium sulfate:* See ¶ 3*a*7) above.

c. Standard solutions:

1) *Stock calibration standard:* Dissolve 11.2 mg tributyl tin chloride in 1000 µL acetone. Dilute 100 µL of this solution with 1000 µL of acetone to obtain a concentration of 1.0 µg/µL. Add 2.5 µL of the 1.0-µg/µL solution to 1000 µL of hexane; final concentration is 2.5 µg tributyl tin/mL.

2) *Stock calibration check standard:* Uses a source of tributyl tin different from source of calibration stock standard. Prepare in the same manner as in ¶ *c*1) above.

3) *Surrogate standard:* Dissolve 11.4 mg tri-*n*-propyl tin chloride in 1000 µL acetone. Dilute 20 µL of this solution with 1000 µL of acetone to obtain a concentration of 200 µg/mL (stock surrogate). From the stock surrogate, prepare a working standard by diluting 50 µL of 200-µg/mL solution to 1000 µL of hexane to yield a final concentration of 10 µg tri-*n*-propyl tin/mL.

4) *Internal standard:* Use a 4-mg/mL solution of d10-phenanthrene, d10-acenaphthene, and d12-chrysene in methylene chloride. Dilute 12.5 µL of 4 mg/mL solution to 1 mL methylene chloride to prepare a concentration of 50 µg/mL.

5) *GC/MS performance check solution:* Use a solution of DFTPP, pentachlorophenol, and benzidine. Prepare a performance check solution containing 5 µg/mL DFTPP, 5 µg/mL pentachlorophenol, and 10 µg/mL benzidine by dilution of a more concentrated stock in methylene chloride.

4. Procedure

a. Standards preparation: Prepare at least five levels of calibration standards. Set lowest calibration standard concentration at three to five times the detection level. Suggested calibration levels are presented in Table 6710:III. Prepare calibration check standard (CC) from a different source and use as an independent calibration check. Prepare standards in 10 mL hexane. Add 20 µL of surrogate standard [¶ 3*c*3)] to each of the calibration standards to yield final concentration of 200 ng/mL.

TABLE 6710:III. CALIBRATION STANDARDS CONCENTRATION LEVELS AND PREPARATION METHOD

Calibration Level	Final Standard Concentration* *ng/mL*	Volume of Stock Standard Added to 10 mL Hexane *µL*	Volume of Surrogate Standard Added to 10 mL Hexane† *µL*
CC‡	100	40	20
1	10	4	20
2	50	20	20
3	100	40	20
4	200	80	20
5	500	200	20

* All calibration standards are concentrated to 1 mL after derivatization and cleanup.
† Yields a surrogate final concentration of 200 ng/mL.
‡ Calibration check.

Carry all prepared stock standards (10 mL each in 40-mL VOA vial) through derivatization and cleanup steps (see ¶s 4*d* and *e*).

b. HMB preparation (not required if reagent is purchased): Prepare reagent no more than 1 d before use. Reagent will react with water. If storing reagent for more than a day, store under a nitrogen blanket or in a desiccator.

Set up reagent vessel as shown in Figure 6710:1. Acid-wash all glassware.

Weigh approximately 2.4 g magnesium turnings into a 50-mL boiling flask. Add 10 mL diethyl ether, and 20 mL 1-bromohexane. Wait for reaction to start — the solution turns cloudy and bubbles are formed. If reaction has not started within 5 min, crush magnesium with a glass stirring rod. Add 20 mL ether, then attach condenser and turn on the cooling water. Moderate reaction, using an ice bath, if flask is about to boil over. If reaction is slow, reflux on hot plate for 5 min. When bubbling stops or slows, swirl flask several times. If bubbling resumes, reaction is not complete. Repeat swirling until no bubbling is observed. Reaction is complete when there are just a few blackened metal fragments left on the bottom of the flask.

