Designation: E 1720 – 01

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.

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

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

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,[3] 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*,[4] 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;[5] and

4.4.2 Capable of measuring CO_2 in gas over the range from 0 to 1 µg carbon.[6]

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.[7]

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,[8] 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,[9] 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

[3] Pierce Chemical Co. 125-mL Hypo-Vials, or equivalent.
[4] New Brunswick Scientific Model G10, or equivalent.
[5] OI Corp. Model 700 TOC analyzer, or equivalent.
[6] Ionics Model 1555b TOC analyzer with Horiba Model PIR2000 NDIR CO_2 detector (approximately $17 000 complete), or equivalent.
[7] Hamilton No. 1001 with No. 81317 tip, or equivalent.
[8] Whatman No. 41, or equivalent.
[9] FisherBrand Catalog No. 11-139A, or equivalent.

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.

4.12 *Magnetic Stirrer(s)*, for media and sample preparation.

4.13 *Automated Pipetting Devices*, to deliver variable volumes up to 100 mL, with an accuracy of ± 1 %.[10]

4.14 *Large Laboratory Oven*, for drying glassware.

4.15 *Ultrasonic Processor* (optional), for dispersing sparingly soluble test chemicals.

5. Reagents

5.1 *Inoculum*—Non-chlorinated secondary effluent from an activated sludge plant treating predominantly domestic sewage is obtained fresh on the day of initiation of the experiment, approximately 200 to 250 mL per test group of 30 vessels. The undiluted inoculum should contain approximately 10^6 organisms per millilitre.

5.2 Alternatively, 40-mL VOA vials or 20-mL serum vials may be substituted.

5.3 *Deionized or Distilled Water*, free from calcium and toxic substances, particularly metals such as copper. It may be desirable to air-saturate the water by aerating strongly for approximately 20 min with clean, filtered, compressed air.

5.4 *Mineral Salts Stock Solutions*—The following stock solutions should be stored in the dark and discarded at the first sign of sediment, turbidity, or biological growth:

5.4.1 *Calcium Chloride Dihydrate*, 3.64 g $CaCl_2 \cdot 2H_2O$/100 mL water.

5.4.2 *Magnesium Sulfate Heptahydrate*, 2.25 g $MgSO_4 \cdot 7H_2O$/100 mL water.

5.4.3 *Ferric Chloride Hexahydrate + EDTA Disodium Salt*, 0.020 g $FeCl_3 \cdot 6H_2O$/100 mL water and 0.040 g EDTA·Na_2/100 mL water.

5.4.4 *Potassium Phosphate, Monobasic + Potassium Phosphate, Dibasic, + Sodium Phosphate, Dibasic·Heptahydrate, + Ammonium Chloride*:

8.50 g	KH_2PO_4/L water
21.75 g	K_2HPO_4/L water
50.30 g	$Na_2HPO_4 \cdot 7H_2O$/L water
0.50 g	NH_4Cl/L water

5.5 *Test Chemical Stock Solutions or Stable Dispersions*—Test chemical stock solutions for a wide range of materials, including sparingly soluble molecules, are normally prepared to contain 25 mg carbon from the test chemical per litre of deionized or distilled water. The dispersion of sparingly soluble test chemicals in the stock solutions may be improved by the use of ultrasonic processing. Two litres of test chemical stock solution is more than sufficient to dose 30 vessels. The pH of the test chemical stock solution may be adjusted with HCl or NaOH to pH 7.2 ± 0.2, provided that no precipitation or reaction of the test material occurs.

5.5.1 Alternatively, some materials, such as insoluble liquids, are better added directly to the test bottles by means of a good-quality microlitre syringe. Very viscous materials may be spread thinly on a tared coverslip that is then added to the test bottle.

5.5.2 Materials known to be toxic to bacteria at 10 ppm carbon (final) may be tested at lower concentrations, to a minimum of 2 to 5 ppm (final), depending on individual instrument sensitivities, by adjusting the stock solution concentrations appropriately.

5.5.3 For analyses of headspace CO_2 in 40mL VOA vials, a suitable headspace auto-sampler (Tekmar M-7000) coupled with a gas chromatograph may be used. For analyses of headspace CO_2 in 20-mL serum vials, a carbon analyzer equipped with an autosampler (ThermoGlas model 1200 carbon analyzer) may be used.

5.6 *Reference Compound Stock Solution*—A reference compound such as sodium benzoate, glucose, or sodium acetate may be prepared as for the test chemicals. A control of similar solubility, for example, sodium stearate, should be used for sparingly soluble or insoluble materials.

