INTERNATIONAL STANDARD

ISO 14592-1

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Water quality — Evaluation of the aerobic biodegradability of organic compounds at low concentrations —

Part 1: Shake-flask batch test with surface water or surface water/sediment suspensions

Qualité de l'eau — Évaluation de la biodégradabilité aérobie des composés organiques présents en faibles concentrations —

Partie 1: Essai en lots de flacons agités avec des eaux de surface ou des suspensions eaux de surface/sédiments

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this part of ISO 14592 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 14592-1 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

ISO 14592 consists of the following parts, under the general title *Water quality — Evaluation of the aerobic biodegradability of organic compounds at low concentrations*:

— *Part 1: Shake-flask batch test with surface water or surface water/sediment suspensions*

— *Part 2: Continuous flow river model with attached biomass*

This corrected version of ISO 14592-1:2002 incorporates corrections to

— the reference given in the third item of the list in 8.2.1;

— the reference given in the penultimate line of 8.2.1;

— the reference given in the last line of the second paragraph of 8.4.1.

Introduction

This International Standard consists of two parts. Part 1 describes a die-away batch test for either surface water with or without added sediment in suspension simulating either a pelagic aquatic environment or a water-to-sediment interface. Part 2 describes a continuous flow system simulating a river with biomass attached to stationary surfaces.

This test has been specifically designed to provide information on the biodegradation behaviour and kinetics of test compounds present in low concentrations, i.e. sufficiently low to ensure that they simulate the biodegradation kinetics which would be expected to occur in natural environmental systems.

Before conducting this test, it is necessary to have information on the biodegradability behaviour of the test compound at higher concentrations (e.g. in standard biodegradation tests), and, if possible, on abiotic degradability or elimination from water, as well as relevant physico-chemical data. This information is necessary for proper experimental planning and interpretation of results.

When this test method is used with a single environmental sample of surface water (either with or without the addition of sediment), a laboratory-derived first-order biodegradation rate can be estimated for one single point in time and space. The test system may be more consistent and provide more reliable biodegradation results if it is adapted to the test compound at a specifically maintained concentration. This may be achieved using the optional semi-continuous procedural variant of the method.

Water quality — Evaluation of the aerobic biodegradability of organic compounds at low concentrations —

Part 1:
Shake-flask batch test with surface water or surface water/sediment suspensions

WARNING AND SAFETY PRECAUTIONS — Activated sludge, sewage and effluent contain potentially pathogenic organisms. Therefore appropriate precautions should be taken when handling them. Toxic and dangerous test compounds and those whose properties are unknown should be handled with care. Radiolabelled compounds, if used, should be handled respecting existing rules and legislation.

1 Scope

This part of ISO 14592 specifies a test method for evaluating the biodegradability of organic test compounds by aerobic microorganisms by means of a shake-flask batch test. It is applicable to natural surface water, free from coarse particles to simulate a pelagic environment ("pelagic test") or to surface water with suspended sediments added to obtain a level of 0,1 g/l to 1 g/l dry mass to simulate a water body with suspended sediment ("suspended sediment test").

This part of ISO 14592 is applicable to organic test compounds present in lower concentrations (normally below 100 µg/l) than those of natural carbon substrates also present in the system. Under these conditions, the test compounds serve as a secondary substrate and the kinetics for biodegradation would be expected to be first order ("non-growth" kinetics).

This test method is not recommended for use as proof of ultimate biodegradation which is better assessed using other standardized tests (see ISO/TR 15462). It is also not well suited to studies on metabolite formation and accumulation which require higher test concentrations.

2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this part of ISO 14592. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 14592 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO/TR 15462, *Water quality — Selection of tests for biodegradability*

3 Terms, definitions and symbols

3.1 Terms and definitions

For the purpose of this part of ISO 14592, the following terms and definitions apply.

