Designation: D6903 – 07 (Reapproved 2013)

Standard Test Method for Determination of Organic Biocide Release Rate From Antifouling Coatings in Substitute Ocean Water[1]

This standard is issued under the fixed designation D6903; 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 test method covers the laboratory determination of the rate at which organic biocide is released from an antifouling coating exposed in substitute ocean water. The test is run entirely in the laboratory under controlled conditions of pH, temperature, salinity, and hydrodynamics. Analytical procedures are provided for the determination of the release rate of 4,5-dichloro-2-n-octylisothiazolin-3-one (DCOIT), zinc and copper pyrithione (ZPT and CuPT), and *N*-cyclopropyl-*N'*-(1, 1-dimethylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine (CDMTD). At predetermined intervals, substitute ocean water samples are analyzed for leached biocide using a suitable analytical technique.

1.2 In cases in which the antifouling coating contains both an organic biocide and a copper-based biocide, the release rate of copper may optionally be concurrently determined according to the procedure found in Test Method D6442.

1.3 The procedure contains the preparation steps for the determination of the release rate of biocide in substitute ocean water from antifouling paints including apparatus, reagents, holding tank conditions, and sampling point details. The procedure calls for the accurate determination of organic biocide concentrations in substitute ocean water at the low $\mu g\ L^{-1}$ (parts per billion, ppb) level. To detect and correct for reagent impurities and allow a suitable level of analytical precision to be achieved, the analytical method to be used for the determination of the concentration of organic biocide in substitute ocean water must meet the acceptability criteria given in Annex A2. Where Annex A2 specifies a limit of quantitation (LOQ), the procedure for determining the LOQ for the organic biocide in substitute ocean water by the analytical method presented in Annex A3 is to be followed.

1.4 Suitable analytical methods that use high-performance liquid chromatography (HPLC) for determining the concentration of DCOIT, ZPT and CuPT, and CDMTD in substitute ocean water are given in Appendix X1 – Appendix X3, respectively. Other methods may be used provided that they meet the appropriate criteria given in Annex A2.

1.5 When the release rate of a highly photosensitive organic biocide is being determined, steps must be taken to protect the apparatus and samples from exposure to natural and artificial visible light sources. Any such requirement for these steps to be taken for a particular biocide is indicated in Annex A2.

1.6 The practical limits for quantifying biocide release rates by this method are from 4.5 to 500 $\mu g\ cm^{-2}\ d^{-1}$ for DCOIT, 0.36 to 500 $\mu g\ cm^{-2}\ d^{-1}$ for CuPT, 0.36 to 500 $\mu g\ cm^{-2}\ d^{-1}$ for ZPT, and 2.7 to 500 $\mu g\ cm^{-2}\ d^{-1}$ for CDMTD. These ranges may be extended to 3.8 to 500 $\mu g\ cm^{-2}\ d^{-1}$ for DCOIT, 0.16 to 500 $\mu g\ cm^{-2}\ d^{-1}$ for CuPT, 0.2 to 500 $\mu g\ cm^{-2}\ d^{-1}$ for ZPT, and 2.2 to 500 $\mu g\ cm^{-2}\ d^{-1}$ for CDMTD if the procedures described in Appendix X1 – Appendix X3 (as appropriate) are followed. The quantitation of release rates lower than these ranges will require the use of analytical methods with lower limits of quantitation than those specified in Annex A2.

1.7 The results of this test method do not reflect environmental biocide release rates for antifouling products, and are not suitable for direct use in the process of generating environmental risk assessments, environmental loading estimates, or for establishing release rate limits for regulatory purposes. See also Section 4.

1.8 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.9 *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:*[2]

[1] This test method is under the jurisdiction of ASTM Committee D01 on Paint and Related Coatings, Materials, and Applications and is the direct responsibility of Subcommittee D01.45 on Marine Coatings.

Current edition approved June 1, 2013. Published June 2013. Originally approved in 2007. Last previous edition approved in 2007 as D6903 – 07. DOI: 10.1520/D6903-07R13.

[2] 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.

D1005 Test Method for Measurement of Dry-Film Thickness of Organic Coatings Using Micrometers
D1141 Practice for the Preparation of Substitute Ocean Water
D1193 Specification for Reagent Water
D6442 Test Method for Determination of Copper Release Rate From Antifouling Coatings in Substitute Ocean Water

2.2 *U.S. Federal Standard:*[3]
40 CFR 136, Appendix B, revision 1.11

3. Summary of Test Method

3.1 The candidate paint system is applied to the cylindrical test specimens. The coated specimens are placed in a tank of substitute ocean water in which the levels of organic biocide and copper (where the coating also contains a biocidal copper compound) are kept below 100 μg L^{-1} by circulating the substitute ocean water through a suitable filtration system (see 5.1.3). At specified intervals, each specimen is placed in 1500 mL of substitute ocean water (see Section 8 for details) and rotated at 60 rpm for 1 h (or less, see 8.7 for further explanation and instruction). The rate of biocide release from the paint is determined by measuring concentrations of the biocide in the substitute ocean water in the individual measuring containers.

3.2 Annex A2 provides acceptance criteria for analytical procedures for measuring the concentration of specific organic biocides in substitute ocean water. Suitable methods are provided in Appendix X1 – Appendix X3. Alternative methods may be used provided that they meet the acceptance criteria given in Annex A2.

4. Significance and Use

4.1 This test method is designed to provide a laboratory procedure to quantify and characterize changes in the release rate of organic biocide from antifouling coatings that occur during a period of immersion under specified laboratory conditions of constant temperature, pH, salinity, and hydrodynamics. Quantitative measurement of biocide release rate is necessary to help in selection of materials, providing quality control, and understanding the performance mechanism.

4.2 Results from this test method establish a pattern of biocide release from an antifouling coating over a minimum of 45 days exposure under controlled laboratory conditions. Biocide release rates of antifouling paints in-service vary over the life of the coating system depending on the formulation and on the physical and chemical properties of the environment. Factors such as differences in berthing locations, operating schedules, length of service, condition of paint film surface, temperature, pH, and salinity influence the actual release rate under environmental conditions. Results obtained using this test method do not reflect actual biocide release rates that will occur in service, but provide comparisons of the release rate characteristics of different antifouling formulations in substitute ocean water under the prescribed laboratory conditions.

4.3 By comparison with published copper and organotin release rate data[4,5] obtained either by direct measurements from ship hulls or release rate measurements from harbor exposed panels, all data indicate that the results of this generic rotating-cylinder test method significantly overestimate the release rate of biocide when compared to release rates under in-service conditions. For example, published results demonstrate that this generic test method produces higher measurements of copper and organotin release rates than from direct in situ measurements for the same coating on in-service ship hulls and harbor-exposed panels. The difference between the results of this test method and the panel and ship studies was up to a factor of about 30 based on copper release rate data for several commercial antifouling coatings.[4,6] No direct release rate data from ship hulls or harbor-exposed panels have been generated to-date for the biocides covered by this method. However, the expectation is that the results of this test method, when compared with the direct measurements from ship hulls and harbor-exposed panels, could follow the same trend. Realistic estimates of the biocide release from a ship's hull under in-service conditions can only be obtained from this test method where the difference between the results obtained by this test method and the release rate of an antifouling coating in service is taken into account.

4.4 Where the results of this test method are used in the process of generating environmental risk assessments, for environmental loading estimates, or for regulatory purposes, it is most strongly recommended that the relationship between laboratory release rates and actual environment inputs is taken into account to allow a more accurate approximation of the biocide release rate from antifouling coatings under real-life conditions. This can be accomplished through the application of appropriate correction factors.[6]

5. Apparatus

5.1 *Sample Generation*—See Annex A2 for guidance on any particular materials restriction and handling requirements relating to each organic biocide.

5.1.1 *Release Rate Measuring Container*—A nominal 2-L (½-gal) container made of an inert material, approximately 13.5 cm (5.3 in.) in diameter and 19 cm (7.5 in.) high, is fitted with three rods also made of an inert material, approximately 6 mm (nominal ¼ in.) in diameter to serve as baffles. Rods shall be evenly spaced on the inside circumference of the container to prevent swirling of the water with the test cylinder during rotation. The rods will be secured to the container walls using an inert adhesive. The material of construction of the release rate measuring container and rods for use with any particular biocide shall be as specified in A2.3. When the release rate of a photosensitive material is to be determined, the container shall be protected from light. The requirement to protect the

[3] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http://www.access.gpo.gov.

[4] Valkirs, A. O., Seligman, P. F., Haslbeck, E., and Caso, J. S., *Marine Bulletin*, Vol. 46 (2003), pp. 763–779.

[5] Champ, M. A. and Seligman, P. F., *Organotin: Environmental Fate and Effects*, Chapter 19 — Measurement and Significance of the Release Rate for Tributyltin, (1996) Chapman and Hall, pp 383–403.

[6] Finnie, A. A., Improved Estimates of Environmental Copper Release Rate From Antifouling Coatings, *Biofouling*, Vol. 22 (2006), pp 279–291.

release rate container from light for any particular organic biocide is indicated in A2.4.

NOTE 1—The results of this test method will be adversely affected if the biocide is strongly adsorbed or absorbed by the release rate measuring container or the test cylinder, or both. Where the release rates of two or more different biocides are to be concurrently determined from a single set of measurements, the release rate measuring container, associated rods and the test cylinders must all be made of a material that is inert to all of the biocides, otherwise repeat testing (different cylinders and measuring containers) for each biocide will be required.

5.1.2 *Constant Temperature Control*—This control is a means of maintaining the release rate measuring containers at a temperature of 25 ± 1°C during the rotation period (see 8.7).

5.1.3 *Holding Tank*—This tank is an inert plastic container of such dimensions so as to permit immersion of four or more test cylinders and must be equipped with a system to circulate the seawater continuously in the tank through an activated carbon filter and, optionally, an absorbent filter.[7] If an absorbent filter is used, regenerate the ion exchange resin following the manufacturer's instructions and wash the resin with substitute ocean water before use. The rate of water flow and the size of the filter shall be selected to maintain the concentration of each organic biocide below 100 μg L^{-1} (100 ppb) and, when the coating contains a biocidal copper compound, the concentration of copper below 100 μg L^{-1}. Flow rates should be set to obtain two to eight turnovers per hour. When the release rate of a photosensitive material is to be determined, the holding tank shall be protected from light. The requirement to protect holding tank from light for any particular organic biocide is indicated in A2.4.

5.1.4 The size and geometry of the tanks as well as the positioning of the inflow and outflow ports for the water circulation system shall be selected to obtain a slow, relatively uniform flow of substitute ocean water past all test cylinders in the tank. Maintain the pH of the substitute ocean water between 7.9 and 8.1, the salinity between 33 and 34 parts per thousand (ppt), and the temperature at 25 ± 1°C (77 ± 2°F).

5.1.5 *Test Cylinders*—Approximately 6.4-cm (nominal 2½-in.) outside diameter by 17.8-cm (nominal 7-in.) long pipe or equivalent cylindrical shapes made of an inert material and coated with a 10-cm (3.94-in.) band of antifouling paint around the exterior circumference of the test cylinder to provide 200 cm^2 of paint film that can be immersed and freely rotated in the release rate measuring container (see Note 1 and Note 2). A top disk, fitted with a shaft of proper diameter for the rotating device, shall be sealed to the cylinder. Seal the bottom of the test cylinder so as to form a watertight joint. Alternatively, prefabricated one-piece test cylinders with an integral sealed bottom-end can be used. Do not coat the lower 1 to 2 cm (0.39 to 0.79 in.) of the test cylinder. The test cylinder shall be of such height so that a rotating device can be attached to and rotate the cylinder with the upper end of the cylinder above the level of the test container immersion liquid to prevent entry of the immersion liquid into the test cylinder (see Annex A1). The material of construction of the test cylinder (including the bottom end-disk) for use with any particular biocide shall be as specified in A2.3. It is advisable to weight the cylinder by filling with water so that the unit does not have buoyancy.

NOTE 2—When coating release rates are very high, it may be desirable to use a 5-cm band (100-cm^2 paint area) to avoid exceeding 200 μ g L^{-1} of organic biocide in the measuring containers (see 8.7.1).

5.1.6 *Test Cylinder Rotating Device*—The device shall be capable of rotating the test cylinder in the release rate measuring container at 60 ± 5 rpm (0.2 ± 0.02 m s^{-1}, velocity of test cylinder surface). No part of the rotating device shall be immersed in substitute ocean water.

5.1.7 *pH Meter,* with a suitable electrode.

5.1.8 *Appropriate Hydrometer or Salinometer.*

5.2 *Analysis of Leachate*—Suitable analytical procedures are provided for the determination of the release rate of 4,5-dichloro-2-n-octylisothiazolin-3-one (DCOIT), zinc and copper pyrithione (ZPT and CuPT), and *N*-cyclopropyl-*N′*-(1, 1-dimethylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine (CDMTD). Refer to Appendix X1 – Appendix X3 for additional apparatus requirements for the analysis of specific organic biocides in which these analytical methods are to be used.

