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INTRODUCTION TO THE OECD GUIDELINES FOR TESTING OF CHEMICALS SECTION 3

PART1: PRINCIPLES AND STRATEGIES RELATED TO THE TESTING OF DEGRADATION OF ORGANIC CHEMICALS

DEGRADATION OF ORGANIC CHEMICALS

1. GENERAL

1. Information on the degradability of organic chemicals may be used for hazard assessment or for risk assessment. Hazard assessment in general, and aquatic hazard classification in particular, are normally based on data obtained in standardised tests for ready biodegradability, but results of tests simulating the 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 include sewage treatment plant simulation data, inherent biodegradability, anaerobic biodegradability, biodegradability in seawater and abiotic transformation.

2. In order to assess the environmental risk of particular chemical, information allowing the estimation of its likely concentrations in the environment is necessary. Such an estimate must 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. Degradation of organic chemicals in the environment influences the exposure and, hence, it is a key parameter for estimating the risk of long-term adverse effects on biota. Degradation rates, or half-lives, may preferably be determined in simulation biodegradation tests conducted with conditions that are realistic for the particular environmental compartment (e.g. sewage treatment plant, surface water, sediment or soil). Simulation tests aim at mimicking the actual environmental conditions such as redox potential, pH, temperature, microbial community, concentration of test substance and occurrence and concentration of other substrates.

4. These are important factors that determine the environmental degradation of organic chemicals in combination with the intrinsic properties of the chemical. The purpose of this introduction is to describe the principles of the different types of degradation tests and to present guidance for the interpretation and use of degradability data.

2. BIODEGRADATION IN WATER, SOILS AND SEDIMENTS

2.1 Introduction

5. Because of the large number of chemicals which are being used in the society an approach is required, which is providing adequate knowledge for decision making as regards environmental protection, but which at the same time enables costs for testing to be reduced as much as possible. Ideally, a system is required that allows preliminary screening of chemicals, using relatively simple tests of ultimate biodegradability, and the identification of those chemicals for which more detailed, and hence more costly, studies are needed. It is possible to organise the examination of the biodegradability of chemicals into a general testing strategy in three steps, consisting of tests of varying complexity, environmental realism and costs:

- First, the aerobic biodegradability should be examined in a screening test for ready biodegradability

- In case of a negative result in a test for ready biodegradability, the biodegradation of the chemical may be examined in a simulation test to obtain data describing the biodegradation rate in the environment. Alternatively or supplementary a screening test for inherent biodegradability may be conducted for generation of data describing

the potential biodegradability under optimised aerobic conditions, such as those which may potentially occur in biological sewage treatment plants (STP).

- Finally, potential biodegradability under anoxic conditions may be examined in a screening test for anaerobic biodegradability.

2.2 Definitions

Ready biodegradability tests

6. 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 the biodegradation rate is measured by non-specific parameters like Dissolved Oxygen Carbon (DOC), Biochemical Oxygen Demand (BOD) and CO_2. In these tests, a positive result can be considered as indicative of rapid ultimate degradation[1] in most environments including biological sewage treatment plants.

7. Aerobic ready biodegradability tests are used for aquatic hazard classification of chemicals (1), and a chemical attaining the pass level in these tests at a certain rate after ended lag phase may be classified as “readily biodegradable”. The pass level depends on the analytical parameter measured.

Simulation tests

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

9. A low concentration of chemical in this type of tests means 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. The degradation rates are measured either by ^{14}C-radiolabelling techniques or by specific chemical analyses. Tests of these types may be subdivided according to the environment, which they are designed to simulate, e.g.: a) soil, b) aquatic sediments c) surface water and d) sewage treatment plants.

Inherent biodegradability tests

10. Tests that possess a high capacity for degradation to take place. The test procedures allow prolonged exposure of the test substance to microorganisms and a low test substance to biomass ratio, which makes the tests powerful. 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 significantly more extensive degradation of the chemical.

11. 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 favourable conditions employed in these tests, a rapid biodegradation in the environment of inherently biodegradable chemicals cannot generally be assumed.

[1] Ultimate degradation is the degradation of the substance to CO_2, biomass, H_2O and other inorganic substances like NH_3

Anaerobic biodegradability screening tests

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

2.3 Ready biodegradability tests

13. Ready biodegradability tests must be 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 the biodegradation rates and the transformation products in cases of high influx into a receiving ecosystem.

