NOTICE:¬This¬standard¬has¬either¬been¬superseded¬and¬replaced¬by¬a¬new¬version¬or discontinued.¬Contact¬ASTM¬International¬(www.astm.org)¬for¬the¬latest¬information.

**Designation: D 6046 – 98a**

An American National Standard

AMERICAN SOCIETY FOR TESTING AND MATERIALS
100 Barr Harbor Dr., West Conshohocken, PA 19428
Reprinted from the Annual Book of ASTM Standards. Copyright ASTM

# Standard Classification of Hydraulic Fluids for Environmental Impact[1]

This standard is issued under the fixed designation D 6046; 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 classification covers all unused fully formulated hydraulic fluids in their original form.

1.2 This classification establishes categories for the impact of hydraulic fluids on different environmental compartments as shown in Table 1. Fluids are assigned designations within these categories; for example PwL, Pwe, and so forth, based on performance in specified tests.

1.3 In the current version of this classification the aspects of environmental impact included are environmental persistence of which biodegradability is one component and acute ecotoxicity. Although environmental persistence is discussed first, this classification does not imply that considerations of environmental persistence should take precedence over concerns for ecotoxicity.

1.4 Another important aspect of environmental impact is bioaccumulation. This aspect is not addressed in the present classification because adequate test methods do not yet exist to measure bioaccumulation of hydraulic fluids.

1.5 The present classification addresses the fresh water and soil environmental compartments. At this time marine and anaerobic environmental compartments are not included, although they are pertinent for many uses of hydraulic fluids. Hydraulic fluids are expected to have no significant impact on the atmosphere; therefore that compartment is not addressed.

1.6 This classification addresses releases to the environment which are incidental to the use of a hydraulic fluid. The classification is not intended to address environmental impact in situations of major, accidental release. Nothing in this classification should be taken to relieve the user of the responsibility to properly use and dispose of hydraulic fluids.

1.7 This classification does not cover any performance properties of a hydraulic fluid which relate to its performance in a hydraulic system.

1.8 *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.*

**TABLE 1 Overview of Extended Classification**

| Environmental Compartment | Categories of Environmental Impact | | |
|---|---|---|---|
| | Environmental Persistence | Ecotoxicity | Bioaccumulation |
| Fresh Water | Pw | Tw | Bw |
| Marine | Pm | Tm | Bm |
| Soil | Ps | Ts | Bs |
| Anaerobic | Pa | Ta | Ba |

## 2. Referenced Documents

2.1 *ASTM Standards:*

D 5291 Test Methods for Instrumental Determination of Carbon, Hydrogen, and Nitrogen in Petroleum Products and Lubricants[2]

D 5864 Test Method for Determining the Aerobic Aquatic Biodegradation of Lubricants or Their Components[2]

D 6006 Guide for Assessing Biodegradability of Hydraulic Fluids

D 6081 Practice for Aquatic Toxicity Testing of Lubricants: Sample Preparation and Results Interpretation[2]

E 943 Terminology Relating to Biological Effects and Environmental Fate[3]

E 1440 Guide for an Acute Toxicity Test with the Rotifer Brachionus[3]

2.2 *ISO Standards:*[4]

International Standard ISO Test 9439:1990, Technical Corrigendum 1, Water Quality—Evaluation in An Aqueous Medium of the “Ultimate” Biodegradability of Organic Compounds—Method by Analysis of Released Carbon Dioxide

2.3 *OECD Standards:*[5]

OECD 301B, $CO_2$ Evolution Test (the Modified Sturm Test)

OECD 301C, Modified MITI Test (I)

OECD 301F, Manometric Respirometry Test

OECD 201, Alga, Growth Inhibition Test

OECD 202, Daphnia sp., Acute Immobilisation Test and Reproduction Test

---

[1] This classification is under the jurisdiction of ASTM Committee D-2 on Petroleum Products and Lubricants and is the direct responsibility of Subcommittee D02.N on Hydraulic Fluids.

Current edition approved Apr. 10, 1998. Published October 1998. Originally published as D 6046–96. Last previous edition D 6046–98.

[2] *Annual Book of ASTM Standards*, Vol 05.03.

[3] *Annual Book of ASTM Standards*, Vol 11.05.

[4] Available from American National Standards Institute, 11 West 42 Street, New York, NY 10036. All standards referenced are from the OECD Guidelines for Testing of Chemicals.

[5] Available from Organization for Economic Cooperation and Development (OECD), 2, Rue Andre Pascal, S-75775, Paris CEDEX 16, France. May also be found in U.S. Federal Register, Vol 50, No. 188, September 27, 1965, paragraph 796.3260.