6. Reagents and Materials

6.1 *Sample Generation:*

6.1.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests, unless otherwise indicated. It is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available.[8] 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.

6.1.2 *Purity of Water*—Distilled water conforming to Type II of Specification D1193.

6.1.3 *Substitute Ocean Water*—Artificial ocean water in accordance with Practice D1141, section on Preparation of Substitute Ocean Water, or a proprietary equivalent with a salinity of 33 to 34 ppt and pH 7.9 to 8.1.

6.1.4 *Extraction Media*—Activated carbon and, optionally, a chelating ion-exchange resin,[7] iminodiacetic (imminodiacetic) acid exchange resin on a styrene support, nominal particle size range approximately 0.300 to 0.850 mm (20 to 50 mesh) (see 5.1.3).

6.1.5 *Hydrochloric Acid (HCl)*—10 % v/v, aqueous solution.

6.1.6 *Sodium Hydroxide (NaOH)*—1.0 *N*, aqueous solution.

6.1.7 *Sodium Chloride (NaCl)*—5 *M*, aqueous solution.

6.2 *Analysis of Leachate*—Refer to Appendix X1 – Appendix X3 for Reagents and Materials requirements for the

[7] A filter cartridge, containing a chelating iminodiacetic (alternative spelling – imminodiacetic) acid ion-exchange resin on a styrene support (nominal particle size range approximately 0.300 to 0.850 mm (20 to 50 mesh)) of sufficient capacity to require regeneration only once a month or less frequently, has been found suitable.

[8] *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. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

analysis of specific organic biocides where these analytical methods are to be followed.

7. Hazards

7.1 **Warning**—Antifouling paints may contain toxic materials that could cause skin and eye irritation on contact and adverse physiological effect if ingested or inhaled. See antifouling coating supplier's Material Safety Data Sheet.

7.2 In the preparation of test specimens and the application of various types of paints, the use of appropriate protective clothing and equipment is required consistent with local, state, and federal government regulations, and recognized industrial and technical standards. Spills, overspray, and unused material shall not be flushed down the drain, but should be disposed of as hazardous waste.

7.3 Additional notes relating to the hazards associated with the analyses of specific organic biocides are given in Appendix X1 – Appendix X3.

8. Procedure

8.1 Prepare the exposure surfaces of three replicate test cylinders to provide a suitable surface for adhesion of the paint system to be applied. The surface area to be painted shall be lightly abraded with 200-grit sandpaper to promote adhesion. Before coating, wipe abraded area to remove dust. Mask the surfaces to remain uncoated (including the bottom 1 to 2 cm of the exterior circumferential surface of the test cylinder). Identify each cylinder to agree with coating sample code or designation.

8.2 Paints shall be manufactured a minimum of seven days before testing. Also, test paints shall not be allowed to age beyond the manufacturer's recommended shelf life. Provide typical storage conditions during aging, that is, sealed in a container commonly used for sale and held at 20 to 30°C (68 to 86°F).

8.3 For each coating, apply antifouling paint to the exterior circumferential surface of a set of three replicate test cylinders to produce a continuous band of antifouling paint with an exposure surface of 200 cm^2. Ensure surface is completely covered with finished dry film coating thickness of 100 to 200 μm (0.004 to 0.008 in.). If, during the test, the film thickness is expected to fall below 50 μm, then a greater thickness of paint shall be applied. Alternative surface areas are allowed when 200 cm^2 is not appropriate; deviations from the 200-cm^2 surface area shall be noted in the final report. Follow manufacturer's instructions with respect to mixing and drying. At a minimum, mechanically shake until the paint appears homogeneous. Apply using a brush, sponge paint applicator, or spray as recommended by the manufacturer. If the paint is marketed only in spray cans, then apply as a spray. If applied by brush, the film shall not show brush marks. After the final application, allow the paint to dry for 7 ± 1 day at 25 ± 2°C (77 ± 36°F) and 30 to 80 % relative humidity. Include application method and coating thickness in report.

8.4 Measure the initial dry film thickness using a suitable nondestructive procedure found in Test Method D1005 or another suitable nondestructive method and report the method used. Remove masking promptly after paint is dry. At the conclusion of the test, allow the test cylinders to dry for at least 12 h at ambient conditions and measure the film thickness again.

8.5 After the drying period, place one or more sets of three replicate coated cylinders coated with test paint and one blank (unpainted) cylinder in a holding tank. The painted surface on the cylinders must be completely submerged. Cylinders must be stationary and positioned so that substitute ocean water moving through the tank will flow around each cylinder.

8.6 Maintain the substitute ocean water within the prescribed range (see 5.1.4) by monitoring and adjusting the pH, salinity, and temperature of the substitute ocean water in the holding tank at least every third day from the start through to the end of the study (see Note 3). Monitor the pH and adjust if necessary using either dilute NaOH or dilute HCl. Monitor the salinity and adjust if necessary by adding distilled water or 5 *M* NaCl. Determine the organic biocide concentration in the holding tank at each sampling point and, where the coating contains a biocidal copper compound, also determine the copper concentration as specified in Test Method D6442 (see Note 4). Replace or regenerate the extraction media before the concentration of organic biocide exceeds 100 $\mu g\ L^{-1}$ and before the concentration of copper exceeds 100 $\mu g\ L^{-1}$.

NOTE 3—More frequent monitoring and adjustment of pH and salinity may be required to maintain the substitute ocean water within the prescribed range during the early stages of a study while the system equilibrates.

NOTE 4—This must be done even if the copper release rate is not being concurrently determined.

8.7 At 1, 3, 7, 10, 14, 21, 24, 28, 31, 35, 38, 42, and 45-day intervals, transfer all cylinders in a given set from the holding tank(s) into individual measuring containers, each containing 1500 mL of substitute ocean water that, before use, has been passed through a filter containing the extraction media. The substitute ocean water may additionally be passed through a 0.2-μm filter if required to remove microbial contamination. On each sampling day, randomly assign cylinders (blank and painted) to measuring containers. When transferring cylinders, lift the cylinders out of the holding tank, allow substitute ocean water to drain off, install the cylinder into the rotating device, and submerge the painted area into the substitute ocean water. Immediately start rotation of the cylinder at 60 ± 5 rpm and continue rotation for 1 h (see 8.7.1). When transferring the cylinders do not touch, or in any way damage the paint film, and do not allow the paint surface to dry. The transfer shall be completed as quickly as possible (generally in less than 5 min).

8.7.1 If, when a measurement is taken, the organic biocide concentration in the individual measuring container is determined to be >200 $\mu g\ L^{-1}$, the rotation period for the next measurement shall be reduced to less than 1 h, with the goal of ultimately building the rotation period back up to 1 h. The amount by which the rotation period is reduced shall be estimated based on familiarity with the coating being evaluated and experience with the test method and shall take into consideration the degree to which the measurement exceeded 200 $\mu g\ L^{-1}$. If the next measurement also exceeds 200 $\mu g\ L^{-1}$, the period of rotation shall be further reduced until the result

falls below 200 μg L^{-1}. Once a measurement has been taken that falls below 200 μg L^{-1}, the period of rotation shall be incrementally readjusted to a maximum of 1 h at the earliest possible point in the testing.

8.7.2 Any measurements taken in which the concentration of organic biocide in the individual measuring container was >200 μg L^{-1} shall be used to calculate release rate and shall be recorded in the final report.

8.7.3 Any measurements taken in which the period of rotation was less than 1 h shall be used to calculate the release rate and shall be recorded in the final report.

8.8 If testing beyond the minimum 45-day period is desired, the study may be extended. During the extended test, remove the cylinders from the holding tank at least once every 7 days to make a measurement of the release rate in accordance with the above procedure.

8.9 At the completion of the cylinder rotation, transfer the cylinder back to the holding tank. Withdraw approximately a 100-mL subsample of the substitute ocean water and follow the sample treatment and storage procedure detailed for each organic biocide in Annex A2. Withdraw a separate 100-mL subsample for each organic biocide to be quantified. If copper release rates are being simultaneously measured, withdraw an additional 100-mL subsample and treat in accordance with Test Method D6442.

8.10 *Sample Treatment*—Store samples in accordance with the procedures found in Annex A2 as appropriate until ready for analysis. Determine the concentration of organic biocide in each subsample using an analytical method that satisfies the acceptance criteria given in Table A2.1.

NOTE 5—It is important that the subsamples used for quantitation of organic biocide release rates are not acidified unless specified in the sample treatment for that biocide in Annex A2.

9. Calculation

9.1 Calculate the organic biocide concentration in each treated subsample (see 8.10) based on the instrument response for samples and blanks.

NOTE 6—If organic biocide is detected in the substitute ocean water used to fill the individual sampling containers, this shall be reflected in the calculation.

9.2 *Calculation of the Release Rate at Each Data Point (Sampling Day):*

9.2.1 Calculate the release rate (μg cm^{-2} d^{-1}) for each individual test cylinder.

$$R_{cyl} = (C_{biocide} \times V \times D)/(T \times A) \quad (1)$$

$$= (C_{biocide} \times 1.5 \times 24)/(1 \times 200)$$

$$= C_{biocide} \times 0.18 \ (for\ 200\ cm^2\ paint\ area)$$

where:

$C_{biocide}$ = concentration of biocide in substitute ocean water, μg L^{-1},
V = substitute ocean water volume in measuring container, L,
D = hours per day (24),
T = rotation period, h, and
A = area of paint, cm^2.

9.2.2 Calculate the mean release rate at each data point (sampling day) for each set of triplicate test cylinders.

9.3 *Calculation of Cumulative Biocide Release:*

9.3.1 Calculate the cumulative release of biocide from the start of the trial through Day 45 as follows:

$$R_{0,45} = \sum \bar{R}_{i,j}\,(j - i) = \sum \frac{(R_i + R_j)}{2}(j - i) \quad (2)$$

where:

$R_{0,45}$ = cumulative release (μg of organic biocide cm^{-2}) from the start of the trial through Day 45,
$\bar{R}_{i,j}$ = mean release rate (μg of organic biocide cm^{-2} d^{-1}) between consecutive sampling Days i and j for all data points between the start of the trial and Day 45,
i *and* j = time elapsed (days) since the start of the trial for each pair of consecutive data points, specifically 0 and 1, 1 and 3, 3 and 7 days, and so forth, respectively, and
R_i *and* R_j = mean release rates (μg of organic biocide cm^{-2} d^{-1}) for each set of triplicate test cylinders for each pair of consecutive data points from the start of the trial through Day 45, specifically Days 0 and 1, Days 1 and 3, Days 3 and 7, and so forth, respectively, and the release rate on Day 0 (R_0) is taken as 0 μg of organic biocide cm^{-2} d^{-1}.

9.3.2 The cumulative release of organic biocide for other periods of time may be calculated if required as follows:

$$R_{x,y} = \sum \bar{R}_{i,j}\,(j - i) = \sum \frac{(R_i + R_j)}{2}\,(j - i) \quad (3)$$

where:

$R_{x,y}$ = cumulative release (μg of organic biocide cm^{-2}) from Day x through to Day y,
$\bar{R}_{i,j}$ = mean release rate (μg of organic biocide cm^{-2} d^{-1}) between consecutive sampling Days i and j for all data points from Day x through Day y,
i *and* j = time elapsed (days) since the start of the trial for each pair of consecutive data points, for example, 0 and 1, 1 and 3, 3 and 7 days, and so forth, respectively, and
R_i *and* R_j = mean release rates (μg of organic biocide cm^{-2} d^{-1}) for each set of triplicate test cylinders for each pair of consecutive data points from Day x through Day y, for example, on Days 0 and 1, Days 1 and 3, Days 3 and 7, and so forth, respectively, and where Day 0 is included, the release rate on Day 0 (R_0) is taken as 0 μg of organic biocide cm^{-2} d^{-1}.

NOTE 7—Previous editions of Test Method D6442 calculated the cumulative (copper) release as follows: $R_1 + 2(R_3) + 4(R_7) + 3(R_{10}) + 4(R_{14}) + 7(R_{21}) + 3(R_{24}) + 4(R_{28}) + 3(R_{31}) + 4(R_{35}) + 3(R_{38}) + 4(R_{42}) + 3(R_{45})$, where R_1, R_3, R_7, R_{10}, and so forth are the release rates for sampling Days 1, 3, 7, 10, and so forth, respectively. The current data treatment provides a more accurate calculation of the cumulative release. However, the formulas presented in 9.3.1 and 9.3.2 are still simple

representations of cumulative release and may not provide a fully accurate estimation of cumulative release under the test conditions, particularly if the rate of release is changing rapidly over the test period.