3.1.1
ultimate aerobic biodegradation
breakdown of a chemical compound or organic matter by microorganisms, in the presence of oxygen, to carbon dioxide (CO_2), water and mineral salts of any other elements present (mineralization) and the production of new biomass

NOTE Total mineralization may be different from ultimate aerobic biodegradation in that total mineralization includes secondary mineralization of biosynthesis products. The kinetics may therefore deviate from first-order kinetics in particular towards the end of the experiment. In this part of ISO 14592, primary aerobic biodegradation is determined when using substance specific analysis and total mineralization when using radiolabelled compounds.

3.1.2
primary biodegradation
structural change (transformation) of a chemical compound by microorganisms resulting in the loss of a specific property of that compound

3.1.3
dissolved organic carbon
DOC
part of the organic carbon in a sample of water which cannot be removed by specified phase separation

NOTE Phase separation may be obtained, for example, by centrifugation of the sample of test water at 40 000 m/s^2 for 15 min or by membrane-filtration using membranes with pores of 0,45 µm diameter.

3.1.4
lag phase
t_{lag}
time from the start of a test until significant biodegradation (about 10 % of the maximum level) can be measured

NOTE Lag phase is expressed in days (d).

3.1.5
maximum level of biodegradation
degree of biodegradation of a chemical compound or organic matter in a test above which no further biodegradation takes place during the test

NOTE The maximum level of biodegradation is expressed as a percentage.

3.1.6
primary substrate
major carbon and energy source which is essential for growth or maintenance of microorganisms

3.1.7
secondary substrate
substrate component present at such low concentrations, that by its degradation, only insignificant amounts of carbon and energy are supplied to the competent microorganisms, as compared to the carbon and energy supplied by their degradation of primary substrates

3.1.8
degradation rate constant
k
rate constant for first-order or pseudo first-order kinetics which indicates the rate at which degradation processes occur

NOTE 1 The degradation rate constant is expressed in inverse days (d^{-1}).

NOTE 2 For a batch experiment, k is estimated from the initial part of the degradation curve obtained after the end of the lag phase. For a continuously operating test system, k can be estimated from a mass balance for the reactor using data collected under steady-state conditions.

3.1.9
degradation half-life
$T_{1/2}$
characteristic of the rate of a first-order reaction and corresponds to the time interval necessary for the concentration to decrease by a factor of two

NOTE 1 The degradation half-life is expressed in days (d).

NOTE 2 The degradation half-life and the degradation rate constant are related by the following equation:

$$T_{1/2} = \ln 2/k$$

NOTE 3 The degradation half-life $T_{1/2}$ for first-order reactions should not be confused with the half-life time, T_{50}, which is often used to describe the environmental behaviour of pesticides and which is simply the time to reach 50 % of total biodegradation. The half-life time T_{50} may be derived from degradation curves without making assumptions about the kinetics.

3.2 Symbols

Symbol	Description	Units
A 1)	activity of the ^{14}C-radiolabelled test compound	becquerels(Bq)
A_I	inorganic ^{14}C-activity ($^{14}CO_2$ evolved as a result of biodegradation)	becquerels(Bq)
A_{TO}	total organic ^{14}C-activity of the residual test compound, metabolites, particulate microbial biomass and dissolved cell constituents, measured in the liquid phase after stripping off $^{14}CO_2$	becquerels(Bq)
A_{DO}	dissolved organic ^{14}C-activity of the residual test compound, metabolites and dissolved cell constituents, measured in the liquid phase after stripping off $^{14}CO_2$ and separation of particles by membrane filtration or centrifugation	becquerels(Bq)
A_{PO}	particulate organic ^{14}C-activity of the sorbed ^{14}C of the test compound and particulate ^{14}C-biomass measured in the particulate residue after filtration or centrifugation	becquerels(Bq)
a 2)	specific activity of the test compound or of a mixture of radiolabelled and “cold” test compound	becquerels per microgram (Bq/µg)
c 3)	residual molar concentration of the test compound	micromoles per litre (µmol/l)
c_0	initial molar concentration of the test compound	micromoles per litre (µmol/l)

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1) A is the symbol for activity, expressed in bequerels, as specified in ISO 31-9-33:1992.