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

15. 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 it should be considered to progress to the next level of testing, i.e. either a simulation test or an inherent biodegradability test. The latter option may be used, if data describing the potential biodegradability under optimised aerobic conditions are sufficient for the particular assessment.

16. The tests which 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 following pass levels of biodegradation, obtained within 28 days, may be regarded as evidence of ready biodegradability: 70% DOC; 60% $ThCO_2$; 60% ThOD; 60% ThOD; 70% DOC; 60% ThOD, respectively, for the tests listed above.

17. These pass levels have to be reached in a 10-day window within the 28-day period of the test. The 10-day window begins when the degree of biodegradation has reached 10% DOC, ThOD or $ThCO_2$ and must end before day 28 of the test. The pass levels of either 60% (ThOD or $ThCO_2$) or 70% DOC 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.

18. Another test for ready biodegradability, which represents an alternative to the CO_2 Evolution Test (TG 301 B), is the CO_2 Headspace Test (draft 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 produced in sealed test bottles, and the pass level has been defined at 60%.

19. As all of the above-mentioned methods are freshwater tests, screening test procedures suitable for marine environments have been described: 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 experienced in freshwater, activated sludge and sewage effluent, and, therefore, a positive

result obtained during 28 days in a Biodegradability in Seawater test (>60% ThOD; >70% DOC) can normally be regarded as an indication of ready biodegradability.

20. The above test guidelines 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.

21. The standardised test duration is 28 days although tests may be prolonged beyond 28 days if the biodegradation has started but has not yet reached a plateau. However, only the extent of biodegradation achieved within 28 days should be used for the evaluation of ready biodegradability.

22. It has been recognised that standardisation 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 recommended to ensure the presence of a variety of degrading organisms in the tests. In view of the stringent requirements to these tests, it was also decided that pre-adaptation of the inoculum to the test substance should not be allowed. Laboratory inter-comparison tests have been carried out for the tests in order to ensure the practicability and validity of the tests.

2.4 Simulation tests

Sewage treatment

23. Simulation tests aim at examining the rate and extent of biodegradation in a laboratory system designed to represent either the activated sludge-based aerobic treatment stage of a sewage treatment plant or environmental compartments, as fresh or marine surface water.

24. The fate of chemicals in sewage treatment plants can be studied in the laboratory by use of 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 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 the biodegradation at suitably low concentrations (<100 µg/L) are presented in the Annex 7 to the TG 303 A.

25. 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 sewage treatment plants and the resulting effluent concentrations for prediction of the concentration in the treatment plant and the receiving aquatic environment.

26. Monod kinetics[2] (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 utilisation of the test substance (e.g. in systems with test substance concentrations above 1 mg/L). If the concentration of the test substance is

[2] 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: $-dS/dt = [(\mu_{max}/Y)\cdot B\cdot S]/[K_s + S]$; 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.

<< K_s, e.g. less than 100 µg/L, the Monod equation may be simplified and the degradation rate expressed by pseudo-first order[3] or first order kinetics[4].

Soil, sediment and water

27. 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 Mineralisation in Surface Water – Simulation Biodegradation Test (TG 309).

28. Generally, a low concentration of the test substance is used in tests designed to determine the biodegradation. A low concentration in this type of tests means a concentration (e.g. more than 1 µg/L to 100 µg/L), 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.

29. The temperature dependence of the kinetic constants must be considered. It is recommended to perform the test at a temperature characteristic of the environment which is simulated.

30. When using ^{14}C-labelled chemicals, the ^{14}C should be located in the most recalcitrant part of the molecule when total mineralization is tested. If the most stable structure does not include the functional or environmentally relevant part of the molecule, it may be considered to use a test chemical with a different ^{14}C-labelling.

31. The results of simulation tests may include:

- First order or pseudo-first order rate constant
- Degradation half-life or DT_{50}
- Half-saturation constant
- Maximum specific growth rate
- Fraction of mineralised ^{14}C, and, if specific analyses are used, the final level of primary degradation
- Identification and concentration of major transformation products

2.5 Inherent biodegradability tests

32. Using favourable conditions, the tests of inherent biodegradability have been designed to assess whether the chemical has any potential for biodegradation under aerobic conditions. This can be measured by specific analysis (primary biodegradation) or by non-specific analysis (ultimate biodegradation).

