



Standard Guide for Assessing Biodegradability of Hydraulic Fluids¹

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1. Scope

1.1 This guide 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 *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:

D 5210 Test Method for Determining the Anaerobic Biodegradation of Plastic Materials in the Presence of Municipal Sewage Sludge²

D 5291 Test Methods for Instrumental Determination of Carbon, Hydrogen, and Nitrogen in Petroleum Products and Lubricants³

D 5480 Test for Motor Oil Volatility by Gas Chromatography³

D 5864 Test Method for Determining the Aerobic Aquatic Biodegradation of Lubricants and Their Components⁴

E 1196 Test Method for Determining the Anaerobic Biodegradation Potential of Organic Chemicals⁴

2.2 ISO Standards:

ISO 9439:1990 Technical Corrigendum I, Water Quality—Evaluation in an Aqueous Medium of the Ultimate Biodegradability of Organic Compounds⁵

ISO 4259:1992(E) Petroleum Products—Determination and Application of Precision Data in Relation to Methods of Test⁵

2.3 OECD Standards:

OECD 301B (the Modified Sturm Test) Guidelines for Testing Chemicals⁶

OECD 301F (the Manometric Respirometry Test) Guidelines for Testing of Chemicals⁶

3. Terminology

3.1 Definitions:

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

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

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

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

3.1.4 *biomass, n*—any material, excluding fossil fuels, which is or was a living organism or component of a living organism.

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

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

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² *Annual Book of ASTM Standards*, Vol 08.03.

³ *Annual Book of ASTM Standards*, Vol 05.03.

⁴ *Annual Book of ASTM Standards*, Vol 11.03.

⁵ Available from American National Standards Institute, 11 W. 42nd St., 13th Floor, New York, NY 10036.

⁶ Available from the Organization for Economic Cooperation and Development, 2 Rue Andre Pascal, S-75775, Paris CEDEX 16.

3.1.6.1 *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.

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

3.1.8 *preadaptation, n*—the incubation of an inoculum in the presence of the test substance which is done prior to the initiation of the test and under conditions similar to the test conditions.

3.1.8.1 *Discussion*—The aim of preadaptation is to improve the precision of the test method by decreasing variability in the rate of biodegradation produced by the inoculum. Preadaptation may mimic the natural processes which cause changes in the microbial population of the inoculum leading to more rapid biodegradation of the test substance, but it is not expected to change the final degree of biodegradation.

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

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

3.1.10.1 *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.

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

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

3.1.12 *ultimate biodegradation, n*—degradation achieved when a substance 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).

3.1.13 *ultimate biodegradation test, n*—a test which estimates the extent to which the carbon in a product is converted to CO₂ or methane, either directly, by measuring the production of CO₂ or methane, or, in the case of aerobic biodegradation, indirectly by measuring the consumption of O₂.

3.1.13.1 *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 CO₂ 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 CO₂. 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);⁷ Test Method D 5864; and the ISO Test 9439:1990. With the exception of Test Method D 5864, 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 CO₂ 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

⁷ The boldface numbers in parentheses refer to the list of references at the end of this standard.

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 CO₂. In this case a biodegradable fluid would have a low measured percent theoretical CO₂. 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 (-CH₂-) 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 CO₂ 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 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 CO₂, 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 CO₂ 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 CO₂ 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 CO₂ produced by the blank is subtracted from the CO₂ produced by the test system to obtain the CO₂ 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 CO₂ (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 D 5210 or E 1196. 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 D 5864, 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 CO₂ production are done frequently in systems that are swept regularly with fresh, CO₂-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 D 5480 or an equivalent method. If the volatility exceeds 5 % at 371°C in accordance with Test Method D 5480, 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. Because of the complex nature of hydraulic fluids, the carbon content of the fluid should be measured, not calculated. Test Method D 5291 or an equivalent procedure may be used.

7.4 The hydraulic fluid should be added gravimetrically and not volumetrically to the test medium. Volumetric measurement of the hydraulic fluid will lead to large variability in the added amount and so to large variability in test results. This error will be prevalent particularly for hydraulic fluids with high viscosity. Because of the small amount of fluid added to the test medium in these procedures, the gravimetric addition may be done in two steps. In other words, the fluid may be weighed into or onto a small container, and then the hydraulic fluid and the container itself added to the much larger test medium container.

7.5 Inocula for aerobic aquatic test methods may be obtained from a well-operated waste water treatment plant that receives predominantly domestic sewage. It also may be obtained from surface waters or from soils after preparation as may be outlined in an appropriate test method. It should be used within 24 h either to initiate preadaptation of the culture or to start the test method itself.

7.6 The inoculum may be preadapted, which is expected to decrease test to test variability. Preadaptation procedures can be found in the U.S. EPA Aerobic Aquatic Biodegradation Test (1) and Ref (13).

