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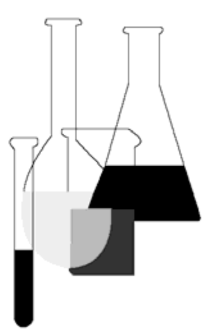
Fate, Transport and Transformation Test Guidelines

OPPTS 835.0001 Principles and Strategies Related to Biodegradation Testing of Organic Chemicals under the Toxic Substances Control Act (TSCA)



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances (OPPTS), United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data to meet the data requirements of the Agency under the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601), the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*), and section 408 of the Federal Food, Drug and Cosmetic (FFDCA) (21 U.S.C. 346a).

OPPTS developed this guideline through a process of harmonization of the testing guidance and requirements that existed for the Office of Pollution Prevention and Toxics (OPPT) in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) in publications of the National Technical Information Service (NTIS) and in the guidelines published by the Organization for Economic Cooperation and Development (OECD).

For additional information about OPPTS harmonized guidelines and to access this and other guidelines, please go to http://www.epa.gov/oppts and select “Test Methods & Guidelines” on the left side menu.

OPPTS 835.0001: Principles and strategies related to biodegradation testing of organic chemicals under the Toxic Substances Control Act (TSCA).

(a) Scope—(1) Applicability.. This guideline is intended for use in testing pursuant to the Toxic Substances Control Act (TSCA) (15) U.S.C. 2601).

(2) Background The source material used in developing this harmonized OPPTS guideline is the Revised Introduction to the Organization for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals, Section 3. Part 1: Principles and strategies related to the testing of degradation of organic chemicals (adopted 23 March 2006), available from Source OECD at http://masetto.sourceoecd.org.

(b) Overview—(1) Degradation tests that may be useful for assessment. Information on the degradability of organic chemicals may be used for hazard assessment or for risk assessment under TSCA. Hazard assessment or risk in general, and aquatic hazard classification in particular, are normally based on data obtained in standardized tests for ready biodegradability, but results of tests simulating biodegradation in water, aquatic sediment and soil may also be used for these purposes. Other types of test data that may be considered in an assessment of the potential environmental hazard or risk include sewage treatment plant (STP) simulation data, inherent biodegradability, anaerobic biodegradability, biodegradability in seawater and abiotic transformation.

(2) Other information that may be useful for assessment. In order to assess the environmental risk of a particular chemical, information allowing the estimation of its likely concentrations in the environment is necessary. Such an estimate should initially be based on knowledge of the likely use and disposal patterns of the chemical, its physical-chemical properties and the characteristics of the receiving environment.

(3) Simulation tests. Degradation of organic chemicals in the environment influences exposure and, hence, it is a key parameter for estimating the risk of long-term adverse effects on biota. Degradation rates, or half-lives, are preferably determined in simulation biodegradation tests conducted under conditions that are realistic for the particular environmental compartment (e.g. STP, surface water, sediment or soil). Simulation tests aim at mimicking actual environmental conditions such as redox potential, pH, temperature, microbial community, concentration of test substance and occurrence and concentration of other substrates.

(4) Purpose of this guideline. Factors such as pH and temperature, in combination with the intrinsic properties of the chemical, are important in determining the environmental degradation of organic chemicals. The purpose of this guideline is to describe the principles of different types of degradation tests and to present guidance for the interpretation and use of degradability data.

(c) Biodegradation in water, soils and sediments—(1) Introduction. (i) Because of the large number of chemicals that are being used in society, an approach is needed that provides adequate knowledge for decision making as regards environmental protection, but which at the same time enables costs for testing to be kept to a minimum. Ideally, a system is needed that allows preliminary screening of chemicals, using relatively simple tests of ultimate biodegradability, with the identification of those chemicals for which more detailed, and hence more costly, studies may be needed. It is possible to organize the examination of the biodegradability of chemicals into a general testing strategy, consisting of tests of varying complexity, environmental realism and cost.

(A) First, aerobic biodegradability should be examined in a screening test for ready biodegradability.

(B) In the case of a negative result in a test for ready biodegradability, biodegradation of the chemical may be examined in a simulation test to obtain data to be used for assessing the biodegradation rate in the environment or in a biological STP. This would also be the case when a positive result for ready biodegradability has been obtained but when a more precise biodegradation half-life or DT_{50} is needed for risk assessment. Alternatively, or as a supplement, a screening test for inherent biodegradability may be conducted for generation of data describing the potential biodegradability under optimized aerobic conditions, such as those which may potentially occur in STPs at long sludge ages.

