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Standard Test Method for Determining Ready, Ultimate, Biodegradability of Organic Chemicals in a Sealed Vessel CO_2 Production Test[1]

This standard is issued under the fixed designation E 1720; 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 procedures for determining the ready, ultimate, aerobic biodegradability of organic chemicals by monitoring CO_2 production in sealed vessels containing the test compound and a dilute sewage inoculum. Because of the stringency of the test conditions, it can be assumed that a chemical that is 60 % or better biodegraded in this test method will biodegrade in most aerobic environmental compartments.

1.2 This test method is derived from the sealed vessel procedures of Birch (**1**),[2] Struijs (**2**), Boatman (**3**), and Peterson (**4**), which were developed as simpler, more economical alternatives to the CO_2 production techniques reported by Gledhill (**5**) and Sturm (**6**), the Sturm report being the basis of the Modified Sturm Test of the Organization for Economic Cooperation and Development (OECD) (**7**).

1.3 The procedures are applicable to pure materials, including sparingly solubles, which can be dissolved or dispersed homogeneously in aqueous stock solutions of at least 25 ppm of carbon, or which can be introduced reproducibly to test bottles as pure test material in 1 to 2-mg portions. The test chemical should be nontoxic to sewage microorganisms at 10 ppm of carbon. The test may be applied to volatile materials with Henry's Law Constants of up to approximately 10^{-2} atm/m^3/mole. The testing of mixtures, extracts, or fully formulated products can lead to serious problems in data interpretation.

1.4 The procedures involve incubation of the test chemical with a dilute inoculum of microbes from domestic wastewater secondary sewage treatment effluent in small, sealed vessels for up to 28 days. Biodegradability is determined by monitoring CO_2 production as dissolved inorganic carbon (DIC) in the liquid phase, and as gaseous CO_2 in the head space. Alternatively, analysis can be performed on just the liquid phase after the addition of alkali, or on just the headspace following acidification. The determinations are made using commercial carbon analyzers based on the IR detection of CO_2. The determination of CO_2 production provides unequivocal proof of biodegradation, barring the unlikely event of abiotic production of CO_2 from the test material.

1.5 For water-soluble materials that do not adsorb to glass or biological solids, biodegradation may be confirmed further by monitoring the disappearance of dissolved organic carbon (DOC) in the liquid phase.

1.6 The simplicity of the sealed vessel method permits ample replicate sampling for rate determination or statistical evaluation, or both.

1.7 For a chemical that fails the test as written, the stringency of the test may be reduced by substituting an acclimated inoculum in order to provide a measure of inherent biodegradability.

1.8 Materials that are toxic to the microbial inoculum at 10 ppm of carbon may not be amenable to testing by this test method, or they may require special method modification such as reducing the test concentration if instrumental sensitivity permits. For some cationics, complexing the test material with a nondegradable anionic may reduce toxicity.

1.9 The values stated in SI units are to be regarded as the standard.

1.10 *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.* For specific precautionary statements, see Section 6.

2. Summary of Test Method

2.1 Biodegradation testing of organic chemicals is performed by monitoring CO_2 production in small sealed vessels inoculated with microbes from secondary sewage treatment effluent obtained from a local domestic sewage treatment plant. The types of test chemicals for which the test is recommended, and those for which special considerations may be required, are summarized in 1.3.

[1] This test method is under the jurisdiction of ASTM Committee E47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.04 on Environmental Fate and Transport of Biologicals and Chemicals.

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[2] The boldface numbers in parentheses refer to the list of references at the end of this standard.

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2.2 Alternatively, smaller vessels (40-mL VOA vials or 20-mL serum vials) containing 25 or 13 mL of medium, respectively may be used if headspace CO_2 is to be measured using a carbon analyzer equipped with an autosampler.

2.3 Vessels (160-mL gas-tight bottles) are charged with the test chemical and sewage inoculum in a dilute mineral salts solution to a volume of 100 mL. The vessels are sealed with butyl rubber or neoprene septa and incubated on a gyrotory shaker at 20°C for up to 28 days.

2.4 Test vessels are sacrificed periodically for analysis of DIC in the liquid phase and analysis of gaseous CO_2 in the headspace, using commercial carbon analyzers.

2.5 The amount of CO_2 resulting from biodegradation of the test chemical is determined by comparing the total CO_2 content of the test vessels with that of blanks containing no test chemical. The extent of biodegradation is determined by comparing the actual CO_2 produced with the theoretical amount that would be produced by complete conversion of the test chemical carbon to CO_2.

