



Standard Test Method for Determining Ready, Ultimate, Biodegradability of Organic Chemicals in a Sealed Vessel CO₂ Production Test¹

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1. Scope

1.1 This test method covers procedures for determining the ready, ultimate, aerobic biodegradability of organic chemicals by monitoring CO₂ 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 CO₂ 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/m³/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 CO₂ production as dissolved inorganic carbon (DIC) in the liquid phase, and as gaseous CO₂ 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 CO₂. The determination of CO₂ production provides unequivocal proof of biodegradation, barring the unlikely event of abiotic pro-

duction of CO₂ 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 CO₂ 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 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.

2.3 Test vessels are sacrificed periodically for analysis of DIC in the liquid phase and analysis of gaseous CO₂ in the headspace, using commercial carbon analyzers.

2.4 The amount of CO₂ resulting from biodegradation of the

¹ This test method is under the jurisdiction of ASTM Committee E-47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.06 on Environmental Fate of Chemical Substances.

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