2) In accordance with ISO 31-9-34:1992, a is defined as the symbol for specific activity, expressed in bequerels per kilogram. It may be common practice sometimes to use the symbol σ for specific activity, but this is not in accordance with ISO 31-10-3:1992 where “σ” is defined as the cross-section for a specified target entity and for a specified reaction or process produced by incident charged or uncharged particles of specified type and energy.

3) In ISO 31-8-13:1992, c is defined as the symbol for “molar concentration”, expressed in moles per litre and in ISO 31-8-11.2:1992, ρ is defined as the symbol for “mass concentration”, expressed in kilograms per litre. Note that in ISO 31, “concentration” of the test compound in solution is expressed in two ways:

— “ρ” refers to the mass of the test compound per unit volume;

— “c” is specifically used to mean the number of moles of the test compound per unit volume.

c_A[4]	residual activity concentration	becquerels per millilitre (Bq/ml)
c_{A0}	initially added activity concentration	becquerels per millilitre (Bq/ml)
$c_{A\text{plateau}}$	activity concentration at the plateau of the transition between the degradation curve and the subsequent “tailing”	becquerels per millilitre (Bq/ml)
F_T	flasks containing the test compound examined	
F_B	flasks containing the blank sample	
F_C	flasks for check the test performance with a reference compound	
F_S	sterile flask for checking possible abiotic degradation or other non-biological removal	
k	biodegradation rate constant	inverse days (d^{-1})
k^*	pseudo first-order rate constant for disappearance of activity	inverse days (d^{-1})
$k_{\text{non-adapted}}$	first-order rate constants and associated half-lives derived from portions of the curve showing no significant growth	inverse days (d^{-1})
k_{adapted}	pseudo first-order rate constants representing adapted environments	inverse days (d^{-1})
t	time	days (d)
t_{lag}	lag phase	days (d)
$T_{1/2}$	degradation half-life	days (d)
V	reaction volume in the reactor	litres (l)
α	fraction of ^{14}C converted to $^{14}CO_2$	
ρ[3]	residual mass concentration of the test compound	micrograms per litre (µg/l)
ρ_0	initial mass concentration of the test compound	micrograms per litre (µg/l)

4 Principle

The test is carried out by batch-wise incubation of the test compound with a sample of either surface water or surface water and sediment. When surface water alone is used, the test is referred to as a “pelagic test” and when sediment is added to obtain a suspension, the test is referred to as a “suspended sediment test”. Incubation takes place at an environmental temperature under agitation by means of a system of flasks on a mechanical shaker.

Test compounds, present in lower concentrations than the natural carbon substrates also present in the system, will serve as secondary substrates. Biodegrading microorganisms obtain the major part of their energy and carbon from primary substrates and not from secondary substrates. Under these conditions, the kinetics for biodegradation would be expected to be first order (“non-growth kinetics”). First-order kinetics implies that the specific rate of degradation is constant and independent of the concentration of the test compound.

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4) c_A is the symbol for volumetric activity, expressed in bequerels per cubic metre, as specified in ISO 31-9-35. a is sometimes used for volumetric activity, but is not in accordance with ISO 31.

The test compound is added at two different concentration levels. The concentrations are chosen to be within the microgram-per-litre range (preferably < 100 µg/l) so as to obtain first-order degradation kinetics and an estimated half-life independent of the test concentration. Concentrations should normally be chosen to be as low as practically possible with respect to the sensitivity of the available measurement techniques. These concentrations need not be as low as those expected in the environment to ensure the same type of degradation kinetics.

The test mixture is transferred to closed flasks with an air headspace. Flasks are incubated in the dark or in diffuse light at either the field temperature or at a temperature of 20 °C to 25 °C as commonly used in biodegradation tests. Agitation by means of continuous shaking or stirring is provided to maintain particles, including microorganisms, in free suspension.

The time-course of degradation is followed by the determination of the residual concentration of test compound at appropriate intervals. The incubation time should be sufficiently long to be able to evaluate the degradation behaviour. If the degradation is found to be significant, the extent of degradation should be sufficiently high (normally greater than 15 % to 20 % degradation) to be able to estimate the first-order rate constant.