9.4 *Mean Release Rate:*

9.4.1 Calculate the mean release rate (μg of organic biocide cm^{-2} d^{-1}) from Day 21 through the end of the trial as follows:

$$\bar{R}_{21,end} = \frac{\sum \bar{R}_{i,j}\,(j-i)}{\sum\,(j-i)} = \frac{\sum \frac{(R_i+R_j)}{2}(j-i)}{\sum\,(j-i)} \quad (4)$$

where:

$\bar{R}_{21,end}$ = mean release rate (μg of organic biocide cm^{-2} d^{-1}) between Day 21 and the last day of sampling,

$\bar{R}_{i,j}$ = mean release rate (μg of organic biocide cm^{-2} d^{-1}) between consecutive sampling Days *i* and *j* for all data points from Day 21 through the last day of sampling,

i and j = time elapsed (days) since the start of the trial for each pair of consecutive data points, specifically Days 21 and 24, 24 and 28, 28 and 31, and so forth, respectively, and

R_i *and* R_j = mean release rates (μg of organic biocide cm^{-2} d^{-1}) for each triplicate set of test cylinders for each pair of consecutive data points from Day 21 through the last day of sampling, specifically Days 21 and 24, Days 24 and 28, Days 28 and 31, and so forth, respectively.

NOTE 8—Eq 4 calculates the weighted mean release rate, taking into account any differences in time between data points, and is a more valid treatment of the data than calculation of the simple arithmetic mean of the data. The calculation may be conveniently done using a suitable computer-generated spreadsheet.

9.4.2 Eq 4 may be modified to calculate the mean release rate over other periods if required.

9.5 If the coating exhibits a pseudo-steady state, calculate the pseudo-steady-state biocide release rate as follows:

$$\bar{R}_{PSS} = \frac{\sum \bar{R}_{i,j}(j-i)}{\sum\,(j-i)} = \frac{\sum \frac{(R_i+R_j)}{2}(j-i)}{\sum\,(j-i)} \quad (5)$$

where:

$\bar{R}_{PSS}$ = mean organic biocide release rate (μg of organic biocide cm^{-2} d^{-1}) over the pseudo-steady-state period, Day *x* to Day *y*,

$\bar{R}_{i,j}$ = mean release rate (μg of organic biocide cm^{-2} d^{-1}) between consecutive sampling Days *i* and *j* for all data points from Day *x* through Day *y*,

i and j = time elapsed (days) since the start of the trial for each pair of consecutive data points, for example, Days 21 and 24, 24 and 28, 28 and 31, and so forth , respectively, and

R_i *and* R_j = mean release rates (μg of organic biocide cm^{-2} d^{-1}), for each set of triplicate test cylinders for each pair of consecutive data points, for example Days 21 and 24, Days 24 and 28, Days 28 and 31, and so forth, respectively.

9.5.1 For the purposes of this test method, a "pseudo-steady state" is defined as being a period of at least 24 days and containing 4 or more data points in which the arithmetic mean of the release rate values for each set of triplicate test cylinders at each data point differs from the weighted mean release rate over the calculation period by no more than 15 %, and the final day of the pseudo-steady state is the final day of the trial.

NOTE 9—Not all coatings will exhibit a pseudo-steady state. When a coating does exhibit a pseudo-steady state, the determined pseudo-steady-state biocide release rate should not be assumed necessarily to reflect a true steady-state release rate under the conditions of the test as the release rate of the coating may continue to change beyond the test period.

10. Report

10.1 Report the following information:

10.1.1 Report the concentration in $\mu g\ L^{-1}$ of organic biocide in the substitute ocean water of the holding tank and the measuring tank and the rate of organic biocide release ($\mu g\ cm^{-2}\ d^{-1}$) for each sampling point (give values for individual replicates as well as the mean). Plot the rate of organic biocide release as a function of time (use linear axes).

10.1.2 Report the cumulative release of organic biocide from the start of the trial through Day 45 (9.3.1), and report the mean organic biocide release rate for Days 21 through the end of the study (9.4.1). Also, when calculated, report the cumulative release over other periods (9.3.2), the mean release rate over other periods (9.4.2), and the pseudo-steady-state release rate (9.5).

10.1.3 Report samples where the concentration of biocide exceeded 200 $\mu g\ L^{-1}$ in the measuring container and samples in which the period of rotation was less than 1 h.

10.1.4 When the coating contains a biocidal copper compound, report samples where the concentration of copper exceeded 100 $\mu g\ L^{-1}$ in the holding tank.

10.1.5 Report the limit of quantitation for the organic biocide in substitute ocean water determined by the laboratory performing the test method in accordance with Annex A3.

10.1.6 Report the coating application method and initial coating dry film thicknesses (8.3) and final coating dry film thickness (8.4).

10.1.7 Report the pH, temperature, and salinity in the holding tank at each monitoring point (8.6).

10.1.8 Report any deviations from this test method or the requirements of this test method.

11. Precision and Bias

11.1 *Precision:*

11.1.1 *Repeatability:*

11.1.1.1 *DCOIT*—The mean DCOIT release rates from Day 21 through 45 for three individual test cylinders using the same batch of paint and concurrently measured in the same laboratory by the same operators using the same equipment were 10.9 $\mu g\ cm^{-2}\ d^{-1}$, 11.3 $\mu g\ cm^{-2}\ d^{-1}$, and 10.4 $\mu g\ cm^{-2}\ d^{-1}$. Based on these results, the determined precision under repeatability condition for this test method is ±4.3 % relative standard deviation.

11.1.1.2 *CuPT*—The mean CuPT release rate from Day 21 through 45 for three individual test cylinders using the same batch of paint and concurrently measured in the same laboratory by the same operator using the same equipment were 1.46 $\mu g\ cm^{-2}\ d^{-1}$, 1.42 $\mu g\ cm^{-2}\ d^{-1}$, and 1.39 $\mu g\ cm^{-2}\ d^{-1}$. Based on

these results, the determined precision under repeatability condition for this test method is ±2.0 % relative standard deviation.

11.1.1.3 *ZPT*—The mean ZPT release rate from Day 21 through 45 for three individual test cylinders using the same batch of paint and concurrently measured in the same laboratory by the same operator using the same equipment were 6.13 μg cm^{-2} d^{-1}, 6.06 μg cm^{-2} d^{-1}, and 6.12 μg cm^{-2} d^{-1}. Based on these results, the determined precision under repeatability condition for this test method is ±0.5 % relative standard deviation. The release rate of ZPT from one paint was tested in one laboratory at two different times, and those tests were separated by five years. In the first test, the Day 21 to 45 mean release rate was calculated to be 6.5 ± 0.9 μg cm^{-2} d^{-1} and in the second test, the Day 21 to 45 mean release rate was calculated to be 6.1 ± 0.4 μg cm^{-2} d^{-1}.

11.1.1.4 *CDMTD*—The mean CDMTD release rate from Day 21 through 45 for three individual test cylinders using the same batch of paint and concurrently measured in the same laboratory by the same operators using the same equipment were 4.31μg cm^{-2} d^{-1}, 4.90 μg cm^{-2} d^{-1}, and 5.15 μg cm^{-2} d^{-1}. Based on these results, the determined precision under repeatability condition for this test method is ±8.9 % relative standard deviation.

11.1.2 *Reproducibility*—The reproducibility of the procedure in this test method for measuring organic biocide release rates from antifouling coating compositions is being determined and will be available on or before December 2011. Participating laboratories will participate in a combined round robin effort on both this test method and Test Method D6442 (copper release rate method). By doing this, participating laboratories will benefit from economy of effort, and the joint round robin will result in reproducibility data for multiple test methods.

11.2 *Bias*—No information can be presented on bias for this procedure for measuring the organic biocide release rate from antifouling coatings because no material having an accepted reference value is available.

11.3 Refer to Appendix X1 – Appendix X3 for information on the precision and bias of the given test methods for quantitation of organic biocide in substitute ocean water.

12. Keywords

12.1 antifouling coating; copper pyrithione (CuPT); *N*-cyclopropyl-*N'*-(1,1-dimethylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine (CDMTD); organic biocide; release rate; zinc pyrithione (ZPT); 4,5-dichloro-2-n-octylisothiazolin-3-one (DCOIT)

ANNEXES

(Mandatory Information)

A1. DESCRIPTION OF PROPOSED TESTING APPARATUS

A1.1 A 200-cm^2 antifouling paint film of specified thickness is applied to the outer curved surface of an inert cylinder closed at one end. This cylinder is suspended with its closed end immersed within and concentric with a larger inert cylinder holding substitute ocean water. The coated internal cylinder is rotated about its axis at 60 ± 5 rpm to produce a peripheral speed of about 0.2 m s^{-1} (about 0.4 knots) (see Fig. A1.1 – required baffles not shown).

Test Container Dimensions:

Capacity, liter	2
Inside Diameter, cm	12.7
Outside Diameter, cm	13.5
Height (without cover), cm	19

Rotating Test Cylinder Dimensions:

D = Approximately 6.4 cm (nominal 2-1/2")
H = 12 cm minimum, immersion depth
L = 10 cm coated section
X = 1 cm uncoated band

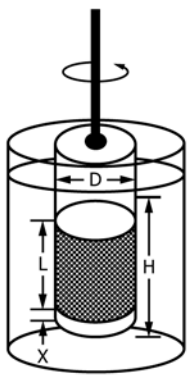

FIG. A1.1 Test Cylinder in Release Rate Measuring Container (Required Baffles Not Shown)

A2. THE ANALYSIS OF ORGANIC BIOCIDES IN SUBSTITUTE OCEAN WATER

A2.1 Scope

A2.1.1 The measurement of the release rate of an organic biocide from an antifouling coating by this test method calls for the quantitation of the biocide in substitute ocean water at low concentrations. The analytical method used to determine the biocide concentration must therefore meet certain acceptance criteria to ensure that an appropriate level of precision and accuracy is achieved.

A2.1.2 This annex describes the acceptance criteria for analytical methods to be used for the determination of the concentrations of DCOIT, CuPT, ZPT, and CDMTD in substitute ocean water test samples, which have been generated in accordance with this test method. These acceptance criteria cover the limit of quantitation for the biocide in substitute ocean water by the analytical method, precision, recovery, linearity, and other parameters as specified in Table A2.1.

A2.1.3 When a LOQ criterion is specified, the LOQ for the biocide in substitute ocean water by the analytical method is determined in accordance with the procedure given in Annex A3.

TABLE A2.1 Acceptance Criteria for Analytical Methods to be Used for the Quantitation of Biocide in Leachate Subsamples

Analytical Parameter	Acceptance Criteria for DCOIT	ZPT	CuPT	CDMTD
Accuracy	Spike recovery at the specified LOQ (25 µg L^{-1}) and 100 µg L^{-1} DCOIT in substitute ocean water shall be between 70 to 125 % of target.	Spike recovery at the specified LOQ (2.0 µg L^{-1}) and 20 µg L^{-1} of ZPT in substitute ocean water shall be between 70 to 125 % at 2.0 µg L^{-1} and 80 to 120 % at 20 µg L^{-1}.	Spike recovery at specified LOQ (2.0 µg L^{-1}) and 20 µg L^{-1} of CuPT in substitute ocean water shall be between 70 to 125 % at 2.0 µg L^{-1} and 80 to 120 % at 20 µg.	Spike recovery at specified LOQ (15 µg L^{-1}) and 50 µg L^{-1} of CDMTD in substitute ocean water shall be between 70 to 125 % at 15 µg L^{-1} and 80 to 120 % at 50 µg L^{-1}.
Repeatability	Repeatability for a minimum of five replicate analyses of calibration standards shall show a relative standard deviation of ±15 % or less.	Repeatability for a minimum of five replicate analyses of calibration standards shall show a relative standard deviation of ±15 % or less.	Repeatability for a minimum of five replicate analyses of calibration standards shall show a relative standard deviation of ±15 % or less.	Repeatability for a minimum of five replicate analyses of calibration standards shall show a relative standard deviation of ±15 % or less.
Reproducibility	Analysis of the seven or more DCOIT spikes used to determine the LOD in accordance with Annex A3 shall show a relative standard deviation of ±15 % or less.	Analysis of the seven or more ZPT spikes used to determine the LOD in accordance with Annex A3 shall show a relative standard deviation of 20 % or less.	Analysis of the seven or more CuPT spikes used to determine the LOD in accordance with Annex A3 shall show a relative standard deviation of ±20 % or less.	Analysis of the seven or more CDMTD spikes used to determine the LOD in accordance with Annex A3 shall show a relative standard deviation of ±15 % or less.
Specificity	When chromatographic methods are used, the retention time of the analyte shall match that of a certified standard.	When chromatographic methods are used, the retention time of the analyte shall match that of a certified standard.	When chromatographic methods are used, the retention time of the analyte shall match that of a certified standard.	When chromatographic methods are used, the retention time of the analyte shall match that of a certified standard.
Limit of Detection (LOD)	The LOD for the quantitation of DCOIT in substitute ocean water by the method shall be 7.8 µg L^{-1} or less, determined in accordanc ewith Annex A3.	The LOD for the quantitation of ZPT in substitute ocean water by the method shall be 0.6 µg L^{-1} or less, determined in accordancewith Annex A3.	The LOD for the quantitation of CuPT in substitute ocean water by the method shall be 0.6 µg L^{-1} or less,determined in accordancewith Annex A3.	The LOD for the quantitation of CDMTD in substitute ocean water by the method shall be 3.5 µg L^{-1} or less, determined in accordancewith Annex A3.
Limit of Quantitation (LOQ)	The LOQ for the quantitation of DCOIT in substitute ocean water by the method shall be 25.0 µg L^{-1} or less, determined in accordance with Annex A3.	The LOQ for the quantitation of ZPT in substitute ocean water by the method shall be 2.0 µg L^{-1} or less, determined in accordance with Annex A3.	The LOQ for the quantitation of CuPT in substitute ocean water by the method shall be 2.0 µg L^{-1} or less, determined in accordance with Annex A3.	The LOQ for the quantitation of CDMTD in substitute ocean water by the method shall be 15.0 µg L^{-1} or less, determined in accordance with Annex A3.
Linearity	A minimum of five calibration standards covering the working range of the method and analyzed in duplicate shall show a correlation coefficient (R^2) of 0.99 or higher.	A minimum of five calibration standards covering the working range of the method and analyzed in duplicate shall show a correlation coefficient (R^2) of 0.99 or higher.	A minimum of five calibration standards covering the working range of the method and analyzed in duplicate shall show a correlation coefficient (R^2) of 0.99 or higher.	A minimum of five calibration standards covering the working range of the method and analyzed in duplicate shall show a correlation coefficient (R^2) of 0.99 or higher.