OECD 203, Fish, Acute Toxicity Test
OECD 207, Earthworm Acute Toxicity Test
OECD 208, Terrestrial Plants Growth Test

2.4 *US EPA Tests:*[6]

Aerobic Aquatic Biodegradation Test, 40 CFR 796.3100. (Also available as EPA publication 560/6-82-003, number CG-2000)

Toxic Substances Control Act (TSCA), 40 CFR Parts 796 and 797—Environmental Effects Testing Guidelines, Federal Register, Vol 50, No. 188, September 27, 1985, p. 39321

2.5 *Environmental Canada Test Methods:*[7]

Biological Test Method: Acute Lethality Test Using Rainbow Trout, Report EPS 1/9, Environment Canada, July 1990

Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout, Reference Method EPS 1/RM/13, Environment Canada, July 1990

Biological Test Method: Growth Inhibition Test Using the Freshwater Alga *Selenastrum capricornutum*, Report EPS 1/RM/25, Environment Canada, November 1992

Biological Test Method: Acute Lethality Test Using *Daphnia* spp., Report EPS 1/RM/11, Environment Canada, July 1990

Biodegradability of Two-Stroke Cycle Outboard Engine Oils in Water, CEC L-33-A-934, Co-Ordinating European Council, 1994 (Formerly L-33-T-82)

Toxic Substances Control Act (TSCA), Good Laboratory Practice Standards, Final Rule, U.S. Federal Register, 40 CFR Part 792, August 17, 1989

2.6 *Other Standards:*

MENVIQ 92.03/800—D.mag. 1.1, March 1992[8]

29 CFR 1910 OSHA Regulated Carcinogens and Potential Carcinogens[6]

## 3. Terminology

3.1 *Definitions:*

3.1.1 *acute ecotoxicity*, *n*—the propensity of a material to produce adverse behavioral, biochemical, or physiological effects in non-human organisms or populations in a short period of time, usually not constituting a substantial portion of the life span of the organism.

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 removed from the environment.

3.1.5 *ecotoxicity*, *n*—the propensity of a material to produce adverse behavioral, biochemical, or physiological effects in non-human organisms or populations.

3.1.6 *effect load XX (ELXX)*, *n*—a statistically or graphically estimated loading rate of test material that is expected to cause one or more specified effects in XX % of a group of organisms under specified conditions for a specified time.

3.1.6.1 *Discussion*—This terminology should be used for hydraulic fluids instead of the standard effect concentration (ECXX) when the hydraulic fluid is not completely soluble under test conditions.

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, aerobic soil, and anaerobic media. The results of test procedures may be applied to environmental compartments, but the test systems do not constitute an environmental compartment.

3.1.8 *fresh water environment*, *n*—the aerobic, fresh water environmental compartment.

3.1.9 *good laboratory practices (GLP)*, *n*— guidelines for the management of laboratory experiments which are published by regulatory agencies or other recognized groups and are concerned with the organizational process and the conditions under which laboratory studies are planned, performed, monitored, recorded, and reported.

3.1.9.1 *Discussion*—The major GLPs used are USEPA–TSCA, USFDA, OECD, and to some extent the MITI version from Japan for submissions in Japan.

3.1.10 *inhibition load XX (ILXX)*, *n*—a statistically or graphically estimated loading rate of test material that is expected to cause a XX % inhibition of a biological process (such as growth or reproduction) which has an analog as opposed to a digital measure.

3.1.10.1 *Discussion*—An example of a digital measure would be alive or dead. This terminology should be used for hydraulic fluids instead of the standard inhibition concentration (ICXX) when the hydraulic fluid is not completely soluble under test conditions.

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

3.1.12 *lethal load XX (LLXX)*, *n*—a statistically or graphically estimated loading rate of test material that is expected to be lethal to XX % of a group of organisms under specified conditions for a specified time.

3.1.12.1 *Discussion*—This terminology should be used for hydraulic fluids instead of the standard lethal concentration (LCXX) when the hydraulic fluid is not completely soluble under test conditions.

3.1.13 *loading rate*, *n*—the ratio of test material to aqueous medium used in the preparation of a water accommodated fraction (WAF) and in interpretation of the results of a toxicity study with a poorly water soluble lubricant or lubricant component.

3.1.14 *mechanical dispersion*, *n*—a mixture produced by the application of mechanical shearing forces to a multi-phase system, one component of which is water, so as to distribute one or more of the materials uniformly throughout the mass of the water with the water existing as a continuous phase.

---

[6] Available from U.S. Government Printing Office, Washington, DC.

[7] Available from CEC, Madou Plaza, Place Madou 1, B-1030 Brussels, Belgium.

[8] Available from Ministere de l'Environment, Gouvernment du Quebec.

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

3.1.15.1 *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 extent of biodegradation.

3.1.16 *primary biodegradation*, *n*—degradation of the test material by microorganisms resulting in a change in its physical or chemical properties, or both.

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

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

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

3.1.18 *terrestrial (or soil) environment*, *n*— the aerobic environmental compartment which is found in and on natural soils.

3.1.19 *theoretical* $CO_2$, *n*—the amount of $CO_2$ which could in theory be produced from the complete oxidation of all the carbon in a material.

3.1.20 *theoretical* $O_2$, *n*—the amount of oxygen which would theoretically be required to completely oxidize a material.