Measurement of the degradation of the test compound is carried out either by a radiotracer technique, normally using ^{14}C-labelling and liquid scintillation counting, or by specific chemical analysis, if a sufficiently sensitive analytical method is available. Using the ^{14}C technique and labelling the most persistent part of the molecule with ^{14}C, total mineralization or ultimate biodegradation can be assessed, while only primary biodegradation can be measured with specific analysis.

5 Reagents and media

5.1 Reagents

Use only reagents of recognized analytical grade and radiolabelled compounds of high radiochemical purity.

5.1.1 Deionized water, for preparing stock solutions of the test compound and the reference substance.

This water shall have a DOC content of no more than 1 mg/l DOC (use e.g. ISO 8245 for determination) and be free from inhibitory concentrations of toxic substances.

5.1.2 Volatile organic solvent (optional), for dissolving test compounds of low solubility.

5.1.3 Mercury(II) chloride ($HgCl_2$) (optional), added to a mass concentration of 100 mg/l in the sample of test water containing the test or reference compound and used for stopping all biological activity.

5.1.4 Sodium azide (NaN_3), (optional), added to a mass concentration of 10 g/l in the sample of test water containing the test or reference compound and used for stopping all biological activity.

5.2 Media

5.2.1 Surface water, for use in the “pelagic test”.

Collect a sample of suitable surface water in a thoroughly cleansed container. Remove coarse particles, for example, by filtration through a nylon filter of about 100 µm mesh size, a coarse paper filter, or by sedimentation. Keep the sample of surface water in an aerobic environment (e.g. by keeping sufficient headspace in the flask) during the transport and until the start of the test in the laboratory. Start the test preferably within 1 d after collection. During transportation and storage, the temperature of the sample of surface water should not be permitted to exceed significantly the temperature to be used in the test. Cool to 4 °C if transportation times exceed a few hours. Ensure this water does not freeze.

Identify the sampling location precisely and describe it in terms of its pollution and nutrient status. Provide the following minimum information for the surface water taken for the test:

a) date and time of collection and delay between collection and use in the laboratory test;

b) depth of collection;

c) appearance of sample (e.g. turbidity);

d) temperature and pH at the place and time of collection;

e) in the case of sea water and brackish water, salinity or conductivity;

f) in the case of a turbid sample, the amount of suspended solids;

g) number of colony-forming microorganisms determined on a suitable growth medium according to standard methods;

and optionally, in addition:

h) DOC and TOC concentration;

i) inorganic nutrients such as total phosphorus, dissolved orthophosphate, total nitrogen, nitrate, nitrite or ammonium nitrogen;

j) chlorophyll-*a* concentration;

k) total microbial number using staining (e.g. by acridine orange) and epifluorescence microscopy after ultrasonic treatment or dispersion by other means;

l) other characteristics relating to the microbial biomass and activity such as ATP (adenosine triphosphate), protein, heterotrophic carbon assimilation activity, and determination of the number of organisms capable of degrading the test compound (e.g. determined using a most probable number method).

5.2.2 Surface water and sediment, for use in the “suspended sediment test”.

Collect a sample of surface water as described in 5.2.1. In addition, collect a grab sample of aerobic surface sediment using an appropriate sampling method. Sample, for example, a number of sediment cores using a tube of transparent plastic and slice off the upper aerobic layer immediately after sampling. Transport the sample in a container with sufficient air headspace to keep the sediment aerobic, and aerate the sample of surface water following arrival at the laboratory until use. First determine the level of suspended solids in the sediment sample, then choose a mass concentration level between 100 mg/l and 1 000 mg/l and adjust the level of suspended solids of the sample to this predetermined level.

Identify the sampling location precisely and describe it in terms of its pollution and nutrient status. Provide the same information for the sample of water medium as described for the pelagic test (5.2.1) and provide the following information for the sediment:

a) date and time of collection and delay between collection and use in the laboratory test;

b) depth of collection;

c) appearance of the sample, such as coloured, muddy, silty, or sandy;

d) dry mass in grams per litre of the suspended solids;

e) TOC concentration or loss of ignition as a measure of the content of organic matter;