(C) In addition, potential biodegradability under anoxic conditions may be examined in a screening test for anaerobic biodegradability.

(ii) Reserved.

(2) Definitions – (i) ***Ready biodegradability tests***. (A) Stringent screening tests, conducted under aerobic conditions, in which a high concentration of the test substance (in the range of 2 to 100 mg/L) is used and biodegradation is measured by non-specific parameters like Dissolved Organic Carbon (DOC), Biochemical Oxygen Demand (BOD) and CO_2 production. Domestic sewage, activated sludge or secondary effluent is the typical source of microorganisms (inoculum) in tests for ready biodegradability. The inoculum should not have been pre-adapted to degradation of the test substance by previous exposure to the test substance or structurally related chemicals. A positive result in a test for ready biodegradability can be considered as indicative of rapid and ultimate degradation in most environments including biological STPs, where ultimate degradation is the degradation of the substance to CO_2, biomass, H_2O and other inorganic substances like NH_3.

(B) A chemical attaining the pass level in these tests at a certain rate after termination of the lag phase may be classified as readily biodegradable. The pass level depends on the analytical parameter measured.

(ii) ***Simulation tests***. (A) Aerobic and anaerobic tests that provide data for biodegradation under specified environmentally relevant conditions. These tests simulate degradation in a specific environment by use of indigenous biomass, media, relevant solids (i.e. soil, sediment, activated sludge or other surfaces) to allow sorption of the chemical, and a typical temperature which represents the particular environment. A low concentration of test substance is used in tests designed to determine the biodegradation rate constant whereas higher concentrations are normally used for identification and quantification of major transformation products for analytical reasons.

(B) A low concentration of chemical in these types of tests refers to a concentration (e.g. less than 1 μg/L to 100 μg/L), which is low enough to ensure that the biodegradation kinetics obtained in the test reflect those expected in the environment being simulated. Biodegradation is measured either by radiolabelling techniques or by specific chemical analyses. Tests of these types may be subdivided according to the environment that they are designed to simulate, e.g.: a) soil, b) aquatic sediments, c) surface water and d) STPs.

(iii) *Inherent biodegradability tests.* (A) Aerobic tests that possess a high capacity for degradation to take place, and in which biodegradation rate or extent is measured. The test procedures allow prolonged exposure of the test substance to microorganisms and a low ratio of test substance to biomass, which offers a better chance to obtain a positive result compared to tests for ready biodegradability. Some of these tests may be conducted using microorganisms that have previously been exposed to the test substance, which frequently results in adaptation leading to a significant increase of the degradation rate.

(B) A substance yielding a positive result in a test of this type may be classified as inherently biodegradable, which, preferably, should be qualified by one of the terms with pre-adaptation or without pre-adaptation as appropriate. Because of the favorable conditions employed in these tests, rapid biodegradation in the environment of inherently biodegradable chemicals cannot generally be assumed.

(iv) *Anaerobic biodegradability screening tests.* Screening tests, conducted under anoxic conditions, in which a high concentration of the test substance (mg/L) is used and biodegradation is measured by non-specific parameters like total inorganic carbon (TIC), CO_2 and CH_4 production. These tests are used for the evaluation of potential anaerobic biodegradability in an anaerobic digester at a given range of concentration of microorganisms.

(v) *Monod kinetics.* The rate of degradation of the test substance in a laboratory study where the substance is the sole source of carbon and energy may be described by: $-dS/dt = [(\mu_{max}/Y)BS]/[K_s + S]$; where: $-dS/dt$ is the degradation rate, μ_{max} is the maximum specific growth rate, Y is the yield coefficient, B is the biomass concentration, K_s is the half saturation constant, and S is the concentration of the test substance.

(vi) *Pseudo-first order.* The rate of degradation is proportional to the concentration of the test substance and biomass, i.e.: $-dS/dt = k_1 SB$; where k_1 is the first order rate constant. It is assumed that the concentration of biomass (B) is constant during the experiment.

(vii) *First order kinetics.* I.e.$-dS/dt = k_1 S$, may be used when the degradation of the test substance is independent of the concentration of biomass.

(viii) ***Half-life***. ($t_{0.5}$(d)) is characteristic of the rate of a first-order reaction and corresponds to the time interval for the concentration to decrease by a factor of two.

(ix) ***DT50.*** (Disappearance Time 50) is the time within which the concentration of the test substance is reduced by 50%; it is different from the half-life t0.5 when transformation does not follow first order kinetics.

