



Designation: D6006 – 23

## Standard Guide for Assessing Biodegradability of Hydraulic Fluids<sup>1</sup>

This standard is issued under the fixed designation D6006; 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 ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

### 1. Scope\*

1.1 This guide covers and provides information to assist in planning a laboratory test or series of tests from which may be inferred information about the biodegradability of an unused fully formulated hydraulic fluid in its original form. Biodegradability is one of three characteristics which are assessed when judging the environmental impact of a hydraulic fluid. The other two characteristics are ecotoxicity and bioaccumulation.

1.2 Biodegradability may be considered by type of environmental compartment: aerobic fresh water, aerobic marine, aerobic soil, and anaerobic media. Test methods for aerobic fresh water, aerobic soil and anaerobic media have been developed that are appropriate for the concerns and needs of testing in each compartment.

1.3 This guide addresses releases to the environment that are incidental to the use of a hydraulic fluid but is not intended to cover situations of major, accidental release. The tests discussed in this guide take a minimum of three to four weeks. Therefore, issues relating to the biodegradability of hydraulic fluid are more effectively addressed before the fluid is used, and thus before incidental release may occur. Nothing in this guide should be taken to relieve the user of the responsibility to properly use and dispose of hydraulic fluids.

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recom-*

*mendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

### 2. Referenced Documents

#### 2.1 ASTM Standards:<sup>2</sup>

[D4175 Terminology Relating to Petroleum Products, Liquid Fuels, and Lubricants](#)

[D5210 Test Method for Determining the Anaerobic Biodegradation of Plastic Materials in the Presence of Municipal Sewage Sludge \(Withdrawn 2016\)<sup>3</sup>](#)

[D5291 Test Methods for Instrumental Determination of Carbon, Hydrogen, and Nitrogen in Petroleum Products and Lubricants](#)

[D5480 Test Method for Engine Oil Volatility by Gas Chromatography \(Withdrawn 2003\)<sup>3</sup>](#)

[D5864 Test Method for Determining Aerobic Aquatic Biodegradation of Lubricants or Their Components](#)

[E1196 Test Method for Determining the Anaerobic Biodegradation Potential of Organic Chemicals \(Withdrawn 1998\)<sup>3</sup>](#)

#### 2.2 ISO Standards:<sup>4</sup>

[ISO 9439:1990 Technical Corrigendum I, Water Quality—Evaluation in an Aqueous Medium of the Ultimate Biodegradability of Organic Compounds](#)

[ISO 4259:1992\(E\) Petroleum Products—Determination and Application of Precision Data in Relation to Methods of Test](#)

#### 2.3 OECD Standards:

[OECD 301B \(the Modified Sturm Test\) Guidelines for Testing Chemicals<sup>5</sup>](#)

[OECD 301F \(the Manometric Respirometry Test\) Guidelines for Testing of Chemicals<sup>5</sup>](#)

<sup>2</sup> For referenced ASTM standards, visit the ASTM website, [www.astm.org](http://www.astm.org), or contact ASTM Customer Service at [service@astm.org](mailto:service@astm.org). For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

<sup>3</sup> The last approved version of this historical standard is referenced on [www.astm.org](http://www.astm.org).

<sup>4</sup> Available from International Organization for Standardization (ISO), ISO Central Secretariat, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, <https://www.iso.org>.

<sup>5</sup> Available from Organisation for Economic Co-Operation and Development (OECD), 2, rue André Pascal, F-75775 Paris Cedex 16, France.

<sup>1</sup> This guide is under the jurisdiction of ASTM Committee D02 on Petroleum Products, Liquid Fuels, and Lubricants and is the direct responsibility of Subcommittee D02.12 on Environmental Standards for Lubricants.

Current edition approved July 1, 2023. Published August 2023. Originally approved in 1996. Last previous edition approved in 2017 as D6006 – 17. DOI: 10.1520/D6006-23.