3.1.21 *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).

3.1.22 *ultimate biodegradation test*, *n*—a test which estimates the extent to which the carbon in a material is converted to $CO_2$ or methane, either directly by measuring the production of $CO_2$ or methane, or, for aerobic biodegradation, indirectly by measuring the consumption of $O_2$.

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

3.1.23 *water accommodated fraction (WAF)*, *n*— the predominately aqueous portion of a mixture of water and a poorly water-soluble material which separates in a specified period of time after the mixture has undergone a specified degree of mixing and includes water, dissolved components, and dispersed droplets of the poorly water soluble material.

3.1.23.1 *Discussion*—The chemical composition of the WAF depends on the ratio of poorly soluble material to water in the original mixture as well as the details of the mixing procedure.

3.1.24 *wppm*—an abbreviation for part per million by weight.

3.1.25 Definitions and terms not given in this classification may be found in the Compilation of ASTM Standard Definitions, 1990 or Terminology E 943.

## 4. Significance and Use

4.1 This classification establishes categories of hydraulic fluids which are distinguished by their response to certain standardized laboratory procedures. These procedures indicate the possible response of some environmental compartments to the introduction of the hydraulic fluid. One set of procedures measures the aerobic aquatic biodegradability (environmental persistence) of the fluids and another set of procedures estimates the acute ecotoxicity effects of the fluids.

4.1.1 Although this classification includes categories for both persistence and ecotoxicity, there is no relationship between the two categories. They may be used independently of each other, that is, a hydraulic fluid can be categorized with respect to both sets of laboratory procedures, or to persistence but not ecotoxicity, or to ecotoxicity but not persistence.

4.1.2 There is no relationship between the categories achieved by a hydraulic fluid for persistence and for ecotoxicity. The placing of a hydraulic fluid with regard to one set of categories has no predictive value as to its placement with regard to the other set of categories.

4.2 The test procedures used to establish the categories of hydraulic fluids are laboratory standard tests and are not intended to simulate the natural environment. Definitive field studies capable of correlating test results with the actual environmental impact of hydraulic fluids are usually site specific and so are not directly applicable to this classification. Therefore, the categories established by this classification can serve only as guidance to estimate the actual impact that the hydraulic fluids might have on any particular environment.

4.3 This classification can be used by producers and users of hydraulic fluids to establish a common set of references that describe some aspects of the anticipated environmental impact of hydraulic fluids which are incidental to their use.

4.4 Inclusion of a hydraulic fluid in any category of this classification does not imply that the hydraulic fluid is suitable for use in any particular hydraulic system application.

4.5 The composition of hydraulic fluids may change with use and any change could influence the environmental impact of a used hydraulic fluid. Therefore, the classification of a hydraulic fluid may change upon use depending on the type and extent of the use.

## 5. Basis of Classification

5.1 This classification consists of two groups of tests, one group addressing the environmental persistence of hydraulic fluids (Category P) and one group addressing acute ecotoxicity of hydraulic fluids (Category T). The ecotoxicity categories are further divided into two environmental compartments, aerobic soil (Ts) and aerobic fresh water (Tw). At this time categories for environmental persistence are limited to aerobic fresh water (Pw).

5.2 All testing shall use as its starting point the unused fully formulated hydraulic fluid.

5.3 The classification of hydraulic fluids for environmental persistence is defined by the hydraulic fluid's biodegradability as measured in tests for either ultimate or primary biodegradation. Pre-adaptation of inoculum for a period of up to two weeks is allowed for all categories. The classification system is given in Table 2.

5.3.1 Table 2 is divided into three parts. Part A, for hydraulic fluids with elemental analyses indicating a contents of less than 10 wt % oxygen, has separate requirements for tests which measure % theoretical $CO_2$ and % theoretical $O_2$. For these hydraulic fluids the different numerical results for the two different types of ultimate biodegradability tests are technically equivalent. Part B for hydraulic fluids with elemental analyses indicating a contents of 10 wt % or more oxygen has the same numerical result for both types of ultimate biodegradation test and these numerical results are also technically equivalent. The difference between hydraulic fluids containing little oxygen and fluids containing relatively abundant oxygen is related to the use of the oxygen present in the base stocks by the microorganisms. Further information is given in Appendix X1.

5.3.1.1 Oxygen content of hydraulic fluids is most commonly determined by difference, that is, by determining the other elemental components of the hydraulic fluid and subtracting that percentage from 100. For purposes of this classification determination of carbon, hydrogen, and nitrogen by Test Method D 5291 and subtraction of that total from 100 is acceptable. Direct determination of elemental oxygen by neutron activation is also acceptable.

5.3.1.2 Table 2 has been constructed assuming that 60 % of the carbon in the hydraulic fluid goes directly to $CO_2$ during biodegradation and that the remaining carbon is converted to biomass.