5.7 *Calibration Gas for Headspace Analysis*, certified standard, approximately 0.25 % (v/v) carbon dioxide, balance nitrogen.

5.8 *Calibration Solution for Liquid Phase DIC Analysis*, standard solutions of sodium hydrogen carbonate in the range from 0 to 20 ppm as TIC.

5.9 *Calibration Solution for Liquid Phase DOC Analysis*, standard solutions of potassium hydrogen phthalate in the range from 0 to 20 ppm as TOC.

6. Safety Precautions

6.1 This procedure involves the use of non-chlorinated sewage treatment plant effluent. Individuals performing this test may consequently be exposed to microbiological agents that are dangerous to human health. Disposable latex gloves and laboratory eyewear with splash guards should be worn during procedures involving the use of the sewage treatment plant effluent. A dust/mist respirator and laboratory footwear are also recommended when large amounts of effluent are being handled, for example, during filtering and sparging operations.

6.2 Those that work with the sewage organisms may opt to keep current with immunizations for polio, typhoid, hepatitis B, and tetanus.

6.3 Sealed vessel test media containing sewage-derived inoculum may be treated with 5 % chlorine bleach during disposal.

7. Sampling and Analytical Procedures

7.1 The carbon analyzer to be used for headspace analysis is calibrated using 0.25 % v/v CO_2 calibration gas.

7.2 The carbon analyzer to be used for liquid phase analysis is calibrated using standard solutions of sodium hydrogen carbonate in the range from 0 to 20 ppm as TIC. Calibration is performed for confirmatory DOC analysis with standard solutions of potassium hydrogen phthalate in the range from 0 to 20 ppm TOC.

NOTE 1—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.

7.3 The time zero samples are analyzed for headspace CO_2 by withdrawing a sample of the headspace gas through the neoprene or rubber septum using a gas-tight syringe and

[10] EM Science Optifix, or equivalent.

injecting the sample into the carbon analyzer as for the gas standard.

7.4 The seals are then removed from the time zero samples, and the liquid phases are assayed for DIC and, optionally, DOC.

7.5 Subsequent samples and blanks are removed from the shaker periodically for analysis, typically in triplicate at semiweekly intervals, up to Day 28, when six sample bottles and a corresponding number of blanks may be analyzed to permit end point statistics.

8. Procedure

8.1 New vessels are rinsed twice with tap water and once with distilled water and dried in an oven at 110°C. Recycled vessels may be used as described in 4.1.

8.2 The day before the test, the final test chemical stock solutions are prepared, diluted in triplicate, and analyzed for test compound concentration and homogeneity by organic carbon analysis or other appropriate method.

8.3 On the day of test initiation, the fresh secondary effluent is vacuum-filtered through coarse filter paper to remove particulates and sparged with CO_2-free air or nitrogen to remove CO_2 and dissolved carbonates and bicarbonates. The CO_2-free sparge is interrupted and the pH measured approximately every 15 min. Sufficient 1 *N* HCl is added to reduce the pH to 6.5, a 12-mL aliquot is removed for TIC/TOC analysis, and sparging is resumed. This procedure is repeated until the DIC is less than 5 ppm. The DOC should not exceed 10 ppm.

8.4 Inoculated mixed mineral salts solution(s) are prepared from the mineral salts stock solutions (5.4) and the sparged inoculum, most conveniently in 6-L batches (sufficient for three and one-third test groups of 30 vessels each). These concentrations and volumes may be adjusted in any convenient manner that results in the same final concentrations (8.6) and that accommodates the properties of the chemicals being tested:

	mL
Deionized or distilled water	5370
Calcium chloride stock solution	10
Magnesium sulfate solution	10
Ferric chloride stock solution	10
Phosphate buffer solution	100
CO_2-free, pH 6.3 inoculum[A]	500
	6000

[A] Inoculum may be varied from 50 to 1000 mL, depending on the inoculum strength, with a corresponding adjustment of the water volume.

8.5 Sixty millilitres of inoculated mixed mineral salts are dispensed into each vessel to be used in the test, typically 30 vessels per test group, plus 30 blanks.

8.6 Forty millilitres of 25 ppm test chemical stock solution at 25 mg/L organic carbon are then added to each test vessel and 40 mL of deionized or distilled water for blanks.