A2.1.4 The results of this test method will be adversely affected if the biocide is strongly adsorbed or absorbed by the release rate measuring container or the test cylinder, or both. Suitable substantially inert materials of construction for these items for use with each biocide are specified in A2.3.

A2.1.5 Some organic biocides in solution in substitute ocean water undergo rapid photodegradation when exposed to natural or synthetic visible light sources. In such cases, the holding tank, the release rate measuring container, and subsamples must be protected from exposure, see 5.1.1, 5.1.3, and 8.9. The requirement for these steps to be taken on any particular organic biocide is indicated in A2.4.

A2.1.6 Additionally, sample treatment and storage requirements for each leachate sample containing each biocide are specified in A2.5.

A2.2 Acceptance Criteria for Analytical Methods

A2.2.1 Analytical methods used for determining the concentration of biocide in leachate subsamples generated in accordance with Section 8 shall meet the acceptance criteria for that biocide given in Table A2.1.

A2.3 Release Rate Measuring Containers and Test Cylinders

A2.3.1 The release rate measuring container and associated rods (5.1.1) and test cylinders (5.1.5) for use with each biocide shall be made of the materials specified in Table A2.2.

A2.3.2 *Polymethyl methacrylate apparatus*—Affix the rods to the release rate measuring container with a minimum quantity of a 2-part epoxy adhesive. Affix the bottom end-disk to the test cylinder using a minimum quantity of a 2-part epoxy adhesive to form a watertight joint, and carefully remove any excess adhesive (see Notes A2.1 and A2.2).

A2.3.3 *Polycarbonate apparatus*—Affix the rods to the release rate measuring container using acetone or methylene chloride. Affix the bottom end-disk to the test cylinder using acetone, methylene chloride or a polycarbonate cement to form a watertight joint (see Note A2.2).

A2.3.4 *Borosilicate glass apparatus*—Affix the rods to the release rate measuring container using glass-blowing techniques or use a minimum quantity of a 2-part epoxy adhesive. Affix the bottom end-disk to the test cylinder using glass-blowing techniques or use a minimum quantity of a 2-part epoxy adhesive to form a watertight joint, and carefully remove any excess adhesive (see Notes A2.1 and A2.2).

TABLE A2.2 Specified Materials for the Release Rate Measuring Container and Associated Rods (5.1.1) and Test Cylinders (5.1.5) for Use with Each Biocide

Biocide	Material
DCOIT	Polymethyl methacrylate or borosilicate glass
ZPT	Polycarbonate or borosilicate glass
CuPT	Polycarbonate or borosilicate glass
CDMTD	Polycarbonate or borosilicate glass

NOTE A2.1—Excess adhesive can be conveniently removed with a solvent-soaked swab when wet, or with a sharp blade when dry.

NOTE A2.2—Prefabricated one-piece test cylinders with an integral sealed bottom-end can be used as an alternative (see 5.1.5).

A2.3.5 Test cylinders and release rate measuring containers for use with DCOIT must be washed and rinsed with water and then rinsed with methanol before use. Test cylinders and release rate measuring containers for use with ZPT, CuPT or CDMTD must be washed and rinsed with water before use.

A2.4 Photosensitivity of Organic Biocides

A2.4.1 When, for the purposes of this test method, a biocide is considered to be photosensitive, the release rate measuring container, the holding tank, and substitute ocean water subsamples shall be protected from exposure to natural and synthetic light.

NOTE A2.3—When, for the purposes of this test method, a biocide is not considered to be photosensitive, the release rate measuring container, the holding tank, and substitute ocean water subsamples do not require protection from exposure to natural and synthetic light.

A2.4.2 *DCOIT*—For the purposes of this test method, DCOIT is not considered to be photosensitive

A2.4.3 *CuPT*—For the purposes of this test method, CuPT is considered to be photosensitive.

A2.4.4 *ZPT*—For the purposes of this test method, ZPT is considered to be photosensitive.

A2.4.5 *CDMTD*—For the purposes of this test method, CDMTD is not considered to be photosensitive.

A2.5 Sample Treatment and Storage

A2.5.1 *DCOIT*—Subsamples shall be transferred directly into a properly labeled glass container—do not filter. Refrigerate the subsample at 2 to 7°C (36 to 45°F) until analyzed. The subsamples may be refrigerated for up to one week if necessary before analysis.

A2.5.2 *ZPT and CuPT*—Subsamples shall be transferred directly into a properly labeled amber glass sample container and a 4-mL sample of this shall be immediately derivatized as specified for working standards in X2.6.1.4 or X2.6.2.4—do not filter. Refrigerate the subsample at 2 to 7°C (36 to 45°F) until analyzed. The subsamples may be refrigerated for up to three days if necessary before analysis.

A2.5.3 *CDMTD*—Subsamples shall be transferred directly into a properly labeled glass or plastic container—do not filter. Refrigerate the subsample at 2 to 7°C (36 to 45°F) until analyzed. The subsamples may be refrigerated for up to two weeks if necessary before analysis.

A3. DETERMINATION OF THE LOQ FOR ORGANIC BIOCIDE IN SUBSTITUTE OCEAN WATER FOR THE ANALYTICAL METHOD

A3.1 Scope

A3.1.1 This procedure is based on the U. S. Environmental Protection Agency Method Detection Limit (MDL) procedure found in Title 40 Code of Federal Regulations Part 136 (40 CFR 136, Appendix B, revision 1.11).

A3.1.2 This procedure was designed for applicability to a broad variety of physical and chemical methods and is device- or instrument-independent. The procedure shall be applicable to any analytical method used to assay organic biocide in substitute ocean water.

A3.1.3 The procedure requires a complete, specific, and well-defined analytical method.

A3.1.4 It is essential that all sample processing steps of the analytical method be included in the determination of the LOQ.

A3.1.5 This procedure shall be performed before organic biocide release rate measurements are started in a laboratory, whenever changes are made to the instrumentation or analytical method, and repeated at least annually.

A3.2 Procedure

A3.2.1 Make an estimate of the detection limit using one of the following:

A3.2.1.1 The concentration value that corresponds to an instrument signal/noise ration in the range of 2.5 to 5.

A3.2.1.2 The concentration equivalent of three times the standard deviation of replicate instrumental measurements of the analyte in substitute ocean water.

A3.2.1.3 That region of the standard curve in which there is a significant change in sensitivity, that is, a break in the slope of the standard curve.

TABLE A3.1 Student's *t* Values at the 99 % Confidence Level

Number of Replicates	Degrees of Freedom (*n* - 1)	$t_{n-1,\ 1-\alpha\ =\ 0.99)}$
7	6	3.143
8	7	2.998
9	8	2.896
10	9	2.821
11	10	2.764
16	15	2.602
21	20	2.528
26	25	2.485
31	30	2.457
61	60	2.390
∞	∞	2.326

A3.2.1.4 Instrumental limitations.

A3.2.2 Prepare substitute ocean water that is as free of organic biocide as possible.

A3.2.3 Prepare a spike in substitute ocean water at a concentration between one and five times the estimated detection limit.

A3.2.4 Take a minimum of seven aliquots of the spike and process each through the entire analytical method, that is, each aliquot shall be subjected to all specified sample treatment, intermediate sample preparation, and processing steps before analysis.

A3.2.5 If a blank measurement is required to calculate the measured level of analyte, obtain a separate blank measurement for each spike aliquot analyzed. The average blank measurement is subtracted from the respective sample measurements.

A3.3 Calculations

A3.3.1 Calculate the standard deviation (S) of the replicate measurements as follows:

$$S = \sqrt{\frac{1}{n-1}\left[\sum x_i^2 - \frac{\left(\sum x_i\right)^2}{n}\right]} \quad \text{(A3.1)}$$

A3.3.2 Calculate the LOQ as follows:

$$LOQ = 10 \times S \quad \text{(A3.2)}$$

A3.3.3 Calculate the limit of detection (LOD) as follows:

$$LOD = t_{(n-1,\ 1-\alpha=0.99)} \times S \quad \text{(A3.3)}$$

where:

$t_{(n-1,\ 1-\alpha\ =\ 0.99)}$ = Student's t-value appropriate for a 99 % confidence level and a standard deviation estimate with n - 1 degrees of freedom. See Table A3.1.

A3.3.4 If the level of organic biocide in the spike used was below the determined LOD or exceeds ten times the LOD, do not use the calculated LOD or LOQ. The procedure must be repeated with a suitable concentration.

A3.3.5 This procedure shall be repeated whenever any changes are made to the instrumentation or analytical method that may affect the performance of the method. If no changes are made to the instrumentation or the analytical method, this procedure shall be repeated at least annually.

APPENDIXES

(Nonmandatory Information)

X1. HPLC ANALYSIS OF SUBSTITUTE OCEAN WATER SUBSAMPLES FOR 4,5-DICHLORO-2-n-OCTYLISOTHIAZOLIN-3-ONE (DCOIT)

X1.1 Scope

X1.1.1 The analytical method used to quantify the concentration of 4,5-dichloro-2-n-octylisothiazolin-3-one (DCOIT) in substitute ocean water must meet the acceptance criteria given in Annex A2 to allow the biocide release rate from an antifouling coating to be determined with an appropriate degree of precision and accuracy.

X1.1.2 The analytical method described in this appendix is direct quantitation of DCOIT in substitute ocean water by high performance liquid chromatography (HPLC). The practical limits for quantifying DCOIT release rates using this analytical method are from 3.8 to 500 $\mu g\ cm^{-2}\ d^{-1}$, provided that the laboratory conducting the test achieves an LOQ for DCOIT in substitute ocean water of 21 $\mu g\ L^{-1}$.

X1.1.3 Leachate test samples for analysis are generated, treated, and stored as specified in A2.3, A2.4, and A2.5.

X1.2 Apparatus (See also Section 5)

X1.2.1 *Liquid Chromatograph (HPLC)*—Equipped with a dual pump capable of gradient program, auto sampler capable of making 200-μL injections, and a variable wavelength ultraviolet (UV) detector capable of monitoring at 275 nm.

X1.2.2 *Analytical Column*—25 cm by 4.6 mm (internal diameter), reverse phase octadecylsilane (ODS, C-18), and 5-μm particle size.

X1.2.3 *Data System*—Use of an electronic data system capable of automated peak area integration is recommended.

X1.2.4 *Dispensers*—Automatic or repeating for reagents.

X1.2.5 *Volumetric pipettes*—Class A.

X1.2.6 *Appropriate Volumetric Flasks.*

X1.2.7 *HPLC Vials*—Glass, of appropriate volume, capped.

X1.3 Reagents and Materials (See also Section 6)

X1.3.1 *Methanol*—HPLC grade.

X1.3.2 *Acetonitrile*—HPLC grade.

X1.3.3 *Water*—Conforming to Type II of Specification D1193.

X1.3.4 *DCOIT Calibration Standard*—Standard of certified purity.

X1.4 Hazards

X1.4.1 Refer to Section 7 for general hazards associated with the handling and use of antifouling paints and coatings.

X1.4.2 Isothiazolones, such as DCOIT, are skin sensitizers and corrosive. Handle with extreme care. Wear safety glasses, goggles, gloves, and a lab coat when handling standards containing over 50 mg L^{-1} of DCOIT.

X1.4.3 Acetonitrile and methanol are volatile, flammable, and toxic organic solvents. Work with these solvents only in a well-ventilated area.

X1.5 Preparation of Reagents

X1.5.1 All reagents are used directly as supplied.