**TABLE 2 Environmental Persistence Classification—Aerobic Fresh Water**

NOTE 1—Test methods are given in Table 4.

| Persistence Designation | Ultimate Biodegradation Test Results | |
|---|---|---|
| | % Theoretical $CO_2$ | % Theoretical $O_2$ |
| | *For Hydraulic Fluids Containing Less Than 10 Wt % $O_2$* | |
| Pw1 | greater than or equal to 60 % in 28 days | greater than or equal to 67 % in 28 days |
| Pw2 | greater than or equal to 60 % in 84 days (12 weeks) | greater than or equal to 67 % in 84 days (12 weeks) |
| Pw3 | greater than or equal to 40 % in 84 days (12 weeks) | greater than or equal to 45 % in 84 days |
| Pw4 | less than 40 % in 84 days (12 weeks) | less than 45 % in 84 days (12 weeks) |

| Persistence Designation | Ultimate Biodegradation Test Results % Theoretical $CO_2$ or % Theoretical $O_2$ |
|---|---|
| | *For Hydraulic Fluids Containing 10 Wt % or More $O_2$* |
| Pw1 | greater than or equal to 60 % in 28 days |
| Pw2 | greater than or equal to 60 % in 84 days (12 weeks) |
| Pw3 | greater than or equal to 40 % in 84 days (12 weeks) |
| Pw4 | less than 40 % in 84 days (12 weeks) |

| Persistence Designation | Primary Biodegradation, Test Results % Loss of Starting Material |
|---|---|
| | *For All Hydraulic Fluids* |
| Pw-C | greater than or equal to 80 % in 21 days |
| Pw4 | less than 80 % in 21 days |

5.3.2 Class Pw1 includes hydraulic fluids which would be expected to be the least persistent in the environment. It may be assumed that such a fluid would rapidly and extensively biodegrade in an aerobic aquatic environment which contains microbial life and the conditions necessary for it.

5.3.2.1 Although a minimum production of 60 % theoretical $CO_2$ or greater up to but not including 100 % theoretical $CO_2$ or consumption of the technically equivalent fraction of theoretical $O_2$ leads to a strong implication that the hydraulic fluid will rapidly and extensively biodegrade, such a finding does not unequivocally rule out the possibility that the biodegradation produces recalcitrant metabolites not normally found in nature. Recalcitrant metabolites could constitute a persistent residue of the hydraulic fluid that may remain in the environment. It is also possible that a very small fraction of the original hydraulic fluid, perhaps an additive, could not be biodegraded and could persist in the environment.

5.3.3 Class Pw2 includes hydraulic fluids which would not be expected to persist in the environment in the long term. It may be assumed that these fluids would most likely be biodegraded eventually in an aerobic aquatic environment which contains microbial life and the conditions necessary for it.

5.3.3.1 As with category Pw1, incomplete biodegradation during the test time does not unequivocally define the extent to which the hydraulic fluid is fully and completely utilized, and so the inclusion of a hydraulic fluid in this class does not unequivocally rule out the possibility that a persistent residue of the hydraulic fluid may remain in the environment.

5.3.4 Class Pw3 includes hydraulic fluids which will not persist in the environment in an unchanged state. It may be assumed that either some or all components of the fluid will biodegrade to some extent, but not necessarily completely, in an aerobic aquatic environment which contains microbial life and the conditions necessary for it.

5.3.5 Class Pw-C includes hydraulic fluids which are not likely to persist in the environment in an unchanged state. This is inferred from results of the primary biodegradation test CEC L-33–A-934. Since primary biodegradation tests do not measure the extent to which the hydraulic fluid is fully and completely utilized and so no longer exists in the environment, inclusion of a hydraulic fluid in this class leaves open the possibility that a persistent residue of the hydraulic fluid may remain in the environment. Because this class is defined by the results of a primary biodegradation test, it is possible for a hydraulic fluid to belong to this class and to one of the other persistence classes.

5.3.6 Class Pw4 includes hydraulic fluids which exhibit low biodegradability and are expected to remain in an aerobic aquatic environment for relatively long periods of time. In the environment these fluids may or may not undergo biologically induced changes. Further testing, such as field studies, would be required to establish biodegradability of materials falling into this category. In the event that results for tests of both ultimate and primary biodegradability are available for a hydraulic fluid, the fluid will be assigned the designation Pw4 only if the criteria for that designation are met for both types of test.

5.3.7 To establish a designation it is necessary to run only one of the recommended methods.

5.3.8 In the event that the results of different tests for one hydraulic fluid are available, the highest result achieved among the Pw classes shall determine the classification. For example, if results for a hydraulic fluid based on a hydrocarbon in a test for theoretical $CO_2$(refer to Table 2, Part A for hydraulic fluids containing less than 10 weight % $O_2$) gave 64 % in 28 days, giving a designation of Pw1, while the results from a test for theoretical $O_2$ gave 62 % in 84 days, for a designation of Pw3, the classification will be Pw1 and not Pw3. That is, the tests are designed so that they are unlikely to find a material to be highly biodegradable if it is not. There is a much higher probability that a test will find a material to have a low biodegradability when in most natural environments it will biodegrade.