(3) Ready biodegradability tests. (i) Ready biodegradability tests are designed so that positive results are unequivocal. Given a positive result in a test of ready biodegradability, it may be assumed that the chemical will undergo rapid and ultimate biodegradation in the environment. In such cases, no further investigation of the biodegradability of the chemical, or of the possible environmental effects of transformation products, is normally required. However, the fact that the chemical is found to be readily biodegradable does not preclude concern about biodegradation rate constants and the transformation products in cases of high influx into a receiving environment. Realizing that ready biodegradability tests may sometime fail because of the stringent test conditions, consistent positive test results from test(s) should generally supersede negative test results. However, when conflicting test results are reported, it is recommended to check the origin of the inoculum in order to check whether or not differences in the adaptation of the inoculum may be the reason.

(ii) When the risk of adverse effects cannot be excluded, as is the case for some high production volume chemicals, it is recommended that the biodegradation rate of the parent substance in a relevant simulation test be determined. If necessary, a risk assessment including the parent substance and possible major transformation products may be performed.

(iii) A negative result in a test for ready biodegradability does not necessarily mean that the chemical will not be degraded under relevant environmental conditions, but it means that the next level of testing, i.e. either a simulation test or an inherent biodegradability test, should be considered.

(iv) The OECD tests that can be used to determine the ready biodegradability of organic chemicals include the six test methods described in the OECD Test Guidelines No. 301 A-F: DOC Die-Away Test (TG 301 A), CO_2 Evolution Test (TG 301 B), Modified MITI Test (I) (TG 301 C), Closed Bottle Test (TG 301 D), Modified OECD Screening Test (TG 301 E) and Manometric Respirometry Test (TG 301 F). The pass levels in this paragraph, obtained within 28 days, may be regarded as evidence of ready biodegradability: 70% DOC removal (TG 301 A and TG 301 E); 60% theoretical carbon dioxide (ThCO2) (TG 301 B); 60% theoretical oxygen demand (ThOD) (TG 301 C, TG 301 D and TG 301 F). Suggestions to decrease the pass level of the respirometric tests of TG301 from 60% to 50% have been put forward by contract laboratories and in the literature. Such a change has, however, not yet taken place.

(v) These pass levels have to be reached in a 10-day window within the 28-day period of the test. The 10-day window does not apply to TG 301 C. The 10-day window begins when the degree of biodegradation has reached 10% DOC removal, ThOD or $ThCO_2$ and has to end before or at day 28 of the test. The pass levels of either 60% ThOD (or $ThCO_2$) or 70% DOC removal practically represent complete ultimate degradation of the test substance, as the remaining fraction of 30-40% of the test substance is assumed to be assimilated by the biomass or present as products of biosynthesis.

(vi) Another test for ready biodegradability, which represents an alternative to the CO_2 Evolution Test (TG 301 B), is the Headspace Test (Ready Biodegradability – CO_2 in sealed vessels; TG 310). In this test, the CO_2 evolution resulting from the ultimate aerobic biodegradation of the test substance is determined by measuring the inorganic carbon (IC) produced in sealed test bottles, and the pass level has been defined as 60% of theoretical maximum IC production (ThIC).

(vii) As all ready biodegradability tests (see paragraphs (c)(3)(iv) and (c)(3)(vi) of this guideline) pertain to conditions in fresh waters, screening test procedures suitable for marine environments have also been developed: The OECD TG 306 on Biodegradability in Seawater includes seawater variants of the Closed Bottle Test (TG 301 D) and of the Modified OECD Screening Test (TG 301 E). Degradation of organic chemicals in seawater has generally been found to be slower than that in freshwater, activated sludge and sewage effluent, and, therefore, a positive result obtained during 28 (Closed Bottle Method) or 60 days (Shake Flask Method) in the biodegradability in Seawater test can be regarded as evidence of a chemical's potential for biodegradation in the marine environment. For example, a result of >20% ThOD or DOC removal is indicative of potential for primary biodegradation in the marine environment, whereas a result of >60% ThOD or 70% DOC removals is indicative of potential for ultimate biodegradation in the marine environment.

(viii) Test guidelines for ready biodegradability and biodegradation in marine environments (see paragraph (c)(3)(vii) of this guideline) are similar in several respects: in all the tests, the test substance providing the sole source of organic carbon (except for carbon associated with the biomass) is diluted in a test medium containing a relatively low concentration of biomass. In all the tests, a non-specific analytical method is used to follow the course of biodegradation. This has the advantage that the methods are applicable to a wide variety of organic substances and there is no need to develop specific analytical procedures. Since these methods also respond to any biodegradation residues or transformation products, an indication of the extent of ultimate biodegradation is provided.