X1.6 Preparation of Calibration Standards

X1.6.1 *Preparation of Stock Standards:*

X1.6.1.1 *1000-mg L^{-1} (1000 parts per million, ppm) DCOIT*—Into a 100-mL volumetric flask, add 0.09 to 0.11 g (±0.1 mg) solid DCOIT. Dilute to the mark with methanol. Calculate actual concentration based on the weight of DCOIT standard added and its certified purity.

X1.6.1.2 *100-mg L^{-1} (100 ppm) DCOIT*—Transfer 10 mL of 1000-mg L^{-1} DCOIT stock standard to a 100-mL volumetric flask. Dilute to the mark with methanol. Calculate actual concentration.

X1.6.1.3 *50-mg L^{-1} (50 ppm) DCOIT*—Transfer 5 mL of 1000-mg L^{-1} DCOIT stock standard to a 100-mL volumetric flask. Dilute to the mark with methanol. Calculate actual concentration.

X1.6.1.4 These stock standards may be stored in a refrigerator at 2 to 7°C (36 to 45°F) for up to one month, after which they must be discarded and fresh stock standards prepared.

X1.6.2 *Working Standards:*

X1.6.2.1 Working standards are prepared fresh daily.

X1.6.2.2 *250-$\mu g\ L^{-1}$ DCOIT*—Using a volumetric pipette, transfer 0.5 mL of 50-mg L^{-1} DCOIT to a 100-mL volumetric flask. Dilute to the mark with water.

X1.6.2.3 *125-$\mu g\ L^{-1}$ DCOIT*—Using a volumetric pipette transfer 25.0 mL of 250-$\mu g\ L^{-1}$ DCOIT to a 50-mL volumetric flask. Dilute to mark with water.

X1.6.2.4 *100-$\mu g\ L^{-1}$ DCOIT*—Using a volumetric pipette, transfer 1.0 mL of 50-mg L^{-1} DCOIT to a 500-mL volumetric flask. Dilute to the mark with water.

X1.6.2.5 *50-$\mu g\ L^{-1}$ DCOIT*—Using a volumetric pipette, transfer 25.0 mL of 100-$\mu g\ L^{-1}$ DCOIT to a 50-mL volumetric flask. Dilute to mark with water.

X1.6.2.6 *20-$\mu g\ L^{-1}$ DCOIT*—Using a volumetric pipette, transfer 10 mL of 100-$\mu g\ L^{-1}$ DCOIT to a 50-mL volumetric flask. Dilute to the mark with water.

TABLE X1.1 Solvent Gradient Program

Time (minutes)	% A	% B
0	100	0
2	100	0
8	20	80
16	20	80
18	100	0
22	100	0

X1.6.3 *DCOIT Control Standard in Substitute Ocean Water:*

X1.6.3.1 On each sampling day (see 8.7), prepare a fresh solution of DCOIT in substitute ocean water (see X1.6.3.2 – X1.6.3.4). This is done to ensure the integrity of DCOIT in substitute ocean water solution during sample storage and analysis.

X1.6.3.2 *250-µg L^{-1} DCOIT Stock Control* —Using a volumetric pipette, transfer 0.5 mL of 50-mg L^{-1} DCOIT to a 100-mL volumetric flask. Dilute to the mark with substitute ocean water (see 6.1.3). Store this sample in a refrigerator at 2 to 7°C (36 to 45°F).

X1.6.3.3 *50-µg L^{-1} DCOIT*—Using a volumetric pipette, transfer 5.0 mL of 250-µg L^{-1} DCOIT stock control to a 25-mL volumetric flask. Dilute to the mark with substitute ocean water (see 6.1.3). Store this sample in a refrigerator at 2 to 7°C (36 to 45°F) and analyze this control standard (duplicate injection) at the same time subsamples are analyzed.

X1.6.3.4 Report any point at which the response of the 50-µg L^{-1} control standard in substitute ocean water varies from the initial calibration by more than 20 %. Prepare a fresh control standard and recalibrate.

NOTE X1.1—Variation by more than 20 % may indicate that the DCOIT has degraded before analysis. This may affect the reliability of the calculated release rate for this data point.

X1.7 Analytical Procedure

X1.7.1 *HPLC Conditions:*

X1.7.1.1 *Wavelength*—275 nm.

X1.7.1.2 *Injection volume*—200 µL.

X1.7.1.3 *Analysis run time*—22 min.

X1.7.1.4 *Solvent Gradient Program*—See Table X1.1 where A is the water, B is the acetonitrile, and the nominal flow rate is maintained at 1.0 mL/min throughout.

X1.7.1.5 *Retention Time*—DCOIT at typically about 12 to 16 min (see Figs. X1.1-X1.4 for example chromatograms). The retention time will vary if the instrument or chromatographic procedure, or both, are changed.

X1.7.1.6 The HPLC parameters provided in X1.7.1.1 – X1.7.1.4 are typical starting points, and the composition of the mobile phase, the flow rate, the injection volume, and the column length may be varied if necessary to improve chromatographic resolution.

X1.7.2 *Column Conditioning*—Fill the solvent reservoir with appropriate solvents and install the HPLC column. Wash the column with 100 % organic for 10 min followed by equilibration of the system at the initial solvent strength for 15 min. Inject 100-µg L^{-1} working standard at least three times and check for peak shape, retention time, and area count reproducibility. Inject calibration standards to establish linearity.

X1.7.3 *Sample Calibration*—Use working standards (20 to 250 µg L^{-1}) to generate a calibration curve that defines the working range of the HPLC system by plotting concentration (*x*-axis) against the peak area (*y*-axis) for each DCOIT standard. DCOIT concentration in substitute ocean water shall be determined using the external standard, linear regression analysis method. Inject each standard at least twice. If the correlation coefficient is less than 0.995, prepare fresh calibration standards and recalibrate.

X1.7.4 *Sample Analysis:*

X1.7.4.1 Analyze a substitute ocean water blank (substitute ocean water that is stored at room temperature and has not been in contact with the painted cylinder) to detect the presence of interfering components. If a major interfering component is

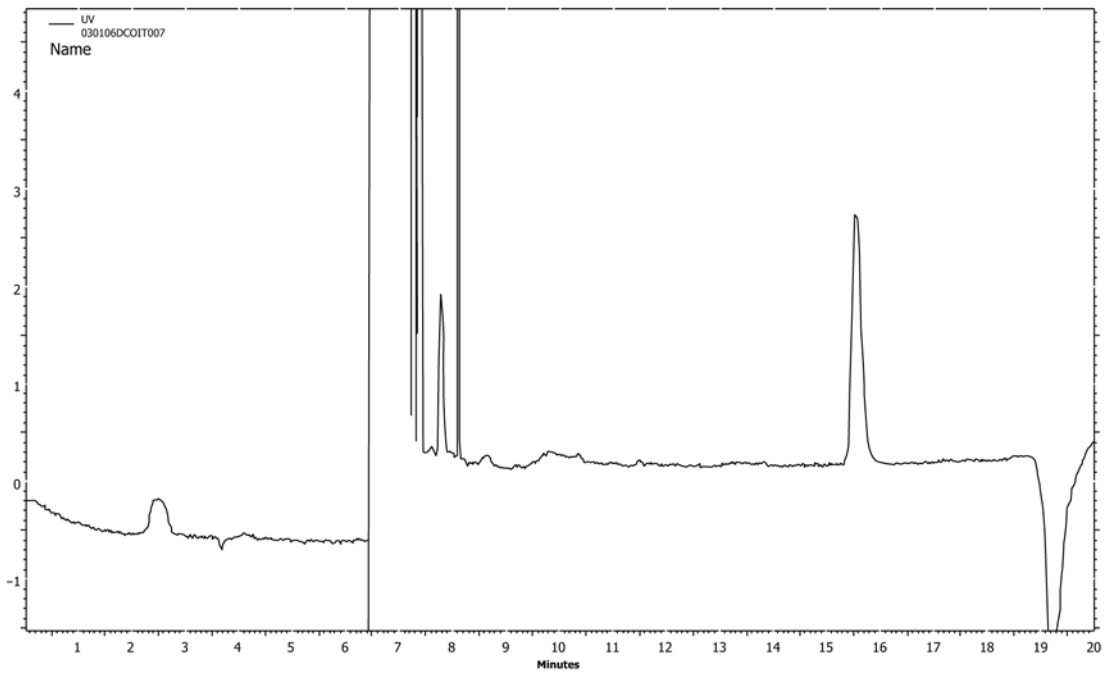

FIG. X1.1 100-µg L^{-1} (100-ppb) DCOIT Standard in Water

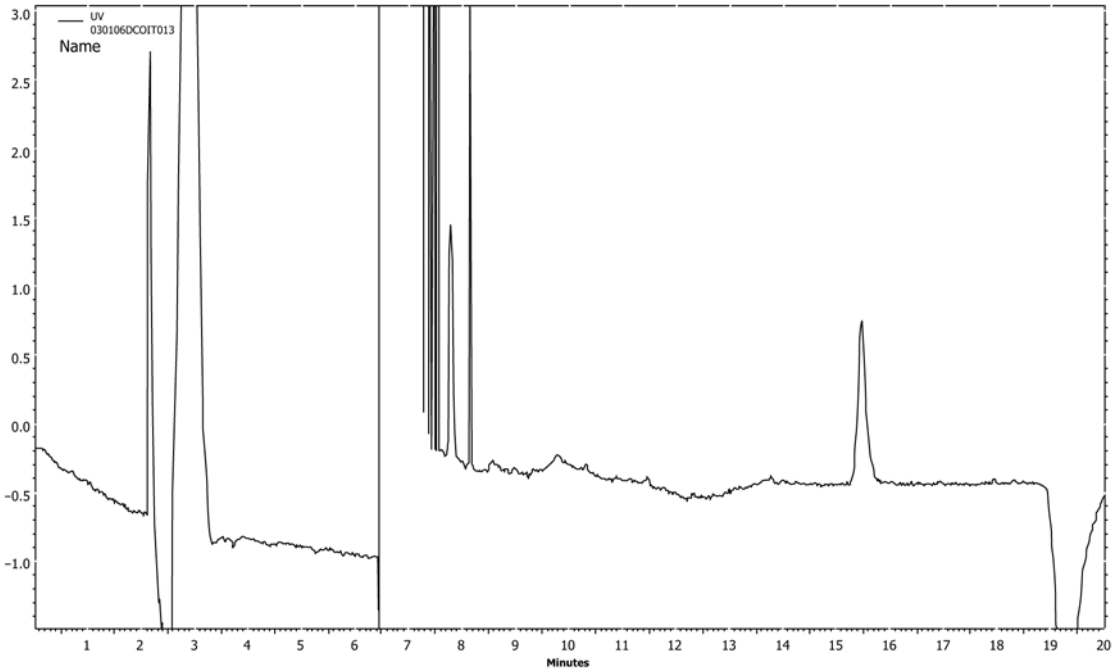

FIG. X1.2 50-μg L^{-1} (50-ppb) DCOIT Spike in Substitute Ocean Water

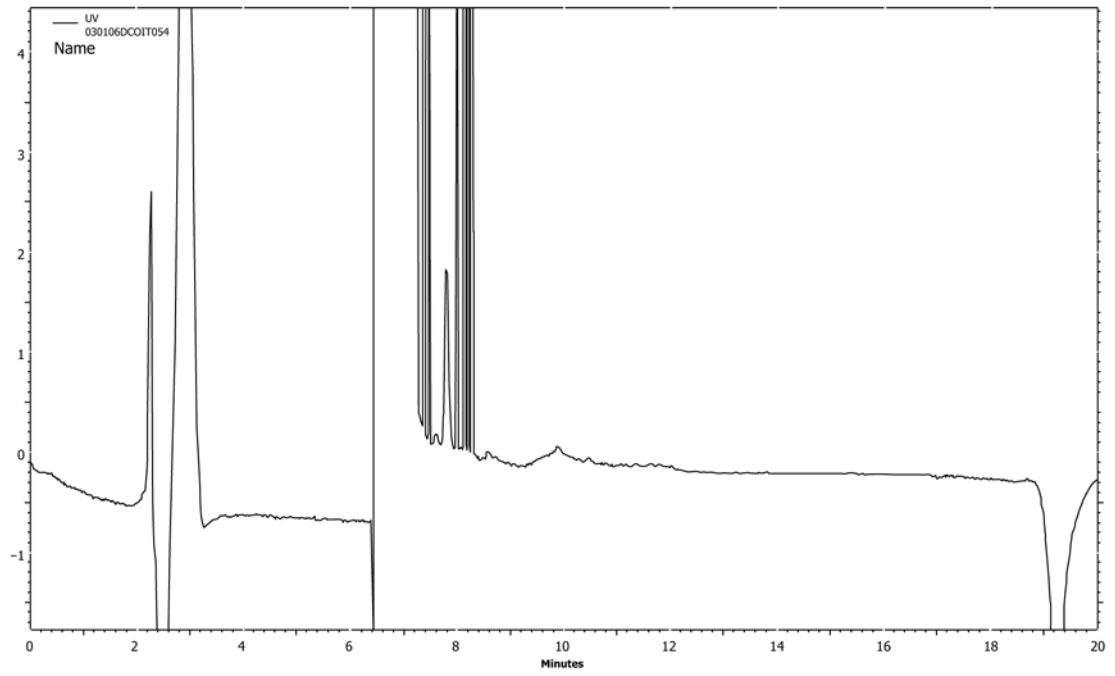

FIG. X1.3 Substitute Ocean Water Blank

detected in the blank, then change the chromatographic conditions to improve resolution and allow accurate quantitation of DCOIT.