5.4 The classification of hydraulic fluids for acute ecotoxicity is given in Table 3. The ecotoxicity classification is further divided into two environmental compartments, aerobic soil, and aerobic fresh water.

5.4.1 Acute ecotoxicity in an aquatic environment shall be tested with at least three tests of acute toxicity, one test being on a plant, one test on a vertebrate, and one test on an invertebrate appropriate for the aquatic compartment.

5.4.2 Acute ecotoxicity in a terrestrial environment shall be tested in at least two tests of acute toxicity, one on an invertebrate and one on a plant. Tests on terrestrial vertebrates usually fall under the category of mammalian toxicity rather than ecotoxicity and so are not included in this classification.

5.4.3 The lowest numerical endpoint shall determine the classification of the hydraulic fluid. For example, if the LL50 for an aquatic vertebrate was 1150 wppm (Tw1) and the IL50 for algal growth was 800 wppm (Tw2), the rating would be Tw2.

5.4.4 If the hydraulic fluid contains materials regarded as carcinogenic (as per 29 CFR, part 1910) in an amount exceeding 0.1 wt %, the hydraulic fluid will not be eligible for the Tw1 or Ts1 designation. If ecotoxicity test results for the hydraulic fluid exceed 1000 wppm LL50, EL50, or IL50, the designation shall be Tw2 or Ts2 as appropriate.

5.5 Ecotoxicity testing of each fully formulated hydraulic fluid to be classified is preferable. However in the case where a hydraulic fluid is formulated at a viscosity grade intermediate to two tested fluids which are both in one designation, then that same designation may be used for the untested fluid provided the following conditions are met.

5.5.1 The change in viscosity grade is achieved by changing the proportions of two or more base stocks which are all present in both tested fluids. No new base stocks are introduced.

5.5.2 The same additives in both the same proportions and overall treat rate are used for all three fluids.

**TABLE 3 Acute Ecotoxicity Classification**

| Ecotoxicity in Soil Designation | Ecotoxicity in Water Designation | Loading Rate, wppm LL50, IL50, or EL50 |
|---|---|---|
| Ts1 | Tw1 | greater than 1000 |
| Ts2 | Tw2 | 1000–100 |
| Ts3 | Tw3 | 100–10 |
| Ts4 | Tw4 | <10 |

## 6. Test Methods

6.1 All testing shall be on unused fully formulated hydraulic fluids. The results of testing the components of hydraulic fluids shall not be used for purposes of this classification.

6.2 The most current version of the test method shall be used when multiple versions are available. The test methods shall be used as sources for test protocol only. Any pass/fail criteria which are implied or explicitly stated do not apply for the purposes of this classification.

6.2.1 The scope or other defining statements for each test method shall be consulted to ensure that the selected test method is appropriate for the hydraulic fluid under consideration; special attention shall be paid to the volatility of the tested fluid and the limitations of the test method reqarding volatility.

6.3 Appropriate quality assurance for all testing must be performed and reported. Good laboratory practice standards, such as OECD GLP and US EPA TSCA GLP, are acceptable.

6.4 The tests to be used for the environmental persistence portion of this classification are given in Table 4. They are ultimate or primary biodegradation tests carried out in aerobic aquatic media. Environmental persistence is a concern in environmental compartments other than aerobic fresh water, but tests for other compartments are not included at this time. See D 6006 for some considerations for these tests and biodegradation testing in general.

6.4.1 The ultimate biodegradation tests listed above have been developed with the intention that they be used to measure biodegradation of pure chemical compounds. Such tests have significant caveats associated with the interpretation of their results. The impact of these caveats may be less for pure chemicals than for mixtures such as hydraulic fluids because pure chemicals are expected to exhibit first order kinetics with respect to biodegradation and to continue biodegrading at one rate until the chemical is substantially depleted. This behavior may not be followed in the biodegradation of a mixture such as

**TABLE 4 Tests of Biodegradability in Aerobic Aquatic Environments**

| Test Title | Measurement | Sponsoring Organization |
|---|---|---|
| *Ultimate Biodegradation Tests:* | | |
| D 5864, Test Method for Determining the Aerobic Aquatic Biodegradation of Lubricants | % theoretical $CO_2$ | ASTM |
| 9429:1990, Technical Corrigendum 1, Water quality—evaluation in an aqueous medium of the “ultimate” biodegradability of organic compounds—Method by analysis of released carbon dioxide | %theoretical $CO_2$ | ISO |
| 301B, $CO_2$ Evolution Test (Modified Sturm Test) | % theoretical $CO_2$ | OECD |
| 301C, Modified MITI Test (I) | % theoretical $O_2$ | OECD |
| 301F, The Manometric Respirometry Test | %theoretical $O_2$ | OECD |
| Aerobic Aquatic Biodegradation Test | % theoretical $CO_2$ | US EPA |
| C.4–C: Carbon dioxide ($CO_2$) evolution | % theoretical $CO_2$ | EUC |
| C.4–D: Manometric Respirometry[A] | % theoretical $O_2$ | EUC |
| *Primary Biodegradation Tests:* | | |
| L-33–A-934, Biodegradability of Two-Stroke Cycle Outboard Engine Oils in Water (Formerly L-33–T-82) | % loss of extractable $CH_2$ groups | CEC |

[A] OECD equivalent test.