(ix) The standardized test duration is 28 days although tests may be prolonged beyond 28 days if the biodegradation has started but not yet reached a plateau. However, only the extent of biodegradation achieved within 28 days should be used for the evaluation of ready biodegradability. Degradation after 28 days would allow the test substance to be classified as inherently biodegradable (see paragraph (c)(5)(iii) of this guideline.

(x) It has been recognized that standardization of the inoculum might also improve the comparability of the methods. However, it was concluded that this is not possible without significantly reducing, at the same time, the number of species present in the test system. A mixed inoculum is therefore test is per definition no longer a test for ready biodegradability, and a positive result may then be used to classify the test substance as inherently biodegradable with pre-adaptation (see paragraph (c)(2)(iii)(B) of this guideline). An OECD inter-laboratory comparison exercise (ring test) (see paragraph (e)(1) of this guideline) took place in 1988 in order to ensure the practicability and validity of the tests.

(4) **Simulation tests—(i) Objective.** Simulation tests aim at assessing the rate and extent of biodegradation in a laboratory system designed to represent either the aerobic treatment stage of STP or environmental compartments, such as fresh or marine surface water.

(ii) Sewage treatment. (A) The fate of chemicals in STPs can be studied in the laboratory by using the Simulation Test - Aerobic Sewage Treatment: Activated Sludge Units (TG 303 A) and Biofilms (TG 303 B). The removal of the test substance is determined by monitoring the changes in DOC and/or Chemical Oxygen Demand (COD). The basic test procedures (TG 303 A and TG 303 B) recommend addition of the test substance at a concentration of DOC between 10 mg/L and 20 mg/L. However, many chemicals are normally present at very low concentrations, even in waste water, and procedures for testing biodegradation at suitably low concentrations (<100 µg/L) are presented in Annex 7 to the TG 303 A.

(B) No specific pass levels have been defined for the elimination of chemicals in aerobic sewage treatment simulation tests. It is noted that such a level would have to be related to the specific operating conditions and plant design. The test results may be used to estimate the removal in STPs, and the resulting effluent concentrations for prediction of the concentration in the treatment plant and the receiving aquatic environment.

(C) Monod kinetics (originally proposed for pure cultures and single substrate systems only) may be used for description of the degradation of a substance when it is assumed that growth is a continuous process, and that the biomass is produced during utilization of the test substance. It follows from Monod kinetics that the effluent concentration is independent of the influent concentration, whereas this is not the case where the test substance serves as a secondary substrate for the degrading biomass. The presence of the test substance as a secondary substrate represents a low concentration scenario, which implies that the degradation rate may be expressed by pseudo-first order or first order kinetics (see paragraphs (e)(2) and (e)(3) of this guideline).

(iii) Soil, sediment and water. (A) The following tests can be used to simulate the biodegradation of organic chemicals under environmentally realistic conditions in soil, sediment or surface water: Aerobic and Anaerobic Transformation in Soil (TG 307); Aerobic and Anaerobic Transformation in Aquatic Sediment Systems (TG 308); and Aerobic Mineralization in Surface Water – Simulation Biodegradation Test (TG 309).

(B) Aerated soils are aerobic, whereas water-saturated or water-logged soils are frequently dominated by anaerobic conditions. The surface layer of aquatic sediments can be either aerobic or anaerobic, whereas the deeper sediment is usually anaerobic. These conditions in soil or sediment may be simulated by using aerobic or anaerobic tests described in the test guidelines (TG 307 and TG 308).

(C) Generally, a low concentration of the test substance is used in tests designed to determine biodegradation. A low concentration in these types of tests means a concentration (e.g. from 1 µg/L to 100 µg/L in TG 309), which is low enough to ensure that the biodegradation kinetics (first order or pseudo-first order) obtained in the test reflect those expected in the environment.

(D) The temperature dependence of the kinetic constants should be considered. It is recommended to perform the test at a temperature characteristic of the environment that is simulated.

(E) When using radiolabelled chemicals, the label should be located in the most recalcitrant part of the molecule when total mineralization is assessed. If the most stable structure does not include the functional or environmentally relevant part of the molecule, it may be appropriate to use a test chemical with a different labeling.