33. The tests which can be used to determine the inherent biodegradability of organic chemicals include four methods described in the OECD Test Guidelines No. 302 A-D: Modified SCAS Test (TG 302 A), Zahn-Wellens/EMPA Test (TG 302 B), Modified MITI Test (II) (TG 302 C) and the draft Concawe Test (draft TG 302D).

[3] The rate of degradation is proportional to the concentration of the test substance and biomass, i.e.: $-dS/dt = k_1 \cdot S \cdot B$; where k_1 is the first order rate constant. It is assumed that the concentration of biomass (B) is constant during the experiment.

[4] First order kinetics, i.e.$-dS/dt = k_1 \cdot S$, may be used when the degradation of the test substance is independent of the concentration of biomass.

34. 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 rates above 20% (measured as BOD, DOC or COD) may be regarded as evidence of inherent, primary biodegradability, whereas biodegradation rates above 70% (measured as BOD, DOC or COD) may be regarded as evidence of inherent, ultimate biodegradability.

35. When the results indicate that inherent, ultimate biodegradability does not occur, it may lead to a preliminary conclusion of environmental persistency and to an evaluation of potential adverse effects of transformation products. An alternative is to examine the ultimate biodegradation at environmentally realistic low concentrations of the chemical in a simulation test. A positive result in a test of inherent biodegradability indicates that the chemical is not likely to persist indefinitely in the environment.

36. A more accurate estimate of the environmental concentration may, however, be required for more detailed assessments. Thus, for risk assessment, it may be necessary to perform a simulation test using a low concentration of the test substance and a sample (inoculum) from a biological sewage treatment plant, natural water, sediment or soil with their indigenous microorganisms to obtain information about degradation kinetics and pathways in one or more of these compartments.

2.6 Anaerobic biodegradability screening tests

37. The potential anaerobic biodegradability of organic chemicals can be determined by using the test: Anaerobic Biodegradability of Organic Compounds in Digested Sludge/Method by Measurement of Gas Production (draft TG 311). 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 in the closed test vessels resulting from the evolution of CO_2 and CH_4.

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

39. The draft TG 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 anoxic environmental compartments such as anoxic sediments and soils.

2.7 Interpretation of results

Ready biodegradability tests

40. 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).

41. However, chemicals that degrade slowly after a short adaptation period and eventually reach the pass level should not pass the test. It is therefore required that the pass level of ready biodegradability is reached within 10 days of the start of biodegradation (10-day window). Biodegradation should be considered to have started when it exceeds the 10% level.

42. 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). As a sequential biodegradation of the individual structures is anticipated, the 10-day window should not be applied to interpret the results of test with mixtures of structurally related chemicals.

43. It should be noticed 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). Tests for ready biodegradability are not applicable for complex mixtures containing different types of chemicals.

44. The results of a ready biodegradability test may be used for aquatic hazard classification of chemicals. According to the principles described in the "Harmonised Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures" (1), 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.

45. 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. First order rate constants may be derived with the purpose of modelling the biodegradation in sewage treatment plants, surface water, sediment and soil by using pragmatic principles.

46. For example, the European Commission Technical Guidance Document on Risk Assessment (2) 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 pass level and 10-day window) in models for estimating the elimination of chemicals in sewage treatment plants (STP models). A lower rate constant of 0.3 $hour^{-1}$, equivalent to a half-life of 2.3 hours, may be used in the STP model, if a chemical reaches the pass level during the 28-day period, but fails the 10-day window (2).

47. The same objective is being addressed in a U.S. Environmental Protection Agency guidance document describing the use of biodegradability data for multimedia models and STP models (3). This document describes that results of ready biodegradability tests may be used to derive activated sludge half-lives as indicated below:

- 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 ($k = 0.069\ hour^{-1}$)

48. If the biodegradability of the chemical does not reach the pass level, it is necessary 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 (1,2).

Simulation tests

49. A chemical that fails to pass the criteria for ready biodegradability, or even inherent biodegradability, may be rapidly degradable when present at low concentrations in the

environment. 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, then the definition of "rapid degradability" in relation to aquatic hazard classification will have been met.

50. If first order kinetics is assumed, which is reasonable at the low substance concentrations prevailing in most aquatic environments, the requirement for "rapid degradability" will be fulfilled, when a chemical is ultimately degraded with a half-life of less than 16 days (1). Results of aquatic simulation tests may be used directly for aquatic hazard classification purposes, when realistic environmental conditions are simulated, i.e.:

- Substance concentration which is realistic for the general aquatic environment (often in the low µg/L range)
- Inoculum from a relevant aquatic environment
- Realistic concentration of inoculum (e.g. 10^3-10^6 cells/mL in surface water)
- Realistic temperature (e.g., 5°C to 25°C)
- Ultimate degradation is determined (i.e., determination of the mineralisation rate or the individual degradation rates of the total biodegradation pathway)

51. 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. 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 (1).