TABLE 5 Tests for Ecotoxicity

| Test Title | Sponsoring Organization |
|---|---|
| *Soil: Invertebrates* | |
| A.8.5 Earthworm survival (*Eisenia foetida*) (reference 2.4.2) | US EPA |
| 207, Earthworm Acute Toxicity Test | OECD |
| *Soil: Plants* | |
| A.8.7 Lettuce Root Elongation (*Lactuca sativa*) (reference 2.4.2) | US EPA |
| A.8.6 Lettuce seed germination (*Lactuca sativa*) (reference 2.4.2) | US EPA |
| 208, Terrestrial Plants Growth Test | OECD |
| 797.2750 of TSCA, Seed germination/root elongation toxicity test | US EPA |
| 797.2800 of TSCA, Early seedling growth toxicity test | US EPA |
| *Water: Plants* | |
| Section 797.1050 of TSCA, Algal acute toxicity | US EPA |
| Section 797.1060 of TSCA, Freshwater algal acute toxicity | US EPA |
| Section 797.1075 of TSCA, Freshwater and marine algae acute toxicity test | US EPA |
| C.3. Algal Inhibition Test | EU |
| Biological test method: Growth inhibition test using the freshwater alga *Selenastrum capricornutum* | Canada |
| 201, Alga Growth Inhibition Test | OECD |
| A.8.4 Algal growth (*Selenastrum capricornutum*) (reference 2.4.2) | US EPA |
| *Water: Invertebrates* | |
| 202, Daphnia sp., Acute Immobilisation Test and Reproduction Test | OECD |
| Biological test method: Acute lethality test using *Daphnia* spp. | Canada |
| C.2. Acute toxicity for *daphnia*$^A$ | EU |
| A.8.2 *Daphnia Pulex* and *Daphnia magna* Survival (reference 2.4.2) | US EPA |
| 797.1300 of TSCA, *Daphnid* acute toxicity test | US EPA |
| E 1440, Acute Toxicity Test with the Rotifer Brachions | ASTM |
| MENVIQ.92.03/800—D. mag. 1.1 Determination de la toxicite lethale CL 50–48 h *Daphnia magna* | Quebec |
| *Water: Vertebrates* | |
| 203, Fish, Acute Toxicity Test | OECD |
| 797.1400 of TSCA, Fish acute toxicity test | US EPA |
| 797.1440 of TSCA, Fish acute toxicity test | US EPA |
| C.1. Acute Toxicity for fish | EU |
| Biological Test Method: Acute Lethality Test Using Rainbow Trout | Canada |
| Biological Test Method: Reference method for determining acute lethality of effluents to rainbow trout | Canada |
| A.8.3 Fathead minnow survival (reference 2.4.2) | US EPA |

a hydraulic fluid. It is possible that a mixture could undergo rapid biodegradation until one or more biodegradable components are depleted, and then the rate could significantly slow as less biodegradable components make up a larger and larger fraction of the remaining fluid.

6.4.2 The tests in Table 4 are to be used as stated in their respective test procedures with one exception. The OECD tests explicitly do not allow the use of a pre-adapted inoculum, while this classification allows the use of pre-adaptation. If a hydraulic fluid is being tested for classification using any of the OECD tests, the pre-adaptation procedure as described in one of the other test methods may be followed.

6.4.3 Biodegradation tests shall include a positive control, for example, low erucic acid rapeseed oil (LEAR) or sunflower oil or another vegetable oil which has been shown to be the equivalent of LEAR in the test being used. The purpose of the positive control is to verify the viability of the test inoculum. Viability of the inoculum is verified if the positive control produces at least 60 % theoretical $CO_2$ or consumes at least 60 % theoretical $O_2$.

6.5 Table 5 gives tests recommended for the acute ecotoxicity classification. Other tests which can be shown to be equivalent are also allowed.

6.5.1 All aquatic toxicity testing of hydraulic fluids which are poorly soluble in water shall be done using a water accommodated fraction (WAF) except that testing on aquatic vertebrates may be done using mechanical dispersion. Practice D 6081 shall be used to prepare WAFs and mechanical dispersions and to interpret these results. All test systems shall contain oxygen levels above 60 % saturation at all times. This may be accomplished with renewals of the test system on a daily basis or at other interval necessary to maintain the required oxygen level. Testing on algae will not be done with daily renewal. Testing of aquatic invertebrates may be done using static methods. Testing for both terrestrial invertebrates and terrestrial plants may be done using direct additions of the hydraulic fluids to the soil. The results shall be calculated and reported in a manner appropriate for the testing method.