Store HMB at 4°C until use (up to 24 h).

c. Sample extraction: Mark the meniscus on side of sample container for later determination of sample volume. Adjust pH of all samples to pH <2 with 1:1 HCl. Pour entire sample into a continuous liquid/liquid extractor. Add 20 µL tripropyl tin surrogate standard [¶ 3*c*3)] to each sample, including method blank (reagent water with all reagents and preservatives added), LCS (reagent water with reagents, preservatives, and TBT), and laboratory-fortified matrix (LFM)/LFM duplicate (LFMD). Add 40 µL stock calibration check standard [¶ 3*c*2)] to the LFM/LFMD. Add 4 µL of the same solution to the LCS sample. If glass sample containers are used, add 250 mL methylene chloride to each extractor. If polycarbonate containers are used, add

‡‡ Millipore MQ or equivalent.
§§ Chemware or equivalent.
|||| EM Science Suprapur Grade or equivalent.
EM Science Omni Solv HRGC or equivalent.
*** Aldrich or equivalent.
††† EM Science GR ACS Grade or equivalent.
‡‡‡ Fluka or equivalent.
§§§ Mallinckrodt AR Grade or equivalent.
|||||| TCI America or equivalent.
Florisil or equivalent.

350 mL methylene chloride to each extractor. If DBT and MBT are target analytes, use 0.2% (w/v) tropolone:methylene chloride as an extraction solvent. If glass containers are used, rinse sample container with 50 mL methylene chloride and add rinsate to extractor. Perform rinse twice. Do not rinse polycarbonate containers. Extract sample for 24 ± 6 h.

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

Prepare drying column containing 50 g muffled granular Na_2SO_4 by pre-wetting it with methylene chloride. Pass extract through column and collect in concentration vessel. Rinse extract holding flask three times with 10 mL methylene chloride and transfer each rinse to drying column. Elute drying column with 30 mL methylene chloride.

Exchange the solvent to hexane, using concentrator (¶ 2*j*). Set temperature of the concentrator at 40°C and set nitrogen gas pressure at 103 kPa (15 psi). Bring extract to a final volume of approximately 2 mL. Transfer extract to 40-mL vial with a Pasteur pipet. Rinse concentrator tube three times with 2 mL hexane and transfer each rinse to the 40-mL vial. The total extract in 40-mL vial after transfer should be approximately 8 to 10 mL.

d. Derivatization: To each extract, slowly add 2.0 mL of 2.0*M* HMB and cap vial. Allow derivatization to proceed for 30 min at room temperature with constant shaking. Place vials in a rack and place rack in a cooling container with ice and water. Add 5 mL 1:1 HCl to each vial. Once vial is cool, shake for 2 min, pull off bottom aqueous layer, and discard. Add another 5 mL 1:1 HCl, shake for 2 min, and pull off and discard aqueous phase as above. Add 5 mL reagent water, shake for 2 min, and pull off and discard aqueous phase. Proceed with extraction cleanup.

e. Extract cleanup:[3] Activate magnesium silica gel by baking overnight at 130°C. Prepare cleanup columns by weighing 20 g activated gel into a glass cleanup column. Cover gel with 2 g of muffled granular sodium sulfate. Wash column with 70 mL hexane. Keep column wet by closing stopcock to keep 2 mm of hexane above sodium sulfate.

Transfer sample extract to a prepared column. Rinse extract vial three times with hexane, adding each rinse to cleanup column. The total hexane rinse volume is approximately 25 mL. Elute column with a total of six 25-mL hexane rinses, collecting all eluent in a concentration tube.

Concentrate extract to a final volume of 1.0 mL using the concentrator. Store extracts at 4°C.

f. Instrument calibration: Set up the gas chromatograph system to operate with parameters shown in Table 6710:IV.

Perform an air/water check as recommended by the mass spectrometer manufacturer. Tune the spectrometer with perfluorotributyl amine (PFTBA) to facilitate meeting the DFTPP ion abundance criteria shown in Table 6710:II.

Inject 1 μL of GC performance check solution. The DFTPP spectrum should meet the criteria outlined in Table 6710:II. The tailing factors for benzidine and pentachlorophenol should be lower than 3 and 5, respectively. Higher tailing factors may indicate system problems: the column may need to be trimmed or baked, or the injector liner may need replacement. Resolve problems before proceeding with sample analysis.

TABLE 6710:IV. GAS CHROMATOGRAPH OPERATING PARAMETERS

Parameter	Setting
Initial temperature	80°C, hold for 2 min
Temperature program	8°C/min to 260°C 50°C/min to 350°C, hold for 5 min
Final temperature	350°C
Injector temperature	230°C
Transfer line	250°C
Detector temperature	200°C
Injector type	Grob-type, splitless; split on 0.7 min after injection
Sample volume	1 μL
Carrier gas	Helium at 30 cm/s (measured at 270°C)

To the calibration standards generated in ¶s 4*c* through *e*, add 5 μL internal standard.