X1.7.4.2 If a major interfering component is detected in the sample, then change the chromatographic conditions of the method to allow accurate quantitation of DCOIT.

X1.7.4.3 Use working standards (20 to 250 μg L^{-1}) at least twice during the sample analysis. Inject samples in duplicate (two injections per sample) without any workup (direct injection of seawater containing leached biocide) into the HPLC.

X1.8 Calculation (see also Section 9)

X1.8.1 If a computerized integrator is used for HPLC data analysis, then set it up to conduct linear regression analysis. Alternatively, using the calibration plot and the derived linear regression equation for concentration versus peak area (X1.7.3), determine the concentration of DCOIT in each sample and express as μg DCOIT L^{-1} of leachate (C_B).

X1.8.2 Calculate the DCOIT release rate in accordance with Section 9.

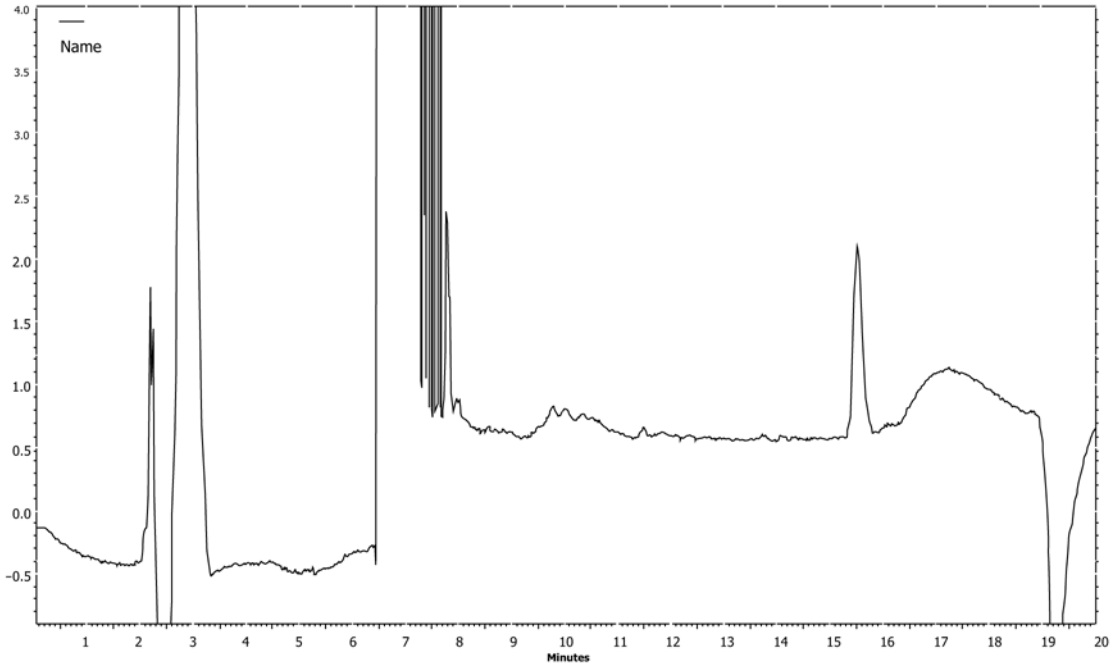

FIG. X1.4 Example of Leachate Subsample in Substitute Ocean Water Sample Taken During a Release Rate Study (Concentration is 55 μg L^{-1} DCOIT)

X1.9 Precision and Bias

X1.9.1 *Accuracy*—Substitute ocean water solutions containing 60, 201, and 502 μg L^{-1} DCOIT were analyzed against freshly prepared linear range of standards on five different days. Results indicate mean recoveries of 58 μg L^{-1} (96.4 % of expected), 181 μg L^{-1} (90.2 % of expected), and 505 μg L^{-1} (100.6 % of expected) DCOIT, respectively, for these solutions.

X1.9.2 *Precision*—The precision of this method was determined by analyzing solutions containing 60, 201, and 502 μg L^{-1} DCOIT in substitute ocean water on five different days. The overall precision of this method is 9.5 % (relative standard deviation) for substitute ocean water solutions containing 60- to 500-μg L^{-1} DCOIT.

X1.10 Limit of Detection and Quantitation

X1.10.1 The LOD and LOQ for DCOIT in substitute ocean water using this method has been determined in a single laboratory using the procedure described in Annex A3. The determined LOD and LOQ were 6 and 21 μg L^{-1} DCOIT in substitute ocean water, respectively.

X1.11 Report (See also Section 10)

X1.11.1 Samples containing ppm level DCOIT in substitute ocean water will be reported to the nearest 1 μg L^{-1}.

X2. HPLC ANALYSIS OF SUBSTITUTE OCEAN WATER SUBSAMPLES FOR COPPER PYRITHIONE AND ZINC PYRITHIONE BIOCIDE

X2.1 Scope

X2.1.1 The analytical method used to quantify the concentration of zinc pyrithione (ZPT) and copper pyrithione (CuPT) in substitute ocean water must meet the acceptance criteria given in Annex A2 to allow the biocide release rate from an antifouling coating to be determined with an appropriate degree of precision and accuracy.

X2.1.2 The analytical method described in this appendix is quantitation of ZPT or CuPT by HPLC following derivitization with pyridine disulfide. The practical limits for quantifying ZPT release rates using this analytical method are from 0.2 to 500 μg cm^{-2} d^{-1} provided that the laboratory conducting the test achieves an LOQ for ZPT in substitute ocean water of 1.1 μg L^{-1}, and the practical limits for quantifying CuPT release rates using this analytical method are from 0.16 to 500 μg cm^{-2} d^{-1} provided that the laboratory conducting the test achieves an LOQ for CuPT in substitute ocean water of 0.9 μg L^{-1}.

X2.1.3 Leachate test samples for analysis are generated, treated, and stored as specified in A2.3 – A2.5.

X2.2 Apparatus (See also Section 5)

X2.2.1 HPLC system consisting of programmable pump, auto injector, and UV detector monitored at 234 nm, column, autosampler capable of injecting 200 μL, and a chromatography data system.

X2.2.2 *Column,* a reverse phase octadecylsilane (ODS, C-18) based packing material with a hydrophilic end capping reagent. Particle size is 5 μm and the dimensions of the column are 2-mm inside diameter (ID) by 250-mm length such as

YMC ODS-AQ column 250 by 2 mm or equivalent. An equivalent reverse phase octadecylsilane-packed guard column can be used.

X2.2.3 *Amber Vials*—Glass, with caps, capacity 4.5 mL or larger.

X2.2.4 *Appropriate Glass or Plastic Pipettes and Amber Glass Volumetric Flasks.*

X2.3 Reagents and Materials (See also Section 6)

X2.3.1 *Acetonitrile*—HPLC grade.

X2.3.2 *Phosphoric Acid*—Reagent grade, 85 %.

X2.3.3 *Dimethyl Sulfoxide (DMSO)*—Minimum purity 99.7 %.

X2.3.4 *Pyridine Disulfide (PDS, also known as 2,2'Dithiopyridine)*—99 % + purity.

X2.3.5 *Ethylenediamine Tetraacetic Acid, Disodium Salt (EDTA)*—ACS grade.

X2.3.6 *Water*—Conforming to Type II of Specification D1193.

X2.3.7 *Mobile Phase*—In a glass container, prepare a mixture of acetonitrile, water, and phosphoric acid in a ratio of 250:750:1 (v/v).

X2.3.8 *ZPT*—Certified standard, minimum purity 97 % by weight.

X2.3.9 *CuPT*—Certified standard, minimum purity 95 % by weight.

X2.4 Hazards

X2.4.1 Refer to Section 7 for general hazards associated with handling and use of antifouling paints and coatings.

X2.4.2 Acetonitrile is a volatile, flammable, toxic, organic solvent. Work with this solvent only in a well-ventilated area.

X2.5 Preparation of Reagents

X2.5.1 *Preparation of the Derivatizing Reagent:*

X2.5.1.1 *PDS solution*—Into a 50-mL glass volumetric flask, weigh 50 ± 2 mg of PDS, add 16 mL acetonitrile, and mix to dissolve. Dilute to volume with water and mix well. Refrigerate the solution. This refrigerated solution is stable for one month.

X2.5.1.2 *EDTA solution*—Into a 50-mL glass volumetric flask, weigh 200 ± 5 mg EDTA. Dissolve the sample in water by mixing or sonicating. Dilute to volume with water and mix well. Refrigerate the solution. This refrigerated solution is stable for two months.

X2.5.1.3 *PDS/EDTA derivatizing agent*—This solution shall be made fresh daily. Mix 5 mL of PDS solution with 5 mL of EDTA solution. Store in a glass amber bottle.

X2.6 Preparation of Calibration Standards

X2.6.1 *ZPT:*

X2.6.1.1 *1000-mg L^{-1} Stock ZPT standard for zinc pyrithione analyses*—Into a 100-mL glass amber volumetric flask, weigh 0.09 to 0.11 g (±0.1 mg) of ZPT, add 50 mL of DMSO, and shake to dissolve and do not sonicate. Dilute to volume with DMSO and mix well. Calculate the actual concentration of the standard based on the purity of the ZPT and weighed standard. Store in a dark place. This standard is stable for two months.

X2.6.1.2 *Intermediate ZPT standard, 4 mg L^{-1}*—Pipette accurately 0.1 mL of 1000-mg L^{-1} ZPT standard into a 25-mL glass amber volumetric flask and dilute to volume with water. Mix well. Store in dark place. This standard shall be made fresh daily.

X2.6.1.3 *Working standards for ZPT*—Aliquot 25, 50, 100, 250, and 500 µL of 4-mg L^{-1} ZPT intermediate standard using a micropipette into separate 10-mL glass amber volumetric flasks. Dilute to volume with water, cap, and mix well. The working standards are nominally 10, 20, 40, 100, and 200 µg L^{-1}, respectively, and the actual concentration shall be calculated from the original standard. Fresh working standards shall be prepared at least every 8 h.

X2.6.1.4 Derivatize each working standard immediately by pipetting 4 mL into an amber autosampler vial and adding 200 µL of PDS/EDTA derivatizing reagent. Cap, mix well, and let it stand for 30 min.

X2.6.2 *CuPT:*

X2.6.2.1 *500-mg L^{-1} stock CuPT standard for CuPT analyses in DMSO*—Into a 100-mL glass amber volumetric flask, weigh 0.045 to 0.055 g (±0.1 mg) of CuPT, add 50 mL of DMSO, and shake to dissolve and do not sonicate. Dilute to volume with DMSO and mix well. Calculate the actual concentration of the standard based on the purity of the CuPT and weighed standard. Store in a dark place. This standard is stable for two months.

X2.6.2.2 *Intermediate CuPT standard, 1 mg L^{-1} (1 ppm) in acetonitrile*—Pipette accurately 0.050 mL of 500-mg L^{-1} CuPT standard into a 25-mL glass amber volumetric flask and dilute to volume with acetonitrile. Mix well. Store in a dark place. This standard shall be made fresh daily.

X2.6.2.3 *Working standards for CuPT in water*—Aliquot 10, 25, 50, 100, 200, and 500 µL of 1-mg L^{-1} CuPT intermediate standard using a micropipette into separate 10-mL glass amber volumetric flasks. Dilute to volume with water, cap, and mix well. The working standards are nominally 1.0, 2.5, 5, 10, 20, and 50 µg L^{-1}, respectively, and the actual concentration shall be calculated from the original standard. Fresh working standards shall be prepared at least every 8 h.

X2.6.2.4 Derivatize immediately each working standard by pipetting 4 mL into an amber glass autosampler vial and adding 200 µL of PDS/EDTA derivatizing reagent. Cap, mix well, and let it stand for 30 min.

X2.7 Analytical Procedure

X2.7.1 *HPLC Conditions:*

X2.7.1.1 *Wavelength*—234 nm.

X2.7.1.2 *Injection volume*—200 µL.

X2.7.1.3 *Run time*—22 min (see Fig. X2.1 for example chromatograms).

X2.7.1.4 *Gradient procedure*—See Table X2.1 where A is the mixed mobile phase acetonitrile, water, and phosphoric acid and B is the acetonitrile.