\*A Summary of Changes section appears at the end of this standard

### 3. Terminology

#### 3.1 Definitions:

3.1.1 For definitions of terms used in this guide, refer to Terminology **D4175**.

3.1.2 *aerobic*, *adj*—1. taking place in the presence of oxygen; 2. living or active in the presence of oxygen.

3.1.3 *anaerobic*, *adj*—1. taking place in the absence of oxygen; 2. living or active in the absence of oxygen.

3.1.4 *biodegradation*, *n*—the process of chemical breakdown or transformation of a material caused by organisms or their enzymes.

3.1.4.1 *Discussion*—Biodegradation is only one mechanism by which materials are transformed in the environment.

3.1.5 *biomass*, *n*—biological material including any material other than fossil fuels which is or was a living organism or component or product of a living organism.

3.1.5.1 *Discussion*—In biology and environmental science, biomass is typically expressed as density of biological material per unit sample volume, area, or mass (g biomass / g (or / mL or / cm<sup>2</sup>) sample); when used for products derived from organisms biomass is typically expressed in terms of mass (kg, MT, etc.) or volume (L, m<sup>3</sup>, bbl, etc.).

3.1.5.2 *Discussion*—Products of living organisms include those materials produced directly by living organisms as metabolites (for example, ethanol, various carbohydrates and fatty acids), materials manufactured by processing living organisms (for example: pellets manufactured by shredding and pelletizing plant material) and materials produced by processing living organisms, their components or metabolites (for example, transesterified oil; also called biodiesel).

3.1.6 *blank*, *n*—*in biodegradability testing*, a test system containing all system components with the exception of the test material.

3.1.7 *environmental compartment*, *n*—a subdivision of the environment based on physical or chemical properties, or both.

3.1.7.1 *Discussion*—Examples of environmental compartments are aerobic fresh water, aerobic marine, and aerobic soil. The results of test procedures may be applied to environmental compartments but the test systems do not constitute an environmental compartment.

3.1.8 *inoculum*, *n*—spores, bacteria, single celled organisms, or other live materials that are introduced into a test medium.

3.1.9 *pre-adaptation*, *n*—the pre-incubation of an inoculum in the presence of the test material under conditions similar to the test conditions.

3.1.9.1 *Discussion*—The aim of pre-adaptation is to improve the precision of the test method by decreasing variability in the rate of biodegradation produced by the inoculum. Pre-adaptation may mimic the natural processes which cause changes in the microbial population of the inoculum leading to more rapid biodegradation of the test material, but not to a change in the final degree of biodegradation.

3.1.10 *primary biodegradation*, *n*—degradation of the test substance resulting in a change in its physical or chemical properties, or both.

3.1.11 *primary biodegradation test*, *n*—a test which follows the disappearance of a test substance by measuring some attribute of the substance.

3.1.11.1 *Discussion*—The extent to which the results of a primary biodegradation test correspond to the biological conversion of the test substance will depend on the attribute which is being measured.

3.1.12 *sonication*, *n*—the act of subjecting a material to the shearing forces of high-frequency sound waves.

3.1.12.1 *Discussion*—Sonication of a two phase liquid system may result in the dispersal of one phase as fine droplets in the other phase.

3.1.13 *ultimate biodegradation*, *n*—degradation achieved when a material is totally utilized by microorganisms resulting in the production of carbon dioxide (and possibly methane in the case of anaerobic biodegradation), water, inorganic compounds, and new microbial cellular constituents (biomass or secretions, or both).

3.1.14 *ultimate biodegradation test*, *n*—a protocol which estimates the extent to which the carbon in a material is converted to CO<sub>2</sub> or methane, either directly, by measuring the production of CO<sub>2</sub> or methane, or, in the case of aerobic biodegradation, indirectly by measuring the consumption of O<sub>2</sub>.

3.1.14.1 *Discussion*—The measurement of new biomass usually is not attempted.

### 4. Summary of Guide

4.1 This guide gives two kinds of information which relate to testing of hydraulic fluids for biodegradability. First, it gives information of a general nature relating to biodegradability. For example, it includes definitions of terms not traditionally used by users or producers of hydraulic fluids (Section 3) and a brief discussion of some of the technical issues which are common to most biodegradability tests when they are applied to hydraulic fluids (Section 7). Second, the guide gives more specific information on the methods, advantages, and disadvantages of several of the biodegradation tests frequently used for hydraulic fluids (Section 6).