52. In relation to risk assessment a similar extrapolation of soil degradation data to surface water has been proposed (2). This approach takes sorption of the test chemical to soil particles into account, but also that the sorbed fraction of the chemical is not available for the degrading microorganisms.

53. Whenever possible, the 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.

54. It should be noted that the 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.

55. 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), the biodegradation of the chemical will frequently support growth of the degrading microorganisms.

56. 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 (2).

57. 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 judgement is required for estimating a degradation half-life, a DT_{50} or half-lives for various sub-compartments (4)

Inherent biodegradability

58. Inherent biodegradability tests are used to assess whether a chemical has any potential for biodegradation. The European Commission Technical Guidance Document (2) 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 of in models for estimation of the elimination of chemicals in sewage treatment plants (2). However, this extrapolation is only allowed, if the inherent biodegradability tests fulfil specific criteria[5].

59. Also the approach of the U.S. Environmental Protection Agency to derive input data for multimedia models and STP models (3) 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 persistency, but it should not be regarded as a definitive evidence of persistency as the high concentration of the test substance may impede ultimate biodegradability by inhibiting the degrading microorganisms.

60. In this case, it is recommended to examine ultimate biodegradability of the chemical in a simulation test by using a realistic source of inoculum, realistic temperature and a realistic low concentration of test substance.

Potential anaerobic biodegradability

61. The draft screening test for potential anaerobic biodegradability (draft TG 311 Anaerobic Biodegradability of Organic Compounds in Digested Sludge / Method by Measurement of Gas Production, Feb 2003) is performed at a high incubation temperature (35°C), which resembles the temperature in heated digesters for anaerobic sludge treatment. This temperature favours anaerobic biodegradation of chemicals with low or moderate toxicity to anaerobic bacteria. On the other hand, the high concentration of test substance may inhibit the ultimate biodegradability of toxic chemicals.

62. The test temperature implies that the results obtained are not necessarily representative of other anoxic environments such as aquatic sediments. 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.

3. ABIOTIC TRANSFORMATION

3.1 Introduction

63. 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 man-made chemicals in the environment. Although abiotic transformation in itself is only primary degradation, the products formed by such abiotic processes may be biodegraded further by microorganisms.

[5] The pass level of 70% degradation in the Zahn-Wellens/EMPA Test must be reached within 7 days, 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, and the log-phase should be no longer than 3 days (2).

64. Generally, the most important processes for the degradation of most chemicals in the aquatic environment are biodegradation or combined degradation by hydrolysis and subsequent biodegradation. In aquatic systems even slow hydrolysis or biodegradation is likely to be more important than phototransformation, because of the limited possibility for exposure to sunlight in aquatic systems (2).

3.2 Hydrolysis

65. 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 the guideline: Hydrolysis as a Function of pH (draft revised TG 111, April 2003). This method is generally applicable to chemical substances (^{14}C-labelled or non-labelled) for which an analytical method with sufficient accuracy and sensitivity is available. The results of a test of hydrolysis may include:

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    - 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)
    - Half-life or DT_{50}

66. 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) (5).

3.3 Phototransformation

67. The potential effects of solar irradiation on the fate of chemicals in surface water and soil, respectively, may be examined by use of the guidelines: Phototransformation of Chemicals in Water – Direct and Indirect Photolysis (draft August 2000) and Phototransformation of Chemicals on Soil Surfaces (draft January 2002).

68. The rate of photolysis is expressed by half-lives or DT_{50}, DT_{75} and DT_{90} values. The exposure of the chemical to sunlight in the environment, including the depth of penetration of solar irradiation in the water column, should be evaluated carefully.

4. LITERATURE

(1) OECD (2001). Harmonised Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures. OECD Series on Testing and Assessment. No. 33.

(2) European Commission (2002). Technical Guidance Document on Risk Assessment in Support of Commission Directive 93/67/EEC on Risk Assessment for new Notified Substances and Commission Regulation (EC) No. 1488/94 on Risk Assessment for Existing Substances and Directive 98/8/EC of the European Parliament and of the Council Concerning the Placing of Biocidal Products on the Market.

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