(F) Measuring disappearance of the parent compound by chemical analysis does not imply mineralization. Simulation tests are especially useful if it is known from other tests that the test substance can be mineralized and that the degradation, which is measured, covers the rate determining process.

(G) The results of simulation tests may include first order or pseudo-first order rate constant; degradation half-life or DT50 half-saturation constant; maximum specific growth rate; fraction of mineralized label, and, if specific analyses are used, the final level of primary degradation; mass balance during and at the end of the study; identification and concentration of major transformation products, where appropriate; and a proposed pathway of transformation, where appropriate. For a complete overview of the result parameters in relation to the simulation degradation test guidelines please refer to these guidelines (TG 303, TG 304, TG 307, TG 308 and TG 309).

(5) Inherent biodegradability tests. (i) Using favorable conditions, the tests of inherent biodegradability have been designed to assess whether the chemical has any potential for biodegradation under aerobic conditions. Tests for inherent biodegradability vary in their degradation capacity (see paragraph (c)(5)(ii) of this guideline), and the differences in test conditions should be considered if the results are used as an indication of potential biodegradation or environmental persistence. Inherent biodegradability can be measured by specific analysis (primary biodegradation) or by non-specific analysis (ultimate biodegradation).

(ii) The tests that can be used to determine the inherent biodegradability of organic chemicals include three methods described in the OECD Test Guidelines No. 302 A-C: Modified SCAS Test (TG 302 A), Zahn-Wellens/EMPA Test (TG 302 B) and

Modified MITI Test (II) (TG 302 C). The biodegradation capacity in these tests generally increases in the order TG 302 C < TG 302 B < TG 302 A.

(iii) Since inherent biodegradability can be considered to be a specific property of a chemical, it is not necessary to define limits on test duration or biodegradation rates. Biodegradation above 20% of theoretical (measured as BOD, DOC removal or COD) may be regarded as evidence of inherent, primary biodegradability, whereas biodegradation above 70% of theoretical (measured as BOD, DOC removal or COD) may be regarded as evidence of inherent, ultimate biodegradability. When results of ready biodegradability tests indicate that the pass level criterion is almost fulfilled (i.e. ThOD or DOC slightly below 60% or 70% respectively), such results can be used to indicate inherent biodegradability. This is also the case when the pass level criterion is fulfilled but the 10-day window criterion is not. Such application of ready biodegradability tests, which may include their incubation beyond 28 days, may in some cases eliminate the need for additional testing of biodegradability in inherent or simulation tests.

(iv) When the results indicate that inherent, ultimate biodegradability does occur, it indicates that the substance has a potential for degradation under favorable conditions, e.g. in well-operated STPs. When a negative result is obtained in a test of inherent biodegradability, it may lead to a preliminary conclusion of environmental persistence and to an evaluation of potential adverse effects of transformation products. An alternative is to examine ultimate biodegradation at environmentally realistic low concentrations of the chemical in a simulation test.

(6) Anaerobic biodegradability screening tests. (i) The potential anaerobic biodegradability of organic chemicals under methanogenic conditions can be determined by using OECD 311, Anaerobic Biodegradability of Organic Compounds in Digested Sludge/By Measurement of Gas Production. The test substance, which is the sole added organic carbon in the test, is exposed to diluted anaerobically digested sludge of a relatively low concentration. Biodegradability of the test substance is followed by measurements of the increase in headspace pressure resulting from the evolution of CO_2, CH_4 and TIC.

(ii) A test duration of 60 days is recommended but the test may be prolonged beyond 60 days or terminated earlier if degradation has reached a plateau, indicating a sufficient degree of biodegradation (>60% of theoretical gas production). No formal decisions on criteria for anaerobic biodegradability have been made but, tentatively, the lowest value (60%) for ready aerobic biodegradability (60% ThOD or 60% $ThCO_2$) has been adopted.

(iii) OECD 311 is designed to assess the ultimate anaerobic biodegradability of organic chemicals in heated digesters for anaerobic sludge treatment. The test is, therefore, not necessarily applicable to environmental compartments such as anoxic sediments and soils.

(7) **Interpretation of results—(i) Ready biodegradability tests.** (A) In

order to interpret the results of a test, the full biodegradation curve should be considered so that the duration of the lag phase, slope and plateau level can be identified. The duration of 28 days in the ready biodegradability tests was defined in order to allow for sufficient time for the microorganisms to adapt to the chemical (lag phase) by an increase in the number of active degrading microorganisms that results in detectable degradation.