### 5. Significance and Use

5.1 This guide discusses ways to assess the likelihood that a hydraulic fluid will undergo biodegradation if it enters an environment that is known to support biodegradation of some substances, for example the material used as the positive control in the test. The information can be used in making or assessing claims of biodegradability of a fluid formula.

5.2 Biodegradation occurs when a fluid interacts with the environment, and so the extent of biodegradation is a function of both the chemical composition of the hydraulic fluid and the physical, chemical, and biological status of the environment at the time the fluid enters it. This guide cannot assist in judging the status of a particular environment, so it is not meant to provide standards for judging the persistence of a hydraulic fluid in any specific environment either natural or man-made.

5.3 If any of the tests discussed in this guide gives a high result, it implies that the hydraulic fluid will biodegrade and

will not persist in the environmental compartment being considered. If a low result is obtained, it does not mean necessarily that the substance will not biodegrade in the environment, but does mean that further testing is required if a claim of biodegradability is to be made. Such testing may include, but is not limited to, other tests mentioned in this guide or simulation tests for a particular environmental compartment.

## 6. Test Methods

6.1 *Aerobic Fresh Water Environment*—The most commonly performed tests cover aerobic biodegradation in fresh water. The tests conducted for this compartment may be ultimate biodegradation tests measuring CO<sub>2</sub> production or primary biodegradation tests measuring the disappearance of the test fluid. The test medium is based on high-grade, carbon-free water. Some salts will be included as necessary for maintenance of solution pH and provision of trace minerals necessary for microbial life.

6.1.1 The majority of ultimate biodegradation tests measure produced CO<sub>2</sub>. Examples of test procedures for ultimate biodegradability in an aerobic aquatic environment are: the Modified Sturm Test (OECD 301B); the Manometric Respirometry Test (OECD 301F); the U.S. EPA Aerobic Aquatic Biodegradation Test, which also is called the EPA Shake Flask Test and was derived from the Gledhill Test (1);<sup>6</sup> Test Method D5864; and the ISO Test 9439:1990. With the exception of Test Method D5864, these tests were originally designed for water-soluble pure compounds and so the test procedures allow some procedural options that are not suitable for water-insoluble substances, such as addition of the test substance in an aqueous solution or calculation, rather than measurement, of carbon content. In other tests, such as the Manometric Respirometry Test, oxygen consumption is measured as a surrogate for CO<sub>2</sub> production. Oxygen consumption is not a direct measure of ultimate biodegradation but is expected to correlate closely with it. The procedures listed are screening tests suitable for laboratory evaluation of the hydraulic fluid. Although all the tests referred to above specify that the length of the test is 28 days, a high level of biodegradation in longer time frames can be taken as evidence that the hydraulic fluid is ultimately biodegradable and nonpersistent in fresh water.

6.1.1.1 If the biodegradability of a hydraulic fluid with a nonnegligible vapor pressure is measured in any one of these tests, except the OECD 301F, a false negative may result. The hydraulic fluid could vaporize from the test solution before conversion to CO<sub>2</sub>. In this case a biodegradable fluid would have a low measured percent theoretical CO<sub>2</sub>. If the aerobic aquatic biodegradability of a volatile hydraulic fluid is to be measured, the OECD 301F test should be used.

6.1.2 Tests for primary biodegradation must be designed for specific classes of test substances. The results of a primary biodegradation test should not be considered equivalent to or substitutable for the results of an ultimate biodegradation test.