## 7. Keywords

7.1 biodegradation; ecotoxicity; environmental persistence; hydraulic fluid; insoluble material

# APPENDIX

### (Nonmandatory Information)

## X1. RELATIONSHIP BETWEEN $CO_2$ PRODUCED AND $O_2$ CONSUMED IN ULTIMATE BIODEGRADATION TESTING

X1.1 The numerical requirements for the various levels of the persistence category shown in Table 2 are different for measurements of $CO_2$ produced by the ultimate biodegradation of a test substance and of $O_2$ consumed by the same process if the hydraulic fluid contains less than 10 % oxygen. This is because different fractions of the carbon and oxygen involved in the process are found in the $CO_2$, water, and biomass which are the process products. The relationship is shown in the chemical Eq X1.1:

$$C_nH_mO_p + aO_2 \rightarrow rCO_2 + s\,H_2O + t\,C_3H_5O \qquad \text{(X1.1)}$$

which gives the chemical reaction for a generalized oxygen-carbon-hydrogen compound undergoing oxidation to $CO_2$, $H_2O$, and biomass. The formula given for biomass is an empirical formula which is documented in X1.2.

X1.1.1 This chemical equation contains too many variables to be solved in a straight forward fashion, so various cases are calculated to arrive at Table 2. One parameter for case selection is the ratio of carbon in $CO_2$ and in biomass. The cases are:

X1.1.1.1 50 % of the carbon going to $CO_2$ and 50 % to biomass ($C_3H_5O$) (see Table X1.1).

X1.1.1.2 60 % of the carbon going to $CO_2$ and 40 % to biomass ($C_3H_5O$) (see Table X1.2).

X1.1.1.3 70 % of the carbon going to $CO_2$ and 30 % to biomass ($C_3H_5O$) (see Table X1.3).

These three selections are equivalent to saying that the % theoretical $CO_2$ produced when biodegradation is complete is 50, 60, and 70 %, respectively. Another parameter for case selection is the specific formula of the test material, $C_nH_mO_p$. Two levels of specificity will be examined. The first level will be the amount of oxygen in the test material:

(*a*) $C_nH_{2n+2}$, a saturated hydrocarbon,

(*b*) $C_nH_{2n-1}O_2$, a single ester linkage in an otherwise saturated hydrocarbon, and

(*c*) $C_nH_{2n-24}O_4$, two ester linkages in an otherwise saturated hydrocarbon.

The second level examined for the test substance is *n*, the number of carbon atoms in the molecule:

$$n = 14 \qquad \text{(X1.2)}$$

$$n = 20$$

$$n = 26$$

**TABLE X1.1 For Cases With 50 % of the Carbon Going to $CO_2$ and 50 % to Biomass**

| Type of Test Compound | | One Ester, $C_nH_{2n-1}O_2$ | Two Ester, $C_nH_{2n-24}O_4$ |
|---|---|---|---|
| Number of Carbons, n | Hydrocarbon, $C_nH_{2n+2}$ | | |
| 14 | 59 | 56 | 53 |
| 20 | 59 | 57 | 54 |
| 26 | 59 | 57 | 56 |

**TABLE X1.2 For Cases With 60 % of the Carbon Going to $CO_2$ and 40 % to Biomass**

| Type of Test Compound | | One Ester, $C_nH_{2n-1}O_2$ | Two Ester, $C_nH_{2n-24}O_4$ |
|---|---|---|---|
| Number of Carbons, n | Hydrocarbon, $C_nH_{2n+2}$ | | |
| 14 | 67 | 65 | 62 |
| 20 | 67 | 66 | 64 |
| 26 | 67 | 66 | 64 |

**TABLE X1.3 For Cases With 70 % of the Carbon Going to $CO_2$ and 30 % to Biomass**

| Type of Test Compound | | One Ester, $C_nH_{2n-1}O_2$ | Two Ester, $C_nH_{2n-24}O_4$ |
|---|---|---|---|
| Number of Carbons, n | Hydrocarbon, $C_nH_{2n+2}$ | | |
| 14 | 76 | 74 | 72 |
| 20 | 75 | 74 | 73 |
| 26 | 75 | 74 | 73 |

X1.1.2 To illustrate the calculation, which involves only principles of chemical stoichiometry and simultaneous equations, one case will be given in detail. Throughout the following the theoretical $CO_2$, *Tc*, will be given in moles of $CO_2$ and theoretical $O_2$. *To*, will be given in mol of atomic oxygen rather than weight to simplify the comparison to the chemical equation.

X1.1.2.1 For a saturated hydrocarbon the *To* is $3n + 1$. With 50 % of the carbon to $CO_2$ and 50 % to biomass, the chemical reaction simplifies to Eq X1.2.

$$C_nH_{2n+2} + aO_2 \rightarrow 0.5n\,CO_2 + sH_2O + 0.17n\,C_3H_5O \qquad \text{(X1.3)}$$

From oxygen balance: $2a = n + s + 0.17n$, which is *To*-(case),

From hydrogen balance: $2n + 2 = 2s + 0.83\,n$, and

Upon simplifying, $To = 1.75n + 1$.