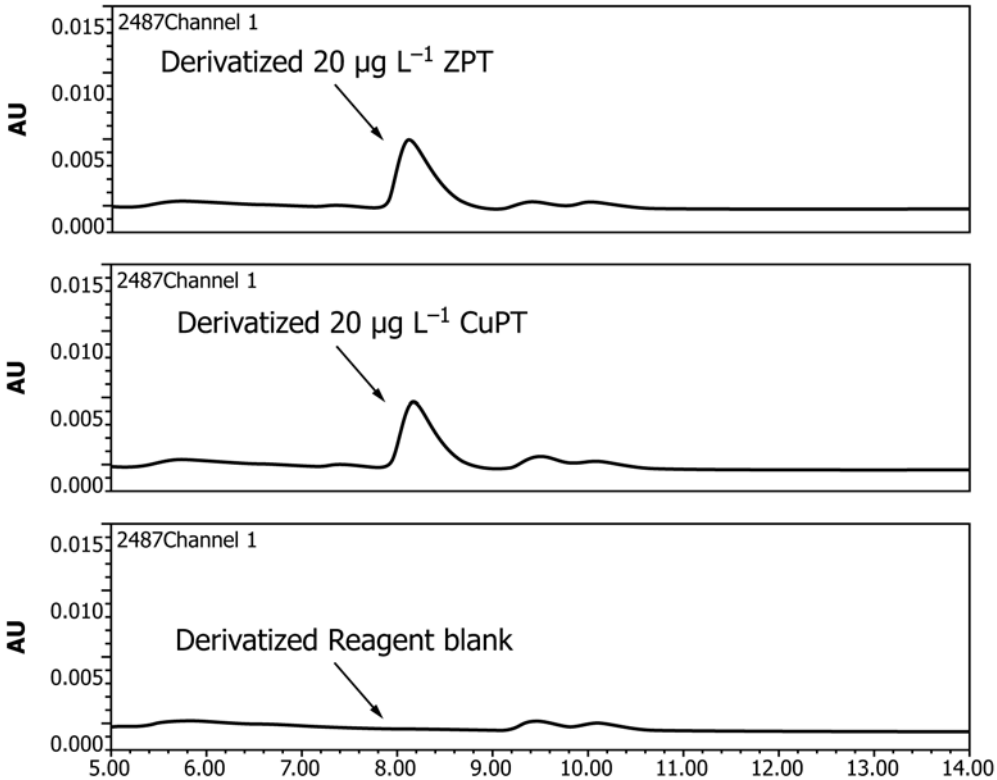

FIG. X2.1 Example Chromatograms for ZPT and CuPT Analysis

TABLE X2.1 Solvent Gradient Profile

Time in minutes	Flow Rate	% A	% B
0	0.3 mL/min	100	0
9.0	0.3 mL/min	100	0
9.1	0.3 mL/min	0	100
15.0	0.3 mL/min	0	100
15.1	0.5 mL/min	100	0
20.0	0.5 mL/min	100	0
20.1	0.3 mL/min	100	0
22.0	0.3 mL/min	100	0

X2.7.1.5 The HPLC parameters provided in X2.7.1.1 – X2.7.1.4 are typical starting points, and the composition of the mobile phase, flow rate, injection volume, and column length may be varied if necessary to improve chromatographic resolution.

X2.7.2 *Column Conditioning*—The UV lamp shall be warmed up for at least 50 min. The 50-μg L^{-1} CuPT standard or 100-μg L^{-1} ZPT standard shall be injected in triplicate. The first injection is used to equilibrate the column. The remaining two injections are used to check for peak shape, retention time, and area count.

X2.7.3 *Calibration*—Use the working standards prepared in X2.6.1.3 for ZPT or in X2.6.2.3 for CuPT to generate a calibration curve that defines the working range of the HPLC system by plotting concentration (*x*-axis) against the peak area (*y*-axis) for each pyrithione standard. The pyrithione concentration in substitute ocean water shall be determined using a linear regression analysis method. If the correlation coefficient is less than 0.995, prepare fresh calibration standards and recalibrate.

X2.7.4 *Sample Analysis:*

X2.7.4.1 Analyze a substitute ocean water blank (substitute ocean water that is stored at room temperature and has not been in contact with the painted cylinder) to detect the presence of interfering components. If a major interfering component is detected in the blank, the chromatographic conditions method shall be changed to improve resolution and allow accurate quantitation of CuPT or ZPT.

X2.7.4.2 Inject the samples after the instrument has been calibrated. It is recommended that standards are injected as sample at the beginning, in between, and at the end of the run of the samples to assure instrument stability and data accuracy in accordance with operating procedure of the testing laboratory.

X2.7.4.3 If a major interfering component is detected in the sample, the chromatographic conditions of the method shall be changed to allow accurate quantitation of CuPT or ZPT or both.

X2.8 Calculation

X2.8.1 Using the calibration plot and the derived linear regression equation for concentration versus peak area (X2.7.3), determine the concentration of the CuPT or ZPT in each sample and express as μg CuPT or μg ZPT per litre of leachate (C_B).

X2.8.2 Calculate the biocide release rate in accordance with Section 9.

X2.9 Precision and Bias

X2.9.1 Precision and accuracy has been determined on replicate (eight) sample substitute ocean water blanks spiked

with 5 and 50 μg L^{-1} of CuPT or ZPT. The mean recovery of 5-μg L^{-1} spikes was 112 % with a precision of ±4.5 % (standard deviation), and the mean recovery of 50-μg L^{-1} spikes was 104 % with a precision of ±2.9 %.

X2.10 Limit of Detection and Quantitation

X2.10.1 The LODs and LOQs for ZPT and CuPT in substitute ocean water using this method have been determined in a single laboratory using the procedure described in Annex A3. Based on the data and concentration of the calibration curve used in this method with a 200-μL sample size, the LOD and LOQ for ZPT in substitute ocean water by the analytical method are 0.35 and 1.10 μg L^{-1}, respectively, and the LOD and LOQ for CuPT in substitute ocean water by the analytical method are 0.28 and 0.90 μg L^{-1}, respectively.

X3. HPLC ANALYSIS OF SUBSTITUTE OCEAN WATER SUBSAMPLES FOR *N*-CYCLOPROPYL-*N'*-(1,1-DIMETHYLETHYL)-6-(METHYLTHIO)-1,3,5-TRIAZINE-2,4-DIAMINE

X3.1 Scope

X3.1.1 The analytical method used to quantify the concentration of *N*-cyclopropyl-*N'*-(1,1-dimethylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine (CDMTD) (CAS No. 28159-98-0) in substitute ocean water shall meet the acceptance criteria given in Annex A2 to allow the biocide release rate from an antifouling coating to be determined with an appropriate degree of precision and accuracy.

X3.1.2 The analytical method described in this appendix is quantitation of CDMTD by HPLC following solid-phase extraction (SPE). The practical limits for quantifying CDMTD release rates using this analytical method are 2.2 to 500 μg cm^{-2} d^{-1}, provided that the laboratory conducting the test achieves an LOQ for CDMTD in substitute ocean water of 12.0 μg L^{-1}.

X3.1.3 Leachate test samples for analysis are generated, treated, and stored as specified in A2.3 – A2.5.

X3.2 Apparatus (See also Section 5)

X3.2.1 A liquid chromatography system (HPLC) that consists of a programmable pump, multiwavelength UV detector capable of monitoring at 243 nm, column, data system and an autosampler capable of making 50-μL injections.

X3.2.2 *Analytical Column*—A reverse phase column, 25 cm by 4.6 mm ID such as ODS, C-18, and 5 μm particle size.

X3.2.3 *Autosampler Vials with Caps*—Capacity 2.5 mL.

X3.2.4 *Appropriate Pipettes and Volumetric Flasks*—Use glass apparatus to avoid adsorption.

X3.2.5 *Appropriate SPE Manifold and Cartridges.*

X3.3 Reagents and Materials (See also Section 6)

X3.3.1 *Methanol*—HPLC grade.

X3.3.2 *Acetic Acid*—Reagent grade, glacial.

X3.3.3 *Water*—Conforming to Type II of Specification D1193.

X3.3.4 *Extraction Media*—Octadecylsilane (C-18) bonded to silica gel (500-mg sorbent bed or larger).

X3.3.5 *CDMTD Standard*—Certified purity ≥98.5 %.

X3.3.6 *Isopropanol*—HPLC grade.

X3.3.7 *Mobile Phase:*

X3.3.7.1 *Mobile phase A*—Methanol:water:acetic acid, 70:30:1 (v/v) filtered through a 0.45-μm filter.

X3.3.7.2 *Mobile phase B*—Methanol filtered through a 0.45-μm filter.

X3.4 Hazards

X3.4.1 Refer to Section 7 for general hazards associated with handling and use of antifouling paints and coatings.

X3.4.2 CDMTD is an eye irritant and a mild skin sensitizer. Handle with care. Wear safety glasses, goggles, gloves, and a lab coat when handling analytical standards.

X3.4.3 Isopropanol and methanol are volatile, flammable, and toxic organic solvents. Work with these solvents only in a well-ventilated area.

X3.5 Preparation of Reagents

X3.5.1 All reagents are used directly as supplied.

X3.6 Preparation of Calibration Standards

X3.6.1 *CDMTD, Standard Stock Solution*—2.0-g L^{-1} nominal stock is prepared for analysis. Into a 100-mL volumetric flask, weigh 0.19 to 0.21 g (±0.1 mg) of CDMTD and dilute to volume with isopropanol. Calculate the actual concentration of the standard based on the purity of the CDMTD and weighed standard. This standard is stable for two months.

X3.6.2 *Intermediate CDMTD Standards:*

X3.6.2.1 *200-mg L^{-1} nominal*—Pipette accurately 5.0 mL of the 2.0-g L^{-1} CDMTD standard stock solution into a 50-mL volumetric flask and dilute to volume with isopropanol.

X3.6.2.2 *10-mg L^{-1} nominal*—Pipette accurately 5.0 mL of the 200-mg L^{-1} CDMTD standard stock solution into a 100-mL volumetric flask and dilute to volume with isopropanol.

X3.6.3 *Working Standards for CDMTD*—Pipette 0.5, 0.75, 1.0, 2.0, and 3.0 mL of the 10-mg L^{-1} nominal intermediate standard into separate 100-mL volumetric flasks. Add 70 mL of isopropanol and dilute to volume with water, cap, and mix well. The working standards are nominally 50, 75, 100, 200, and 300 μg L^{-1}, respectively, and the actual concentration shall be calculated from the original standard.

NOTE X3.1—Since the samples are concentrated by a factor of five during extraction (see X3.7), the nominal concentrations of the calibration standards correspond to 10, 15, 20, 40, and 60 μg L^{-1} CDMTD in substitute ocean water.

X3.6.4 *Control Standard*—Prepare a 20-μg/L spike of CDMTD in substitute ocean water by pipetting 1.0 mL of the

10-mg L^{-1} stock standard into a 500-mL volumetric flask and bring to volume with substitute ocean water.

X3.7 Preparation of Extracts

X3.7.1 Run a spike (X3.6.4) with each batch of extractions.

X3.7.2 *Extraction*—Place the desired number of C-18 columns on the SPE manifold.

X3.7.3 Condition the SPE columns with a 5-mL portion of methanol and two 5-mL portions of water.

X3.7.4 Attach a 75-mL reservoir with adapter to each column. Aspirate 50 mL of sample through at a rate less than 5 mL/min.

X3.7.5 Wash the sample with two 5-mL portions of water.

X3.7.6 Put a 10-mL volumetric flask into each position of the rack as necessary. Remove the manifold cover and dry the needles with a tissue. Place the rack inside the manifold and replace the cover.

X3.7.7 Elute with two approximately 3.5 mL portions of isopropanol.

X3.7.8 Remove the volumetric flasks and dilute to volume with water.

X3.8 Analytical Procedure

X3.8.1 *Chromatography (HPLC) Conditions:*

X3.8.1.1 *Wavelength*—243 nm.

X3.8.1.2 *Injection volume*—50 μL.

X3.8.1.3 *Analysis run time*—25 min.

X3.8.1.4 *Gradient procedure*—See Table X3.1 where A is the mixed mobile phase methanol, water and acetic acid, B is the methanol and the nominal flow rate is maintained at 1.0 mL/min throughout.

X3.8.1.5 The HPLC parameters provided in X3.8.1.1 – X3.8.1.4 are typical starting points, and the composition of the mobile phase, flow rate, injection volume, and column length may be varied if necessary to improve chromatographic resolution.

X3.8.2 *Column Conditioning*—The UV lamp shall be warmed up for at least 50 min. The 100-μg L^{-1} standard shall be injected in triplicate. The first injection is used to equilibrate the column. The remaining two injections are used to check for peak shape, retention time and area count.

X3.8.3 *Calibration*—Use working standards prepared in X3.6.3 to generate a calibration curve that defines the working range of the HPLC system by plotting concentration (*x*-axis) against the peak area (*y*-axis) for each CDMTD standard. The concentration in substitute ocean water shall be determined using a linear regression analysis method. If the correlation coefficient is less than 0.995, then prepare fresh calibration standards and recalibrate.