6.1.2.1 The most commonly performed primary biodegradation test for lubricants is the CEC L-33-A-94 test, developed

by the Coordinating European Council in the early 1980s and approved by the CEC in 1993. This test, which was called the CEC L-33-T-82 test prior to approval, measures the IR absorption spectrum of saturated carbons found in the test materials. It was designed specifically for two-stroke outboard engine lubricants; however, it is frequently used for measuring the biodegradability of other lubricants. It is suitable for measuring the primary biodegradation of hydraulic fluids if they have methylene hydrogens (-CH<sub>2</sub>-) in their chemical structures. CEC results for some materials have been found to correlate with the results of ultimate biodegradation tests, but for some substances results from the CEC L-33-A-94 test over-predict ultimate biodegradability results (2, 3, 4, 5). The test is not suitable for either volatile or water soluble test materials.

6.2 *Aerobic Marine Environment*—Tests for oil biodegradation in a marine environment are cited in the literature and OECD has published a standardized method, OECD 306 (6). At the present time the standardized method has not been widely used, and there are significant uncertainties regarding the test methods cited in literature. Each case must be examined individually.

6.2.1 The OECD 306 test method has two options for the test procedure: the Shake Flask Method (which is not the same as the U.S. EPA Shake Flask Test) and the Closed Bottle Method. The Shake Flask Method measures dissolved organic carbon (DOC) and is not suitable for substances with low water solubility (less than 2 mg/L). If poorly soluble substances are tested with this test method, unchanged and undissolved carbon will be removed from the test system during a filtration step and the test substance will appear to have biodegraded when it has not. The Closed Bottle Method measures oxygen content of the test system and OECD states in the method that it is not recommended for substances with low water solubility.

6.3 *Aerobic Soil Environment*—Test procedures in aerobic soils are not as well developed as test procedures for aerobic aqueous environments (7). It is not possible to sterilize soil without drastically changing its physical properties, so a sterile starting soil similar in function to high-grade, carbon-free water, is not possible. The inoculum for these test procedures is typically the microbial community that naturally resides in the soil sample used for the test. No further augmentation generally is required. The sources of the soil samples should be reported with test results. A low result in any soil test may not mean that the hydraulic fluid will persist in an aerobic soil environment, but does mean that more testing is required.

6.3.1 With modifications, published tests for aerobic biodegradation in soils could be suitable for hydraulic fluids, but none of the available standardized tests can be used as written. In some cases only minor changes are necessary, such as development of a method for introduction of a water insoluble substance. Tests of soil biodegradation that currently are available fall into three categories.

6.3.1.1 First are those tests that follow CO<sub>2</sub> production by chemical means. An example of this kind of test has been published by the U.S. FDA (8). These tests are suitable for adaptation to assess the biodegradability of a hydraulic fluid in aerobic soils. Such adaptation may include different sample

<sup>6</sup> The boldface numbers in parentheses refer to the list of references at the end of this standard.

handling procedures or changes in sample concentration. The U.S. FDA test is not suitable for testing volatile hydraulic fluids.

6.3.1.2 Second are those that use test substances labeled with radioactive tracers and follow the production of radioactive CO<sub>2</sub>, for example OECD 304A (6). These tests have the advantage of allowing the use of very low concentrations of test substance, but are inappropriate for hydraulic fluids because hydraulic fluids are complex mixtures of many chemical compounds, so the selection of an appropriate site for the label is impossible (7).

6.3.1.3 Third are soil slurry tests that make a well-mixed aerobic slurry of the soil and follow CO<sub>2</sub> production. A consideration for soil slurry tests is that they are not a direct comparison to a soil system. The fungi normally present in soil will not be active in a slurry and the microbial activity may or may not be representative of that found in soil.

6.3.2 A major reason for the difficulty of testing for biodegradation in soils is that soils are complex mixtures of living organisms and nonliving substances, some of which are sources of food for the organisms. Soils vary greatly between locations, and the range of variation is large enough to affect the biodegradation of hydraulic fluids in soil. Because of the large variation in soil properties, demonstration of the biodegradation of hydraulic fluids in soils should be done using at least three different types of soil from three different locations. The characteristics of the soil should be reported with the test results.

6.3.2.1 Some soil characteristics that are important in biodegradation are pH, percent water holding capacity, moisture content, percent organic matter, percent silt and clay, nitrogen content and phosphorus content.