8.6.1 For 40-mL VOA vials or 20-mL serum vials, medium is delievered to vials in 25 mL or 13 mL aliquots, respectively.

8.6.2 For test materials that neither precipitate in the presence of mineral salts, nor adsorb to inoculum, large batches (approximately 3 L) of inoculated mineral salts plus test chemical stock solution (60:40) may be prepared and delivered to the bottles in 100-mL aliquots.

8.6.3 The final concentrations of all components of the test system are as follows:

Calcium chloride dihydrate, $CaCl_2 \cdot 2H_2O$	36.4 mg/L
Magnesium sulfate heptahydrate, $MgSO_4 \cdot 7H_2O$	22.5 mg/L
Ferric chloride hexahydrate, $FeCl_3 \cdot 6H_2O$	0.2 mg/L
EDTA disodium salt, $EDTA \cdot Na_2$	0.4 mg/L
Potassium phosphate, monobasic, KH_2PO_4	85.0 mg/L
Potassium phosphate, dibasic, K_2HPO_4	217.5 mg/L
Sodium phosphate, dibasic heptahydrate, $Na_2HPO_4 \cdot 7H_2O$	503.0 mg/L
Ammonium chloride, NH_4Cl	5.0 mg/L
2° treatment effluent	5.0 %
Organic carbon from test chemical	10.0 mg/L

The final pH test medium should be 7.2± 0.2.

8.7 The test vessels are sealed immediately with neoprene or butyl rubber septa and aluminum crimp caps. The appropriate number of replicate vessels (typically three) from each test group and an equal number of blanks are set aside for time zero analysis.

8.8 The remaining vessels are packed in boxes, covered tightly to maintain light-free conditions, and rotated on the gyrotory shaker at approximately 150 r/min at 20 ± 1°C (68°F).

9. Calculation

9.1 The amount of carbon, as evolved CO_2, appearing in the water phase is determined by the following equation:

$$C_w = [(\text{SampleDIC}_t - \text{BlankDIC}_t) - (\text{SampleDIC}_0 - \text{BlankDIC}_0)] \times V_w \quad (1)$$

where:

C_w = total micrograms inorganic carbon in liquid phase,
SampleDIC_t = sample DIC at Time *t* (all units are µg C/mL),
BlankDIC_t = blank DIC at Time *t* (mean of all replicates),
SampleDIC_0 = sample DIC at Time 0 and $(\text{SampleDIC}_0 - \text{BlankDIC}_0) \sim 0$,
BlankDIC_0 = blank DIC at Time 0, and
V_w = volume of water phase (mL) (100 mL).

9.2 The amount of carbon as evolved CO_2 appearing in the gas phase is determined by the following equation:

$$C_g = (\text{SampleIC}_t - \text{BlankIC}_t) \times V_g \quad (2)$$

where:

C_g = total micrograms inorganic carbon in headspace,
SampleIC_t = sample IC at Time *t* (all units are µg C/mL),
BlankIC_t = blank IC at Time *t* (mean of all replicates), and
V_g = volume of headspace (mL) (60 mL).

9.3 The total percent theoretical CO_2 production is determined by the following equation:

$$\text{total \% ThCO}_2 = \frac{(C_w + C_g) \times 100}{\text{TOC}_0 \times V_w} \quad (3)$$

where:

TOC_0 = test chemical TOC in solution at Time 0 (µg C/mL) (TOC_0 may be obtained by either measurement or calculation).

9.4 The mean % $ThCO_2$, sample standard deviation, and 95 % confidence limits are calculated for each data set of three

or more replicates, using the usual statistical equations:

$$s = \sqrt{\frac{\Sigma_i (x_i - \bar{x})^2}{n - 1}} \qquad 95\ \%\ CI = \bar{x} \pm \frac{s \cdot t}{\sqrt{n}} \tag{4}$$

where:

$\bar{x}$ = mean % $ThCO_2$,
x = % $ThCO_2$,
s = sample standard deviation,
n = number of replicates, and
t = "t" value (two-tailed test) at $n - 1$ degrees of freedom at 0.05 level.

10. Interpretation

10.1 The sealed vessel test would be interpreted similarly to the Sturm and other CO_2-production ready tests (see 3.1). Specifically, pure test chemicals yielding a result of 60 % of theoretical CO_2 production within 28 days would be regarded as readily biodegradable. This level must be attained within ten days of biodegradation exceeding the 10 % level.