Inject a solvent blank followed by the calibration standards. Process calibration standards with instrument data system and prepare a calibration curve. A sample mass spectrum for tributyl tin is shown in Figure 6710:2. Suggested quantitation ions and internal standard assignments are outlined in Table 6710:V.

Acceptance criteria for the calibration curve are outlined in Table 6710:VI. If the %RSD for the calibration curve is <20%, the response can be assumed to be linear through 0 and response factors (RFs) may be used. If the %RSD is >20%, do not use RFs for quantitation purposes, but choose an appropriate regression technique.

g. Sample analysis: Add 5 μL internal standard to all samples. Inject and process all the samples in the batch, including QC samples, using the calibration curve obtained according to ¶ *f* above. Ensure that all reported concentrations are bracketed by the calibration standards. If a sample concentration exceeds the highest standard, rerun sample at the appropriate dilution to bring the concentration within the calibration range. Before rerunning the diluted sample, add required volume of internal standard to make up the loss caused by dilution. Accept or reject the sample batch on the basis of QC criteria given in ¶ 5 below.

TABLE 6710:V. ASSIGNED QUANTITATION ION AND INTERNAL STANDARDS

Analyte/Internal Standard (IS)	Analyte/IS Quantitation Ions	Analyte/IS Qualitative Ions
Tripropyl tin/ d10-acenaphthene	207/164	164, 165/165
Tributyl tin/ d10-phenanthrene	179/188	177, 207, 319/ none

TABLE 6710:VI. CALIBRATION ACCEPTANCE CRITERIA

Calibration Technique	Acceptance Criteria
Response factors (RF)	≤20% relative standard deviation (RSD)
Linear or quadratic regression	R^2> 0.998

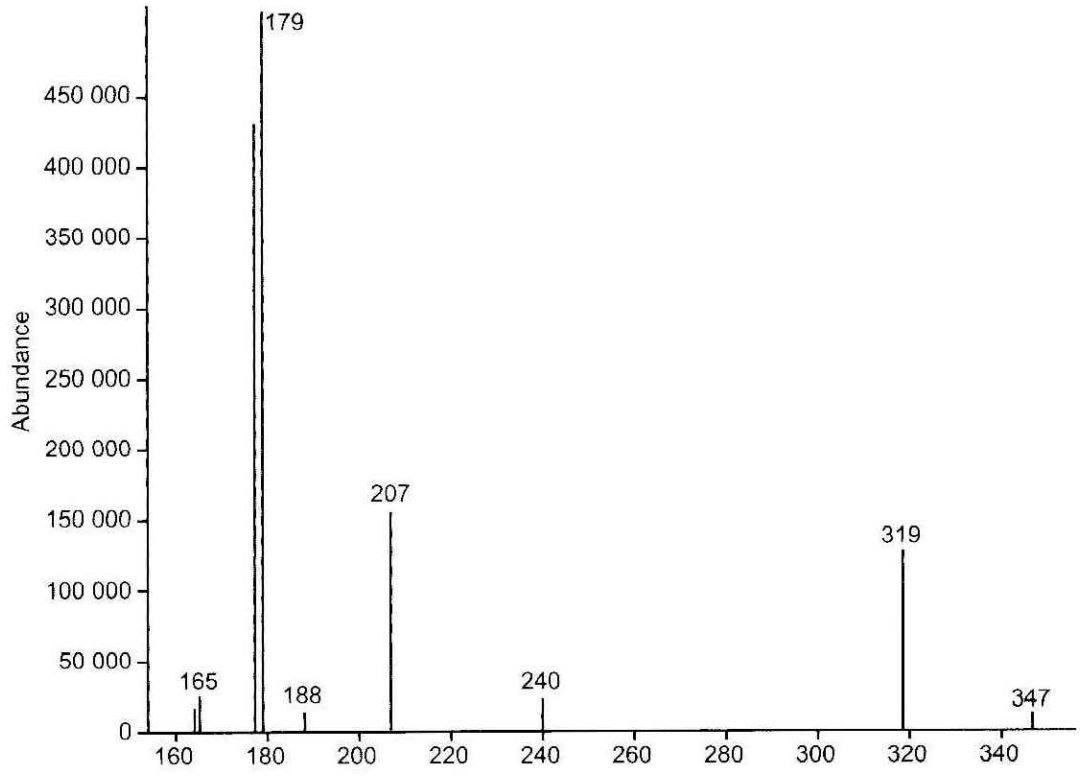

Figure 6710:2. Tributyl tin spectrum with selected ion monitoring.