6.3.2.2 Soil tests commonly are run using blank systems to account for the metabolism and subsequent production of CO<sub>2</sub> that would occur in the absence of the test substance. The blank system contains everything the test system contains with the exception of the test substance. The amount of CO<sub>2</sub> produced by the blank is subtracted from the CO<sub>2</sub> produced by the test system to obtain the CO<sub>2</sub> produced during metabolism of the test substance.

6.3.2.3 Implicit in the use of a blank to account for intrinsic metabolism is the assumption that the addition of the test substance will not affect that metabolism. There have been some reports that some test substances, especially very easily biodegraded substances, may stimulate the metabolic transformation of materials which occur naturally in the soil and so lead to measured biodegradabilities over 100 % theoretical CO<sub>2</sub> (9).

6.4 *Anaerobic Environment*—Testing for biodegradation in an anaerobic environment may be done with the U.S. EPA Anaerobic Biodegradation test procedure (10) or with the method recommended by ECETOC (11). Other test procedures may be modeled after Test Methods D5210 or E1196. The test procedure and inoculum source, if not the naturally occurring microbial community, should be reported with the test results. A high result indicates that the fluid will be biodegradable in many natural anaerobic environments, such as swamps and sediments and in manmade anaerobic environments, such as

anaerobic waste water digesters. As with the other test procedures cited, a low result may not mean that the fluid is anaerobically persistent, but does mean that further testing is needed.

6.5 Table 1 summarizes the test methods discussed in Section 6.

## 7. Discussion

7.1 Hydraulic fluids are difficult to test for biodegradability because usually they are insoluble in water and consist of complex mixtures of chemicals. With the exception of Test Method D5864, currently available tests for biodegradation have been developed for water-soluble pure chemicals. In some cases, the tests contain procedural options not suitable for testing hydraulic fluids, and in other cases the procedures are not specific enough to give good, reproducible results for hydraulic fluids. Guidelines for the performance of test procedures for biodegradability testing of hydraulic fluids are given in this section. All test procedures for regulatory submission or advertising purposes should be run under Good Laboratory Practice (GLP) standards as outlined in the Toxic Substances Control Act (12).

7.2 Tests for aerobic biodegradation which measure CO<sub>2</sub> production are done frequently in systems that are swept regularly with fresh, CO<sub>2</sub>-free air to maintain the aerobic environment. Any test substance in the air space of the system will be removed, so hydraulic fluids with high vapor pressure above the test medium could give a false negative result.

7.2.1 The volatility of a petroleum-based hydraulic fluid above a test system can be estimated by its vapor pressure as measured by Test Method D5480 or an equivalent method. If the volatility exceeds 5 % at 371 °C in accordance with Test Method D5480, then the OECD 301F test should be used to measure aerobic aquatic biodegradability.

7.3 The amount of hydraulic fluid to be added is determined by calculating the weight of the hydraulic fluid that will contain the amount of carbon specified in the selected test method.

TABLE 1 Biodegradation Test Methods

Environmental Conditions	Test Method	Section
Aerobic aquatic	OECD 301B, the Modified Sturm Test	6.1 to 6.1.1.1
	OECD 301F, the Manometric Respirometry Test	6.1 to 6.1.1.1
	U.S. EPA Aerobic Aquatic Biodegradation Test (EPA Shake Flask Test)	6.1 to 6.1.1.1
	ISO Test 9439:1990	6.1 to 6.1.1.1
	Test Method D5864	6.1 to 6.1.1.1
	CEC L-33-A-94 (formerly CEC L-33-T-82)	6.1, 6.1.2
Aerobic marine	OECD 306, Biodegradability in Seawater	6.1.2
Aerobic soil	U.S. FDA, Aerobic Biodegradation in Soil	6.3 to 6.3.2.3
	OECD 304A	6.3 to 6.3.2.3
Anaerobic	U.S. EPA Anaerobic Biodegradation	6.4
	ECETOC Evaluation of Anaerobic Biodegradation	6.4
	Test Method E1196	6.4
	Test Method D5210	6.4