2.6 The duration of the sealed vessel test is typically four weeks, with periodic sacrifice of the vessels for analysis. Preadapted inoculum may be used in a subsequent test for test chemicals that fail to degrade within that time, but a positive result would classify the chemical only as "inherently" biodegradable rather than "readily" biodegradable.

3. Significance and Use

3.1 As a ready biodegradability test, when using non-adapted inoculum, the sealed vessel method provides only a limited opportunity for biodegradation and acclimatization to occur. It may therefore be assumed that a chemical yielding a positive result in this stringent test will biodegrade rapidly and ultimately in the environment. Generally, no further biodegradability testing would be required for a chemical that passes this test unequivocally.

3.2 The sealed vessel test is applicable to the testing of volatile test chemicals because the biodegradative formation of CO_2 occurs in a closed system.

3.3 The sealed vessel test is also appropriate for testing sparingly soluble chemicals and for chemicals that bind to inoculum, since biodegradability is based on the analysis of a soluble formation product rather than on the disappearance of the sparingly soluble substrate.

3.4 Ample replicate sampling for rate determination or statistical evaluation, or both, is feasible because of the speed, economy, and space efficiency of the sealed vessel test.

3.5 The sealed vessel test is ideal for the comparative testing of groups of chemicals and for generating structure-activity data bases also because of its speed, economy, and space efficiency.

4. Apparatus

4.1 The apparatus, reagent concentrations, and procedures described in the following sections are appropriate for testing both soluble and sparingly soluble materials, and for volatile materials with Henry's Law Constants of up to approximately 10^{-2} atm/m^3/mole. Stock solution concentrations and volumes can be varied in practice in any convenient manner that results in the final concentrations indicated in 10.6 and permits the accurate and reproducible introduction of test chemical to the reaction vessels. Some materials, such as insoluble or viscous liquids, are more effectively added directly to the test bottles by the alternative techniques described in 5.5.

4.2 *Gas-Tight Glass Vessels*, 160-mL capacity, with aluminum crimp caps and neoprene or butyl rubber septa. Approximately 30 vessels per test group, plus an additional 30 for blanks, will provide triplicate sampling at time 0 and seven semiweekly time points, plus six bottles for Day 28 to permit end point statistics. The actual number of bottles will depend on the objectives of the particular experiment since there can be great flexibility both in the sample timing and sample replication needs.

4.2.1 Bottles may be reused after thorough cleaning, for example, in a 60°C ultrasonic bath, rinsing with copious amounts of water (final distilled) and drying.

4.3 *Large, Heavy-Duty Gyrotory Shaker,* equipped with a universal platform.

4.4 *Carbon Analyzer(s)*:

4.4.1 Capable of measuring DIC and DOC in aqueous media over the range from 0 to 20 ppm; and

4.4.2 Capable of measuring CO_2 in gas over the range from 0 to 1 µg carbon.

4.4.2.1 The same analyzer, for example, the Ionics 1555b, can be used for both analyses, with some loss of speed and convenience.

4.4.2.2 Alternatively, analysis can be performed on just the liquid phase after the addition of 1 mL 10*N* NaOH, or on just the headspace following acidification with 1 mL 10*N* HCl (**4**).

4.5 *Gas-Tight Cemented Needle Syringe*, 1000 µL with a 22° beveled bent point, for piercing the butyl rubber or neoprene septa and injecting into the gas phase analyzer.

4.5.1 *Spring-Loaded Hamilton Syringe*, with a "square" end for injecting liquid samples into Ionics-type analyzers, if used.

4.6 *Filter Apparatus*—Two- or three-litre filter flask, 20-cm Buchner funnel, 18.5-cm coarse filter paper, and a vacuum source, for filtering sewage effluent inoculum.

4.7 *Compressed CO_2-Free Air or Nitrogen*, for sparging the inoculum free of CO_2. The delivery line should be equipped with a large gas diffusing stone, for maximum sparging efficiency.

4.8 *pH Meter*.

4.9 *Volumetric Flasks*, three 100-mL and one 1-L capacity for preparation of mineral salts stock solutions.

4.10 *Glass Bottles or Flasks*, 6-L capacity, for preparation of mineral salts solution. Sufficient media is provided by 6 L of mineral salts for approximately 99 test vessels (that is, approximately three and one-third test groups) for this test method as written.

4.11 *Volumetric Flasks*, 2-L capacity, one flask per test material, for preparation of test material stock solutions.

4.11.1 More concentrated stock solutions may be used for soluble test chemicals that do not precipitate in the presence of the mineral salts medium; that is, smaller volumetric flasks will be appropriate. In this case, volumes and concentrations of the mineral salts must also be adjusted accordingly, or an appropriate volume of pure water must be added to each test vessel to bring the total to 100 mL.

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