7.7 It may be advantageous to disperse poorly water soluble hydraulic fluids in addition to that dispersal that would be achieved by the mixing prescribed in each test method. In the event that additional dispersal of the hydraulic fluid is desired, physical means of dispersal are preferred. The applicability of various dispersal methods to hydraulic fluids is a matter still under investigation. Special care should be taken with the dispersal of viscous fluids. The method of dispersal should be reported with the test results. Examples of some dispersal methods follow.

7.7.1 Sonication of the hydraulic fluid in water may create a dispersion or emulsion of very fine droplets. If this technique is used, the container holding the sonicated mixture during sonication should be added to the preadaptation mixture or test mixture. Addition of the container in which sonication is done

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 D 5864	6.1 to 6.1.1.1
	CEC L-33-A-94 (formerly CEC L-33-T-82)	6.1, 6.1.2
	OECD 306, Biodegradability in Seawater	6.1.2
Aerobic marine	U.S. FDA, Aerobic Biodegradation in Soil	6.3 to 6.3.2.3
	OECD 304A	6.3 to 6.3.2.3
Aerobic soil	U.S. EPA Anaerobic Biodegradation	6.4
	ECETOC Evaluation of Anaerobic Biodegradation	6.4
	Test Method E 1196	6.4
	Test Method D 5210	6.4

to the test flask will ensure that all the hydraulic fluid is added to the test flask.

7.7.2 Coating of the hydraulic fluid onto glass beads, or other fine inert substrate, will present a large surface area to the inoculum. If this test method is used, the container holding the test material and the inert carrier should be added to the mixture.

7.7.3 It may be acceptable to introduce a hydraulic fluid to the test flask in a volatile nonbiodegradable carrier solvent and then allow the solvent to evaporate. In order for this procedure to be acceptable, the blank system must be treated in exactly the same manner. It is advisable to conduct separate experiments to determine that the carrier solvent does evaporate under the conditions used for this experiment. It also is advisable to have available data that demonstrates that the use of the carrier solvent does not affect the biodegradation of substances with known biodegradability.

7.8 Nonvolatile reference substances must be used in all test methods. The successful biodegradation of a substance known to be biodegradable demonstrates the viability of the inoculum. It is advisable to use a reference substance with water solubility characteristics and, if possible, viscosity similar to the test substance. The suggested reference substance for a water-insoluble hydraulic fluid with low viscosity is low erucic acid rapeseed (LEAR) oil, for example canola oil. The fatty acids in the low erucic acid rapeseed oil are a maximum of 2 % by weight erucic acid. The reference substance for a water-soluble hydraulic fluid may be glucose, phenol or another reference substance that is cited in the standard tests referenced in 6.1.1. The reference substance and the test substance should be dispersed in the test medium with the same procedures. The results of the reference substance should be reported with the results of the hydraulic fluid. The results obtained with the reference substance should not be used to generate a correction factor for interpretation of test results because the test substance does not necessarily respond in a test procedure in the same way as the reference substance.

7.9 In order to interpret tests that measure produced CO₂, the ratio between the CO₂ and the biomass produced by metabolism of the hydraulic fluid under the conditions of the test must be known or assumed. The Modified Sturm Test

(OECD 301B) referenced in 6.1 mentions 60 % of theoretical CO₂ in 28 days as being required for a passing result for a test substance. The U.S. EPA Aerobic Aquatic Biodegradation Test says that if the reference substance produces 60 % of theoretical CO₂ in 28 days, the inoculum is acceptable, and the ISO test 9439:1990 requires that the reference reach 50 % of theoretical CO₂ in 14 days. An inference that could be drawn from these three test procedures is that good biodegradability has been demonstrated if the test substance reaches the level set by the Modified Sturm Test, 60 % of theoretical CO₂ in 28 days. The 60 % level assumes that the carbon mass ratio of CO₂ to biomass produced during the biodegradation is greater than or equal to 60:40. If this ratio is exactly 60:40, a result of 60 % theoretical CO₂ means that all of the test substance has been biodegraded. If the ratio is 50:50, however, the test substance will not pass even though it has completely biodegraded, and so will give a false negative. Conversely, if the true ratio is 80:20, a result of 60 % means that the test substance has not completely biodegraded, and in these time-limited tests, gives a false positive. The ratio of produced CO₂ to biomass is under study **(14,15,16)**.

7.9.1 The biodegradation tests published by OECD as tests in the 301 series have as part of their pass criteria a requirement commonly referred to as the 10-day window. This requirement states that the percent theoretical CO₂ must reach 60 % within 10 days after reaching the 10 % level. The OECD currently is studying this requirement. It was based on data for water soluble substances, and there are questions as to its applicability to substances that are poorly soluble in water.

7.10 At least duplicate test mixtures of all hydraulic fluids, reference substances and blanks, should be made and run. Triplicates are preferred. The average of all replicates and their standard deviation are to be reported. In the case that one or more replicates are excluded on statistical grounds as given in ISO 4259, the excluded data and the reason for exclusion should be reported. More elaborate analysis of the statistics of test results is part of test method development.

8. Keywords

8.1 biodegradation; hydraulic fluids; persistence; water-insoluble

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