NOTE 2—It is possible that the potential for passing the ten-day window rule may be increased somewhat in the sealed vessel method relative to that in the Sturm test since all of the CO_2 produced in the sealed vessel is measured instantaneously, while there is a lag in transporting the gaseous CO_2 to the Sturm test traps.

10.2 Like other CO_2 production methods, the sealed vessel test provides unequivocal proof of biodegradation, excluding the occurrence of abiotic production of CO_2 from the test material. The probability of CO_2 formation occurring by nonbiological mechanisms depends on the reactivity of the test chemical, presence of reactive substrates in the medium, and energy sources, particularly solar radiation.

10.3 Information on the purity of the test chemical is important in interpreting the results, particularly for cases in which the result lies close to the pass level. It should be emphasized that this test method is recommended only for single compounds of reasonable purity.

10.4 Information on the microbial toxicity of the test chemical or potential toxic degradation products may be helpful in determining whether a reduced test concentration should be used or may be useful in the interpretation of low or erratic results. Information regarding toxicity may be gained from standard microbial toxicity tests or by adding approximately 10 ppm total organic carbon (TOC) from glucose to some additional sealed vessels with the test chemical and observing the effect of the test chemical on the glucose biodegradation.

10.5 If preadapted inoculum is used in the sealed vessel test, test chemicals yielding a result of 60 % of theoretical CO_2 production within 28 days would be classified only as inherently biodegradable because of the less-stringent conditions.

11. Report

11.1 A protocol providing a general overview of the study goals and procedures must be prepared before the study is initiated. If a substantive modification of this test method is deemed necessary for the test chemical, deviation from the test method should be documented in the protocol.

11.2 The final results of this study should be documented in the final report. Report the following information:

11.2.1 Names of study, investigator(s), and laboratory.

11.2.2 Brief description of the test material, including its log number, chemical name(s), composition, and other appropriate parameters.

11.2.3 Summary of the test method, including deviations from the written protocol.

11.2.4 Brief description of any supplementary tests performed, such as microbial toxicity or analyses to verify test chemical concentration or homogenicity, or both, and the results of these tests.

11.2.5 Tabular and graphical presentation of % $ThCO_2$ production data (if determined) as a function of time after test initiation. The final % $ThCO_2$ production is expressed as the mean and standard deviation or 95 % confidence limits, or both, of all results determined after significant differences in semiweekly determinations no longer occur, or as the mean and standard deviation or 95 % CI, or both, of the end point values. Also indicate whether or not the test chemical passed the ten-day window rule.

11.2.6 Listing of relevant references, including all notebook pages and computer files containing raw data from the study.

12. Quality Assurance

12.1 To ensure the integrity of data developed using this test method and to comply with current regulatory requirements, a quality assurance program meeting the Environmental Protection Agency, Food and Drug Administration, or OECD guidelines should be followed.

13. Precision and Bias

13.1 A precision and bias statement cannot be made at this time, although an indication of the within-test precision of the test method does appear in published work **(1)**. It is possible that the precision and bias may vary depending on the choice of analytical conditions. A recommendation to perform an interlaboratory comparison on this test method has been made to the sponsoring committee.

14. Keywords

14.1 aerobic; biodegradation; CO_2 production; ready; sealed vessel

APPENDIX

X1. ADDITIONAL INFORMATION ON USING SMALLER VIALS

X1.1 The Astm E 1720 sealed vessel CO_2 production test can be conducted in smaller test vessels (40–mL VOA vials or 20-mL serum vials) with headspace gas analysis using a suitable gas chromatograph linked to a headspace sampler (for example, Tekmar M-7000) or using a carbon analyzer equipped with an autosampler (for example, ThermoGlas Model 1200). Other than vessel size, all of the test procedures outlined for the 160-mL serum vials are followed for the smaller vials. Smaller vials will also maintain an approximate 60:40 medium to headspace ratio as is used for the 160-mL serum vials with the addition of 25 mL or 13 mL of medium, respectively. Smaller vessels provide more of a challenge for testing water insoluble or volatile chemicals. For non-volatile, non water soluble liquids or solids, the test material can be dissolved in a suitable solvent (for example, methylene chloride), added to the test vessel and the solvent removed under a gentle stream of nitrogen. Volatile organic chemicals (for example, gasoline) may be added to vessels containing inoculated medium by use of a suitable microliter syringe (Hamilton 1.0µL or equivalent) and the vessels quickly sealed. When using the latter procedure, it is recommended that multiple replicates (5 to 10) be set up for analysis at each sampling interval to improve the statistical precision and overcome potential variations in test substance dosing.

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