5. Quality Control

Include a method blank, LCS, and LFM/LFMD in all analytical batches. The required QC sample types and frequencies and batch acceptance limits calculated from a single-laboratory data set are summarized in Table 6710:VII.

Develop laboratory batch acceptance limits by calculating the average percent recovery (p) and the standard deviation (s) for each of the laboratory-fortified matrix compounds after analysis of 15 to 20 laboratory-fortified matrix samples of the same matrix. Calculate average percent recovery (p) and the standard deviation (s) for each of the surrogates after analysis of 15 to 20 field samples of the same matrix, in a similar fashion. Calculate upper and lower control limit for each matrix known addition or surrogate compound:

Upper control limit = $p + 3s$
Lower control limit = $p - 3s$
Calculate warning limits as:
Upper warning limit = $p + 2s$
Lower warning limit = $p - 2s$

For laboratories employing statistical software to determine these limits, the control limits approximate a 99% confidence interval around the mean recovery, while the warning limits approximate a 95% confidence interval.[4]

TABLE 6710:VII. MINIMUM QC SAMPLES FOR EACH BATCH AND RESPECTIVE ACCEPTANCE LIMITS

QC Type	Frequency	Acceptance Range
Internal standard (IS)	Every sample	70–130% of IS response from previous CC and 50–150% of mean IS response from initial calibration
Surrogate	Every sample	29–129% recovery
Method blank	1/batch	All analytes less than MDL
LCS at 3–5 times MDL	1/batch	50–150% recovery
Calibration check (CC)	Every 10 samples; minimum 2/batch; at start and end	80–120% recovery
Laboratory-fortified matrix	10% of samples; minimum 1/batch	30–132% recovery
Laboratory-fortified matrix duplicate	10% of samples; minimum 1/batch	30–132% recovery and ≤40 relative percent difference (RPD)

NOTE: Ensure that laboratory meets acceptance criteria above for internal standard, method blank, LCS, and calibration check. Establish acceptance limits for recoveries of surrogate, LFM, LFM duplicate, and precision for LFM – LFMD in the laboratory.

Ensure that laboratory meets the acceptance limits in Table 6710:VII for the internal standard, method blank, LCS, and calibration check. These QC types are used to establish that the method performance is met. Recoveries for the surrogate, LFM, LFM duplicate, and precision for the LFM-LFMD are matrix-dependent; establish these in the laboratory.

6. Precision and Bias

Single-laboratory precision and bias in a wastewater matrix are presented in Table 6710:I.

7. References

1. U.S. Environmental Protection Agency. 1984. Definition and procedure for the determination of the method detection limit. 40 CFR Part 136, Appendix B. *Federal Register* 49, No. 209.
2. U.S. Environmental Protection Agency. 1995. Determination of organic compounds in drinking water by liquid-solid extraction and capillary column gas chromatography/mass spectrometry. Method 525.2, Methods for the Determination of Organic Compounds in Drinking Water, Supplement III. EPA-600/R95-131. National Service Center for Environmental Publications, Cincinnati, Ohio.
3. Unger, M.A., W. G. MacIntyre, J. Greaves & R. J. Hugget. 1986. GC determination of butyl tins in natural waters by flame photometric detection of hexyl derivatives with mass spectrometric confirmation. *Chemosphere* 15: 461.
4. U.S. Environmental Protection Agency. 1997. Test Methods for Evaluating Solid Waste. Physical/Chemical Methods (SW846) (CD ROM Version 2). National Technical Information Serv., Springfield, Va.

8. Bibliography

U.S. Environmental Protection Agency. 1996. DBP/ICR Analytical Methods Manual, Section 10. EPA 814-B-96-002, Off. Water, Cincinnati, Ohio.

6710 C. Gas Chromatographic/Flame Photometric Detector Method

This method is applicable to the determination of tributyl tin (TBT) in drinking water, marine water, and wastewater. It is an adaptation of Method 6710B, modified for the analysis of tributyl tin with a flame photometric detector (FPD).

1. General Discussion

a. Principle: The principle is identical with that given in 6710B.1*a*, except that a flame photometric detector (FPD) is used for separation and analysis.

b. Interferences: See 6710B.1*b*. Because detection with GC/FPD can be subject to interferences, confirmation on a secondary column or by mass spectrometry is desirable.

c. Minimum detectable concentration: The method detection level (MDL)[1] for TBT in seawater is 1 ng/L. Table 6710:VIII contains results of a single-laboratory detection level study performed in an artificial seawater matrix.