(B) While the test duration of 28 days allows time for adaptation, chemicals that degrade slowly after a short lag phase should not be considered as readily biodegradable in tests employing the 10-day window (the 10-day window does not apply to TG 301 C). The 10-day window is the 10-day period that starts when biodegradation exceeds 10%. A chemical can only be described as readily biodegradable if the pass level for ready biodegradability is reached within the 10-day window.

(C) Although these tests are intended for pure chemicals, it is sometimes relevant to examine the ready biodegradability of mixtures of structurally similar chemicals like oils and surface-active substances (surfactants). Such substances often occur as mixtures of constituents with different chain lengths, degree and/or site of branching or stereo-isomers, even in their most purified commercial forms. Testing of each individual component may be costly and impractical. If a test on the mixture is performed and it is anticipated that a sequential biodegradation of the individual structures is taking place, then the 10-day window should not be applied to interpret the results of the test. A case by case evaluation should however take place on whether a biodegradability test on such a complex mixture would give valuable information regarding the biodegradability of the mixture as such (i.e. regarding the degradability of all the constituents) or whether instead an investigation of the degradability of carefully selected individual components of the mixture is would be more useful.

(D) It should be noted that such mixtures are here regarded as technical materials of similar types of chemicals (e.g. homologues of surfactants composed of fatty alcohols of varying chain length, or poly(oxyalkylene) polyol materials having defined molecular weight distributions). Tests for ready biodegradability are not generally applicable for complex mixtures containing different types of chemicals.

(E) The results of a ready biodegradability test may be used for aquatic hazard classification of chemicals. According to the principles described in the "Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures" (see paragraph (e)(4) of this guideline), a positive result in one of the OECD tests for ready biodegradability can be considered as indicative of rapid degradation in most environments. Positive results obtained by the TG 306, which is more suitable for marine environments, can also be considered as evidence of rapid degradability.

(F) Results from ready biodegradability tests may be used for assessment of biodegradation in a specific environmental compartment, when no data from tests simulating the conditions in that compartment are available (see paragraphs (e)(5) and (e)(6) of this guideline). First order rate constants may be derived with the purpose of modeling biodegradation in STPs, surface water, sediment and soil by using pragmatic principles (see examples in paragraphs (c)(7)(i)(G) and (c)(7)(i)(H) of this guideline).

(G) For example, the European Commission Technical Guidance Document on Risk Assessment (see paragraph (e)(5) of this guideline) prescribes that a rate constant (k) of 1.0 $hour^{-1}$, and a half-life of 0.69 hours, may be assigned to readily biodegradable chemicals (fulfilling the pass level and the 10-day window) in models for estimating the elimination of chemicals in STPs (STP models). A lower rate constant of 0.3 $hour^{-1}$, equivalent to a half-life of 2.3 hours, may be used in STP models, if a chemical reaches the pass level during the 28-day period, but fails the 10-day window (see paragraph (e)(5) of this guideline).

(H) The same objective is addressed in a U.S. Environmental Protection Agency guidance document describing the use of biodegradability data for multimedia models and STP models (see paragraph (e)(6) of this guideline). This document describes how results of ready biodegradability tests may be used to derive activated sludge half-lives as indicated in this paragraph:

Readily degradable chemicals: ....1 hour (k = 0.69 $hour^{-1}$);
Chemicals attaining 40% degradation: ....3 hours (k = 0.23 $hour^{-1}$);
Chemicals attaining 20 but <40% degradation:.......10 hours (k = 0.069 $hour^{-1}$).

(I) If the biodegradability of the chemical does not reach the pass level, it is recommended to examine whether it was inhibitory to microbial activity at the concentration used in the test. If the test substance was inhibitory, it may be re-tested at low, non-inhibitory concentrations in a relevant simulation test (TG 303, TG 307, TG 308 or TG 309). Re-testing in a modified ready biodegradability test at a much lower concentration (i.e. more than 10 times lower than prescribed) cannot generally be recommended, as suitable simulation test methods are available (see paragraphs (e)(4) and (e)(5) of this guideline).

(ii) Simulation tests. (A) In simulation tests, a chemical that fails to meet the criteria for ready biodegradability, or even inherent biodegradability, may be rapidly degradable when present at low concentrations in the environment. Simulation tests may be used to examine the biodegradation of organic chemicals in STPs (TG 303 A and TG 303 B), soil (TG 307), aquatic sediment (TG 308) and surface water (TG 309). If it can be demonstrated that the chemical is ultimately degraded by more than 70% in 28 days under realistic conditions in the aquatic environment (e.g. by using TG 308 or TG 309), then the definition of rapid degradability in relation to aquatic hazard classification will have been met (see paragraph (e)(4) of this guideline).