In the following *To* (ratio) = *To*(case)*To* and is equal to the % theoretical $O_2$ which would be measured in an experiment meeting the conditions of the case. For this case:

$$\text{If } n = 14,\ To\ (\text{ratio}) = 59\ \% \qquad \text{(X1.4)}$$

$$\text{If } n = 20,\ To\ (\text{ratio}) = 59\ \%$$

$$\text{If } n = 26,\ To\ (\text{ratio}) = 59\ \%$$

These calculations contain rounding errors and should be considered accurate to only two significant figures with ±1 in the final figure.

X1.1.3 The following tables summarize the results for calculations of the other cases. Listed results are *To*(ratio).

X1.1.4 As more oxygen is present in the test substance, the *To*(ratio), which is measured in ultimate biodegradation tests using consumed oxygen, approaches the amount of carbon going into $CO_2$, which is the *Tc*(ratio), measured in ultimate biodegradation tests using produced $CO_2$. The largest discrepancy between results for the two types of tests will exist for hydrocarbons.

X1.2 The empirical formula for biomass used above, $C_3H_5O$, has been estimated assuming that the biomass is bacteria and is on a dry weight basis assuming that C, H, and O constitute 99 % of the weight of the cell. The estimate further assumes that the major types of macromolecules present are protein, carbohydrate, and lipid. A model molecule for each of these groups was estimated, and then all the groups were normalized against the proportion of the biomass which consists of that group. References used to develop the empirical formula are given below.

X1.2.1 Protein typically ranges from 40 to 80 % (dry weight) of the cell, of which 5 to 30 % is nucleoprotein. The composition of the model protein macromolecule was taken to be the average of all 26 amino acids, which assumes that all amino acids are used equally. This assumption is not likely, but no general rule exists for the occurrence of amino acids in bacteria. The final result is an empirical formula of $C_6H_{10}O_{2.7}$ for proteins. Nucleoproteins also exist in a range of compositions, but average to 50 % GC and 50 % AT for an empirical formula of $C_{19.5}H_{20.5}O_4$.

X1.2.2 Carbohydrates are 1.5 to 36 % (dry weight) of bacteria. Carbohydrate composition was calculated on the basis of it being polymers of glucose, glycogen, and cellulose, with an empirical formula of $C_6H_{10}O_5$.

X1.2.3 Lipids are 0.4 to 39 % (dry weight) of bacteria. The empirical formula for palmitic acid as a free acid was used since this is the most common lipid, that is, $C_{16}H_{32}O_2$.

X1.2.4 When calculating the empirical formula of the biomass the midpoint of the above ranges, normalized to 99 %, was assumed to be the composition of the bacteria. These midpoints were 49 % protein, 11 % nucleoprotein, 19 % carbohydrate, and 20 % lipid.

## REFERENCES

(**1**) Mandelstam, Joel and McQuillen, Kenneth, eds., *Biochemistry of Bacterial Growth*, 2nd edition, 2nd printing, Halsted Press, John Wiley & Sons, New York, 1973.

(**2**) Lamanna, Carl, and Mallette, M. Frank, *Basic Bacteriology—Its Biological and Chemical Background*, Third Edition, The Williams and Wilkens Company, Baltimore, 1965.

(**3**) Morrison, Robert T., and Boyd, Robert N., *Organic Chemistry* , 3rd edition, Fifth Printing, Allyn and Bacon, Inc. Boston, 1973.

(**4**) Davis, Bernard D., Dulbecco, Renato, Eisen, Herman N., Ginsberg, Harold S., Wood, W. Barry, Jr., and McCarty, Maclyn, *Microbiology*, 2nd edition, Harper and Row Publishers, Inc., Hagerstown, MD, 1973.

(**5**) Greene, J. C., Bartels, C. L., Warren-Hicks, W. J., Parkhurst, B. R., Linder, G. L., Peterson, S. A., and Miller, W. E., "Protocols for Short Term Toxicity Screening of Hazardous Waste Sites, EPA/600/3-88/029, Environmental Research Laboratory, US EPA, Corvallis, OR.

(**6**) *Official Journal of the European Communities, No. L383 A/1, Dec. 29, 1992.*

*The American Society for Testing and Materials takes no position respecting the validity of any patent rights asserted in connection with any item mentioned in this standard. Users of this standard are expressly advised that determination of the validity of any such patent rights, and the risk of infringement of such rights, are entirely their own responsibility.*

*This standard is subject to revision at any time by the responsible technical committee and must be reviewed every five years and if not revised, either reapproved or withdrawn. Your comments are invited either for revision of this standard or for additional standards and should be addressed to ASTM Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend. If you feel that your comments have not received a fair hearing you should make your views known to the ASTM Committee on Standards, 100 Barr Harbor Drive, West Conshohocken, PA 19428.*