Table 6710:VIII. Single-Laboratory Method Detection Level in Artificial Seawater*

Replicate No.	Concentration *ng/L*	Recovery %
1	1.3	120
2	1.3	120
3	0.9	82
4	0.6	55
5	0.8	73
6	1.4	79
7	0.9	82
Average	1.0	
Standard deviation	0.3	
Calculated MDL	0.96	

* Known addition: 1.1 ng TBT/L.

2. Apparatus

a. Gas chromatograph, equipped with an FPD using a >600-nm band pass filter, capable of temperature programming, and equipped for splitless injection.

b. Data system: A computer interfaced with the GC with adequate software to allow the continuous acquisition, storage, and processing of all data.

c. Other apparatus: See 6710B.2*d* through *j*.

3. Reagents

a. Reagents specified in 6710B. 3a1) through c3).

b. Internal standard solution: Dissolve 44.3 mg tri-*n*-pentyl tin chloride* in 1000 µL hexane. Dilute 20 µL of this solution with 1000 µL hexane to obtain a concentration of 800 µg/mL (stock internal standard). Dilute 50 µL of this solution with 1000 µL hexane. This will yield an internal standard solution with a concentration 40 µg/mL. Add 5 µL of this solution to the calibration standards and sample extracts before derivatization and cleanup. The final concentration is 200 ng/mL of tri-*n*-pentyl tin in calibration standards and sample extracts.

4. Procedure

a. Standards preparation: Prepare at least five levels of calibration standards. Set lowest calibration standard concentration at three to five times the detection level, at minimum reporting level. Suggested calibration levels are presented in Table 6710:III. Prepare calibration check standard (CC) from a different source and use as an independent calibration check. Also use CC

* Organometallics, Inc., East Hampstead, NH, or equivalent.

TABLE 6710:IX. GAS CHROMATOGRAPH OPERATING PARAMETERS

Parameter	Setting
Initial temperature	135°C hold for 2 min
Temperature program	10°C minute to 300°C, hold 5 min
Final temperature	300°C
Injector temperature	280°C
Detector temperature	280°C
Injector type	Grob-type, splitless; split on 0.7 min after injection
Sample volume	1 μL
Carrier gas	Helium at 30 cm/s (measured at 300°C)
Make-up gas	Helium at 90 mL/min
Detector gases	Hydrogen at 152 mL/min; air1 at 97 mL/min; air2 at 188 mL/min

for making known additions to samples and to prepare the laboratory control standard (LCS).

Add 5 μL of 40 μg/mL internal standard solution to all stock standards. Carry all prepared stock standards (10 mL each in 40-mL vials) through the derivatization and cleanup steps (see ¶s 6710B.4*d* and *e*).

b. HMB preparation: See 6710B.4*b*.

c. Sample extraction: Follow procedure given in 6710B.4*c*. When extraction is complete, add 5 μL of 40 ng/μL internal standard (¶ 3*b* above) to every sample extract.

d. Derivatization: See 6710B.4*d*.

e. Extract cleanup: See 6710B.4*e*.

f. Instrument calibration: Set up the gas chromatograph system to operate with the parameters shown in Table 6710:IX.

Inject a solvent blank followed by the calibration standards. Process calibration standards with instrument data system and prepare a calibration curve.

Acceptance criteria for the calibration curve are outlined in Table 6710:VI. If the %RSD for the calibration curve is ≤ 20%, the response can be assumed to be linear through 0 and response factors may be used. If the %RSD is > 20%, do not use RFs for quantitation purposes but choose an appropriate regression technique.

g. Sample analysis: Inject and process all the samples in the batch, including QC samples, using the calibration curve obtained according to ¶ 4*f* above. Ensure that all reported concentrations are bracketed by the calibration standards. If a sample concentration exceeds the highest standard, rerun sample at the appropriate dilution to bring the concentration within the calibration range. Accept or reject the batch on the basis of QC criteria given in 6710B.5.

5. Quality Control

See 6710B.5.

6. Precision and Bias

Single-laboratory precision and bias in an artificial seawater matrix are presented in Table 6710:VIII.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of the method detection limit. 40CFR Part 136, Appendix B. *Federal Register* 49, No. 209.

8. Bibliography

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U.S. ENVIRONMENTAL PROTECTION AGENCY. 1996. DBP/ICR Analytical Methods Manual, Section 10. EPA 814-B-96-002, Off. Water, U.S. Environmental Protection Agency, Cincinnati, Ohio.