(B) The results of a simulation test may show a rapid transformation of the parent compound, whereas ultimate degradation, measured by e.g. CO_2 gas production, is limited due to the formation of recalcitrant transformation products. It is, therefore, necessary to distinguish between primary and ultimate biodegradation, when the rate and extent of degradation are calculated.

(C) If first-order kinetics are assumed, which is reasonable at the low substance concentrations prevailing in most aquatic environments, the definition of rapid degradability in relation to aquatic hazard classification (see paragraph (e)(4) of this guideline) will have been satisfied when a chemical is ultimately degraded in a simulation test with a half-life of less than 16 days. Results of aquatic simulation tests (e.g. TG 308, TG 309) may be used directly for aquatic hazard classification purposes, when realistic environmental conditions are simulated; i.e. when the following conditions are met: the substance concentration is realistic for the general aquatic environment (often in the low µg/L or µg/kg range); the inoculum is from a relevant aquatic environment; the inoculum concentration is realistic (e.g. 10^3-10^6 cells/mL in surface water); the temperature is realistic (e.g. 5°C to 25°C); and ultimate degradation is determined (i.e. determination of the mineralization rate or the individual degradation rates of all relevant transformation products).

(D) If no data are available from aquatic simulation or screening tests, the degradation rate of a chemical in surface water may be estimated by using results of a simulation test for degradation in soil (e.g. TG 307). In relation to aquatic hazard classification, a chemical may be considered rapidly degradable in the aquatic environment, if it is ultimately degraded in soil with a half-life of less than 16 days provided that no pre-exposure has taken place and that a realistic concentration of the test chemical has been employed (see paragraph (e)(4) of this guideline). In relation to risk assessment, similar approaches have been proposed for extrapolation of results from biodegradation tests to rate constants in surface water, sediment and soil (see paragraph (e)(5) of this guideline).

(E) Whenever possible, assessment of biodegradation in the environment should be based on results from tests simulating the conditions in the relevant environmental compartment. Degradation half-life and kinetic constants determined in a simulation test should be corrected to the average outdoor temperature or it should be documented that the difference between test temperature and outdoor temperature is negligible.

(F) It should be noted that results of a simulation test should only be extrapolated to degradation in the real environment if the concentrations used in the test were in the same order of magnitude as the concentrations expected in the environment.

(G) Man-made organic chemicals will normally be present at low concentrations (i.e. low µg/L level) in the environment compared to the total mass of biodegradable carbon substrates. This implies that the anticipated biodegradation kinetics are first-order (non-growth kinetics). If a higher concentration is used in a test (e.g. to examine transformation products), biodegradation of the chemical will frequently support growth of the degrading microorganisms.

(H) Degradation kinetics in soil or sediments may often deviate from first-order kinetics because sorption/desorption processes take place simultaneously with degradation processes. In such cases expert judgment should be applied in estimating a degradation half-life or half-lives for various sub-compartments (see paragraph (e)(7) of this guideline).

(I) When results from more than one simulation test are available, a suitable half-life or DT_{50} in the higher end of the observed range should be used for estimating environmental degradation, taking into account the realism, relevance, quality and documentation of the studies in relation to the environmental conditions (see paragraph (e)(5) of this guideline).

(iii) Inherent biodegradability tests. (A) Inherent biodegradability tests are used to assess whether a chemical has any potential for biodegradation. The European Commission Technical Guidance Document (see paragraph (e)(5) of this guideline) proposes that results of the Zahn-Wellens/EMPA Test (TG 302 B) and the Modified MITI Test (II) (TG 302 C) may be used for extrapolation to a rate constant in models for estimation of the elimination of chemicals in STPs. However, this extrapolation is only allowed, if the inherent biodegradability tests fulfill specific criteria. The pass level of 70% degradation in the Zahn-Wellens/EMPA Test must be reached within 7 days, including the lag-phase and the log-phase, the log-phase should be no longer than 3 days, and the percentage removal in the test before biodegradation occurs should be below 15%. The pass level of 70% in the Modified MITI Test (II) must be reached within 14 days, including the lag-phase and the log-phase, and the log-phase should be no longer than 3 days. During the lag-phase, growth of specific microorganisms may be exponential, but due to their small number, it takes several doubling times before biodegradation becomes detectable above the background value of the inoculum. During the log-phase, exponential growth of specific microorganisms continues and the maximum growth rate μ_{max} remains constant. The log-phase ends when depletion of the parent substance leads to a decrease of the growth rate below μ_{max}.