X3.8.4 *Sample Analysis:*

X3.8.4.1 Analyze a substitute ocean water blank (substitute ocean water that is stored at room temperature and has not been in contact with the painted cylinder) to detect the presence of interfering components. If a major interfering component is detected in the blank, the chromatographic conditions method shall be changed to improve resolution and allow accurate quantitation of CDMTD.

X3.8.4.2 Inject the samples after the instrument has been calibrated. It is recommended to inject standards as sample in the beginning, in between, and in the end of the run of the samples to assure instrument stability and data accuracy in accordance with operation procedure of the testing laboratory.

X3.8.4.3 If a major interfering component is detected in the sample, the chromatographic conditions of the method shall be changed to allow accurate quantitation of CDMTD.

X3.8.4.4 The peak of interest elutes after approximately 12.2 min (see Figs. X3.1-X3.4 for example chromatograms).

X3.8.4.5 *Spiked substitute ocean water samples*—Run a control standard (X3.6.4) with each batch of extractions. The control standard shall be extracted and analyzed as specified for the test samples to determine the extraction recovery. Recovery for the 20-μg L^{-1} spike must be 100 ± 15 %. If the recovery is greater than 115 % or less than 85×%, repeat the batch of extractions.

TABLE X3.1 Solvent Gradient Profile

Time (minutes)	% A	% B
0.0	100	0
18.00	100	0
18.01	0	100
20.00	0	100
20.01	100	0
25.00	100	0

X3.9 Calculation

X3.9.1 Use any suitable chromatographic software to evaluate the calibration plot and the derived linear regression equation for concentration versus peak area. Determine the concentration of CDMTD in each sample as follows:

$$C_B = C_E \times \frac{V_L}{V_E} \quad \text{(X3.1)}$$

where:

C_B = concentration of CDMTD in the leachate subsample, in μg L^{-1},

C_E = determined concentration of CDMTD in the extracted subsample, in μg L^{-1},

V_L = volume of leachate subsample that is extracted, in mL (= 50 mL), and

V_E = final makeup volume of the extracted sample, in mL (= 10 mL).

X3.9.2 Calculate the biocide release rate in accordance with Section 9.

X3.10 Precision and Bias

X3.10.1 Precision and accuracy were determined on replicate (9) substitute ocean water blanks spiked with 19.2 μg L^{-1} of CDMTD. The mean recoveries of the 19.2-μg L^{-1} spikes were 101.4 % with a precision of ±6.2 % (relative standard deviation).

X3.11 Limit of Detection and Quantitation

X3.11.1 The LOD and LOQ for CDMTD in substitute ocean water using this method has been determined in a single

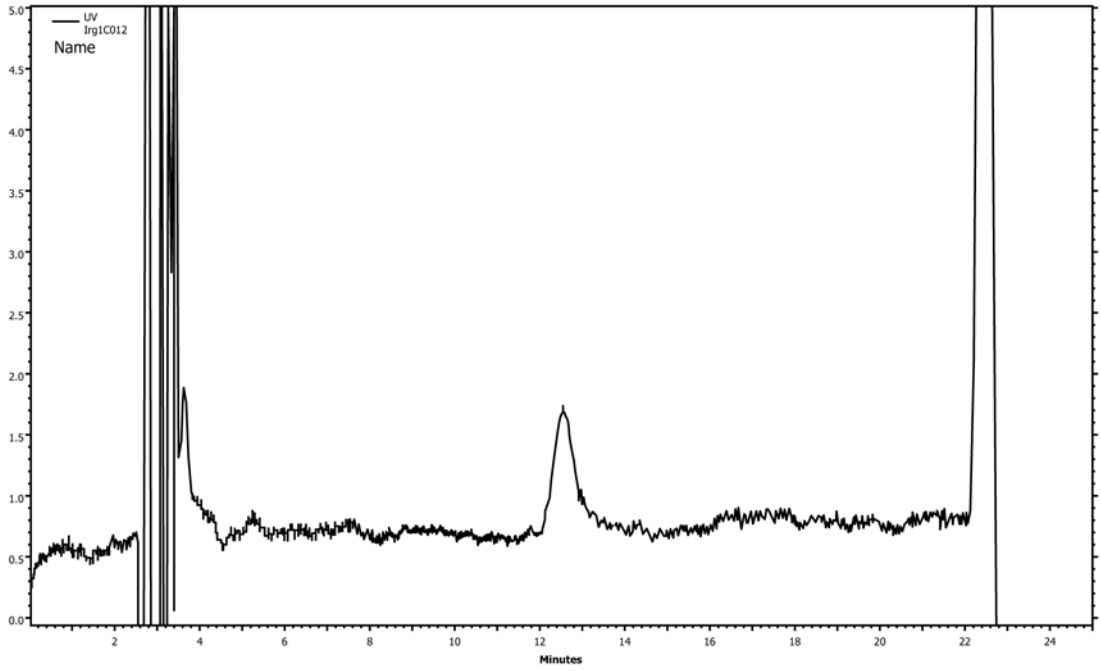

FIG. X3.1 200-μg L^{-1} (200-ppb) Calibration Standard CDMTD

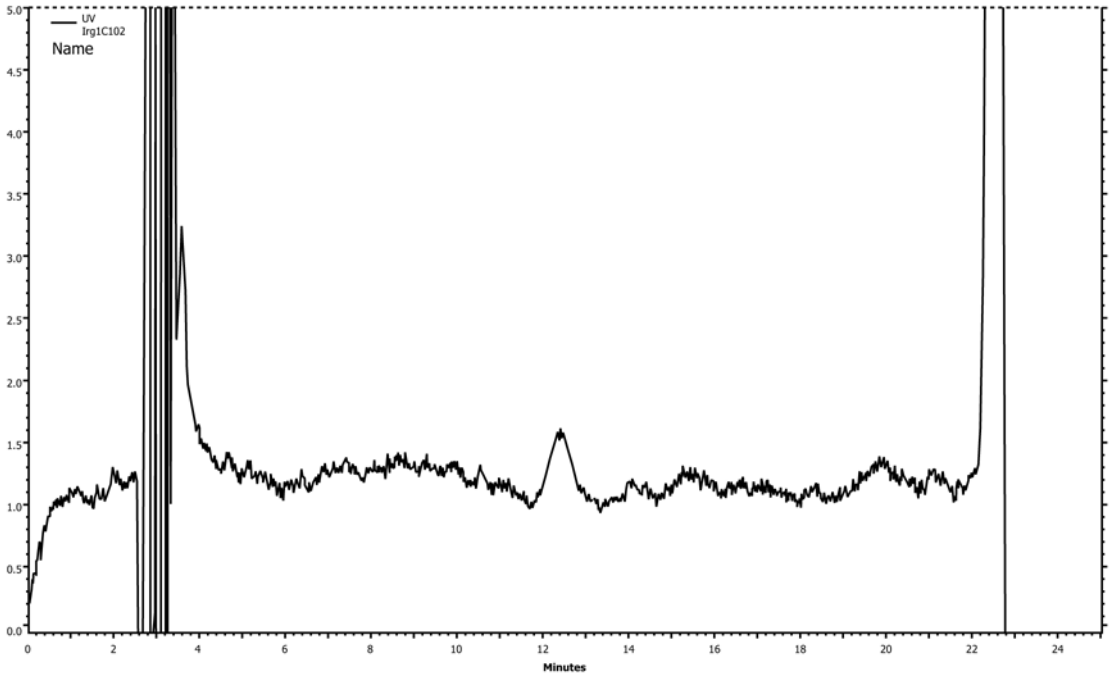

FIG. X3.2 22-μg L^{-1} Spike of CDMTD in Substitute Ocean Water

laboratory using the procedure described in Annex A3. Based on the data and calibration curve, using 50-mL leachate subsamples, the LOD and LOQ for CDMTD in substitute ocean water by this method were found to be 3.5 and 12.0 μg L^{-1}, respectively.

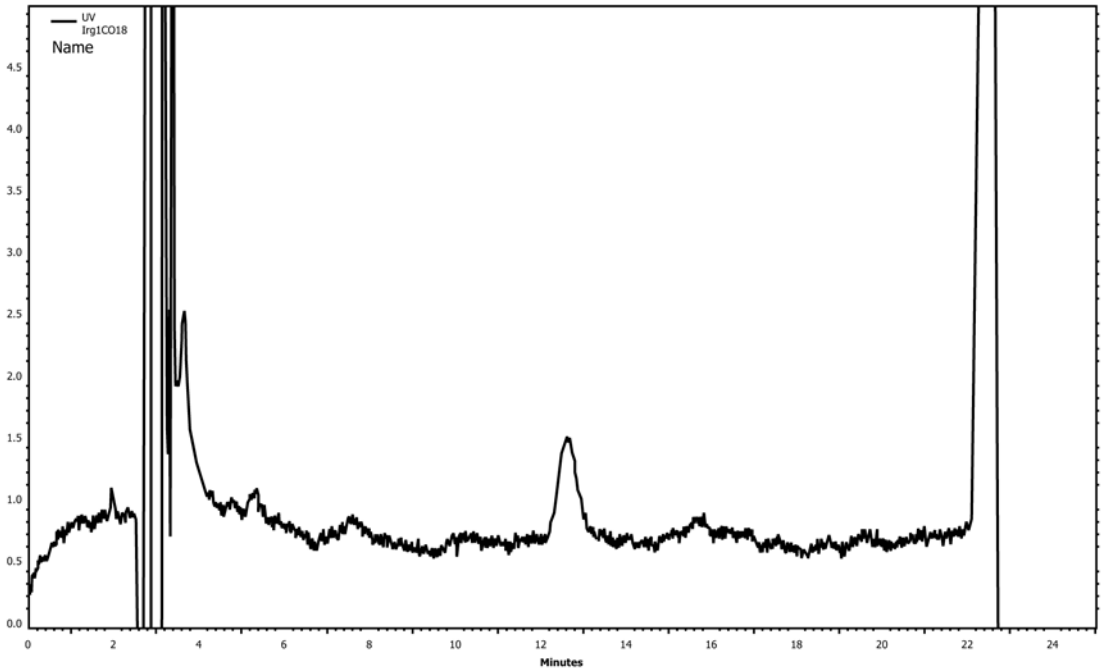

FIG. X3.3 Example of a Typical Leachate From a Paint Sample Containing CDMTD

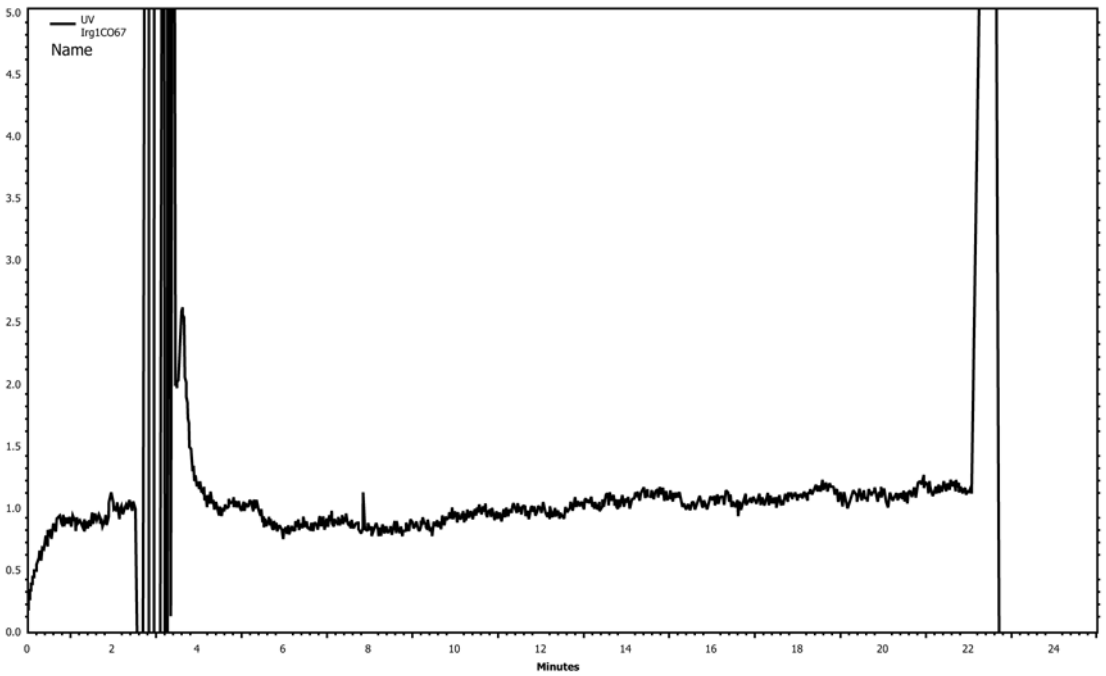

FIG. X3.4 Substitute Ocean Water Blank