UNGER, M. A. 1996. A Manual for the Analysis of Butyltins in Environmental Samples. Virginia Inst. Marine Science, College of William and Mary, Gloucester Point.

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Abbreviations

The following symbols and abbreviations are used throughout *Standard Methods:*

Abbreviation	*Referent*
AA	atomic absorption
A or amp	ampere(s)
AC	alternating current
ACS	American Chemical Society
amu	atomic mass units
APHA	American Public Health Association
ASTM	American Society for Testing and Materials
AWWA	American Water Works Association
BOD	biochemical oxygen demand
°C	degree(s) Celsius
c	counts
Ci	curie(s)
cm, cm^2, cm^3	centimeter(s), square centimeter(s), cubic centimeter(s)
COD	chemical oxygen demand
conc	concentrated
cpm	counts per minute
cps	counts per second
d	day(s)
DC	direct current
diam	diameter
DO	dissolved oxygen
DOX	dissolved organic halogen
dpm	disintegrations per minute
g	gram(s)
g	gravity, unit acceleration of
GC	gas chromatograph
GC/MS	gas chromatograph/mass spectrometer
h	hour(s)
HPLC	high-performance liquid chromatography
IC	ion chromatograph
ICP	inductively coupled plasma
ID	inside diameter
IU	international unit(s)
keV	kiloelectron volt(s)
kg	kilogram(s)
kPa	kilopascal
L	liter(s)
M	mole or molar
m, m^2, m^3	meter(s), square meter(s), cubic meter(s)
MCL	maximum contaminant level
MDL	method detection level
me	milliequivalent(s)
MeV	megaelectron volt(s)
mg	milligram(s)
min	minute(s)
mL	milliliter(s)
mm, mm^2, mm^3	millimeter(s), square millimeter(s), cubic millimeter(s)
mol wt	molecular weight
MPN	most probable number
MS	mass spectrometer
mV	millivolt(s)
μA	microampere(s)
μCi	microcurie(s)
μg	microgram(s)
μL	microliter(s)
μm	micrometer(s)
N	normal
nCi	nanocurie(s)
ng	nanogram(s)
NIST	National Institute of Standards and Technology
No.	number
NTU	nephelometric turbidity unit(s)
OD	outside diameter
Pa	pascal
pCi	picocurie(s)
pg	picogram(s)
PTFE	polytetrafluoroethylene
PVC	polyvinyl chloride
rpm	revolution(s) per minute
rps	revolution(s) per second
SD	standard deviation
SDI	sludge density index
s	second(s)
sp., spp.	species
sp gr	specific gravity
ST	standard taper
SVI	sludge volume index
TFE	tetrafluoroethylene
THM	trihalomethane(s)
TOC	total organic carbon
TON	threshold odor number
TOX	total organic halogen
Toxicity terms	*see* Section 8010B
U	unit(s)
USGS	U.S. Geological Survey
USP	United States Pharmacopoeia
UV	ultraviolet
V	volt(s)
v/v	volume ratio
W	watt(s)
WEF	Water Environment Federation
WPCF	*see* WEF

Abbreviations of periodical titles in reference lists and bibliographies are based on those given in *Biosis. List of Serials with Title Abbreviations*, 1970. Biosciences Information Service of Biological Abstracts, Philadelphia, Pa.

General Information

TABLE A: UNIT PREFIXES

Symbol	Prefix	Multiples and Submultiples
M	mega-	10^6
k	kilo-	10^3
m	milli-	10^{-3}
	micro-	10^{-6}
n	nano-	10^{-9}
p	pico-	10^{-12}

TABLE B: METRIC ENGLISH EQUIVALENTS

Metric Unit	Multiplied by	English Unit
m	3.2[illegible]	ft
lux	0.0929	ft-c
L	0.2642	gal
cm	0.394	in
kg	2.205	lb
g	0.0353	oz
kPa	0.145	psi

Since 1905, analysts, researchers, and regulatory officials have relied on *Standard Methods for the Examination of Water and Wastewater* as a trusted source of accurate, proven methodology for analysis of natural waters, water supplies, and wastewaters. In a single, convenient compilation, the publication includes methods for measuring the biological, chemical, and physical attributes of waters and offers guidance for choosing among available methods for specific elements and compounds. Methods have been reviewed and approved by a wide consensus of experts in water analysis.

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