(B) Also the approach of the U.S. Environmental Protection Agency and Environment Canada to derive input data for multimedia models and STP models (see paragraphs (e)(6), (e)(8) and (e)(9) of this guideline) includes principles for extrapolation of inherent biodegradability test results to activated sludge half-lives in STP models and surface water. A negative result in tests for inherent biodegradability may lead to a preliminary conclusion of environmental persistence, but it should not be regarded as a definitive evidence of persistence, as the high concentration of the test substance may impede ultimate biodegradability by inhibiting the degrading microorganisms.

(C) If the microorganisms in the test are inhibited by the test substance, it is recommended that ultimate biodegradability of the chemical be examined in a simulation test by using a realistic source of inoculum, realistic temperature and a realistic low concentration of test substance.

(iv) Anaerobic biodegradability test. (A) The screening test for potential anaerobic biodegradability (TG 311, Anaerobic Biodegradability of Organic Compounds in Digested Sludge/By Measurement of Gas Production) is performed at a high incubation temperature (35°C), which approximates the temperature in heated digesters for anaerobic sludge treatment. This temperature favors anaerobic biodegradation of chemicals with low or moderate toxicity to anaerobic bacteria. On the other hand, because this test uses a high concentration of test substance, negative results may be observed for some chemicals that would otherwise be biodegradable at lower, presumably more realistic concentrations.

(B) The test temperature implies that the results obtained are not necessarily representative of what might be observed in other anoxic environments such as aquatic sediments, as does the use of digester sludge as the inoculum. If the test substance was inhibitory in the screening test, it may be re-tested at low, non-inhibitory concentrations in a relevant simulation test, e.g., TG 308, which can be conducted under strictly anaerobic conditions.

(d) Abiotic transformation—(1) Introduction. (i) Chemicals in aquatic environments, soil and air may be transformed by abiotic processes such as hydrolysis, oxidation and photolysis. Abiotic transformation can be an important step in the pathway for degradation of anthropogenic chemicals in the environment. In the atmosphere, abiotic transformation (oxidation by hydroxyl radicals) is the most important process for the complete destruction of airborne chemicals. Although abiotic transformation in water, sediments and soil in itself usually is only primary degradation, the products formed by such abiotic processes may be biodegraded further by microorganisms.

(ii) Generally, the most important processes for the degradation of most chemicals in the aquatic environment are biodegradation and combined degradation by hydrolysis and subsequent biodegradation. In aquatic systems, sediments and soil, even slow hydrolysis or biodegradation is likely to be more important than phototransformation, because of the limited possibility for exposure to sunlight. Depending on environmental conditions, time of the year and latitude, phototransformation may be an important step in the initial transformation of some chemicals, which may lead to biodegradable transformation products.

(2) Hydrolysis. (i) Abiotic hydrolytic transformation of chemicals in aquatic systems may be examined at pH values normally found in the environment (pH 4-9) by use of available hydrolysis test guidelines (see paragraphs (e)(10) and (e)(11) of this guideline. These methods are generally applicable to chemical substances (^{14}C-labeled or non-labeled) for which an analytical method with sufficient accuracy and sensitivity is available. The results of a test of hydrolysis may include repeatability and sensitivity of the analytical methods; recoveries; mass balance during and at the end of the study (when ^{14}C-labelled test substance is used); and half-life or DT_{50}.

(ii) Most hydrolysis reactions follow apparent first-order reaction rates and, therefore, half-lives are independent of the concentration. This usually permits the extrapolation of laboratory results determined at 10^{-2} to 10^{-3} M to environmental conditions ($\leq 10^{-6}$ M) (see paragraph (e)(12) of this guideline).

(3) Phototransformation. (i) The potential effects of solar irradiation on the fate of chemicals in surface water may be examined by use of guidelines referenced in paragraphs (e)(13) and (e)(14) of this guideline.

(ii) The rate of photolysis of a chemical in the environment depends on several factors. These include the attenuation of solar light in natural water bodies and the intensity of solar radiation, the latter of which is itself dependent on factors such as latitude and season. Any data on half-lives or DT_{50}, DT_{75} and DT_{90} values should be reported along with calculations associated with these data, and the results of any outdoor experiments, if the latter have been conducted. Where possible, information on transformation products should be provided as well.

(e) References.

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