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Water quality — Sampling —

Part 16: Guidance on biotesting of samples

Qualité de l'eau — Échantillonnage —

Partie 16: Lignes directrices pour les essais biologiques des échantillons

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Contents

Page

Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by ISO/TC 147, *Water quality*, Subcommittee SC 6, *Sampling (general methods)*.

This second edition cancels and replaces the first edition (ISO 5667-16:1998), which has been technically revised.

A list of all parts in the ISO 5667 series can be found on the ISO website.

Introduction

Biological tests are suitable for determining the effect of environmental samples or chemical substances on the respective test organism under the specific standardized test conditions. Environmental samples are e.g. treated communal and industrial waste water, fresh water, aqueous extracts of solid material (e.g. leachates, eluates), pore water of sediments. The effect can be stimulative or inhibiting, and can be determined by the reaction of the test organism (e.g. death, growth, morphological and physiological changes or generally, changes in molecular mechanisms of action). Inhibiting effects can be triggered by toxic water constituents or by other noxious influences.

The toxicity measurable in the biological test is the result of the interaction between a single toxic substance, a mixture of substances or the constituents of an environmental sample and the test organism. The protective potential of the biological system, i.e. the test organism, for instance by metabolic detoxification and excretion, is an integral part of the biological test.

Apart from the direct toxic effect of one or more sample constituents, biological effects can be exerted by the combined action of all constituents of a sample. Such a combined effect includes the impact of, for example, substances which are not toxic *per se* but affect the chemical or physical properties of the test batches by interfering with the test specific additives (e.g. nutrients, salts) and, consequently, the living conditions for the test organisms. This applies for instance to oxygen-depleting substances, coloured substances or turbid matter which reduce light exposure.

Biological tests also include those tests which examine the effect of organisms on substances (e.g. microbial degradation studies).

The results of the biological test refer primarily to the organism used in the test and the defined conditions stipulated for the test procedure. A harmful effect stated by means of standardized biological tests can justify concern that aquatic organisms and biocoenosis might be endangered. The results, however, do not permit direct or extrapolative conclusions as to the occurrence of similar effects in the aquatic environment. This applies in particular to suborganismic tests, as important properties and physiological functions of intact organisms (e.g. protective integuments, repair mechanisms) are removed or deactivated.

In principle there is no test organism which can be used to test all the effects on the biocoenosis or the ecosystem possible under the various combinations of abiotic and biotic conditions. Only a few ("model") species representing relevant ecological functions can be tested in practice.

Besides these fundamental and practical limitations in the selection of test organisms some issues should be taken into account during sampling and sample treatment in order to avoid a change in the sample properties. This applies to the method of sampling, including the sampling equipment and sample container as well as the transport to the laboratory. The method of sample pre-treatment and storage, as well as the preparation of, for example, stock solutions, may have an influence on the test result as well.

Furthermore, the sample to be tested can pose experimental problems on biotesting. Environmental samples (e.g. waste water, eluates) are complex mixtures and may contain, for example, sparingly soluble, volatile, unstable, coloured substances or suspended, sometimes colloidal, particles. The complexity and heterogeneity of materials give rise to a variety of experimental problems when performing biotests.

Special problems are related to the instability of the test material due to reactions and processes such as

— physical (e.g. phase separation, sedimentation, volatilization),

— chemical (e.g. hydrolysis, photodegradation, precipitation), and/or

— biological (e.g. biodegradation, biotransformation, biological uptake in organisms).

Other problems, especially if spectrometric measurements are applied, relate to turbidity and colour of the test batch.

The statistical analysis of the data from biological testing of environmental samples should be conducted according to the current state of the art if not stipulated by the specific biotest standard.

Finally, it is recommended to implement and maintain a quality management system regardless if a laboratory is involved in testing of substances or environmental samples.

This document is one of a group of International Standards dealing with the sampling of waters and sediments and is intended to be read in conjunction with the other parts of the ISO 5667 series, in particular with ISO 5667-1, ISO 5667-3 and ISO 5667-15.

Water quality — Sampling —

Part 16:
Guidance on biotesting of samples

1 Scope

This document gives practical guidance on sampling, pre-treatment, performance and evaluation of environmental samples in the context of performing biological tests. Information is given on how to cope with the problems of biotesting arising from the sample and the suitability of the test design.

It is intended to convey practical experience concerning precautions to be taken by describing methods successfully proven to solve or to circumvent some of the experimental problems of biotesting of, for example, waters.

Primarily dealt with are substance-related problems concerning sampling and pre-treatment of environmental samples (e.g. waste water samples) for the performance of biotests.

This guidance is on ecotoxicological testing with organisms (single-species biotests; *in vivo* and *in vitro*). Some features addressed in this document also apply to biotests using single-cell systems (*in vitro* bioassays) and biodegradation studies as far as sampling and sample preparations are concerned. Testing of substances in the water solubility range is also addressed.

Reference has been made as far as possible to existing International Standards and guidelines. Information taken from published papers or oral communication has been utilized as well.

This document is applicable to biological tests for determining the effect of environmental samples like treated communal and industrial waste water, groundwater, fresh water, aqueous extracts (e.g. leachates, eluates), pore water of sediments and whole sediments. This document is also applicable to chemical substances.

This document is not applicable to bacteriological examination of water. Appropriate methods for bacteriological examination are described in other documents (see ISO 19458[17]).

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
blank
mixture of water and nutrients without test organism

3.2
cell density
x
number of cells per unit volume of medium

Note 1 to entry: Cell density is expressed in cells per millilitre.

[SOURCE: ISO 10253:2016, 3.1][6]

3.3
control
control medium (3.4), or *control sediment* (3.5), including organisms used in the test, without test sample

3.4
control medium
combination of dilution water and/or nutrient medium used in the test

[SOURCE: ISO 20079:2005, 3.6][18]

3.5
control sediment
defined artificial or natural sediment used in the test

3.6
dilution level
D
reciprocal value of the volume fraction of test sample in *dilution water* (3.7) in which the test is conducted

EXAMPLE 250 ml of waste water in a total volume of 1 000 ml (volume fraction of 25 %) represents dilution level $D = 4$.

[SOURCE: ISO 15088:2007, 3.2, modified — "waste water" replaced by "test sample"][13]

3.7
dilution water
water added to the test sample to prepare a series of defined dilutions

Note 1 to entry: The composition of the water is specified in the respective standard.

[SOURCE: ISO 20079:2005, 3.7, modified — "Note 1 to entry" has been added][18]

3.8
effective concentration
EC_x
concentration of the test material in water or sediment that causes *x* % change in response during a specified time interval

[SOURCE: ISO/TS 20281:2006, 3.8.1, modified — "quantal" has been removed from the term and abbreviated term; "soil" and "(e.g. immobility)" have been removed from the definition; the EXAMPLE and Notes 1 and 2 to entry are not included][20]

3.9
field blank
container prepared in the laboratory using reagent water or other blank matrix and sent with the sampling personnel for exposure to the sampling environment to verify possible contamination during sampling

[SOURCE: ISO 11074:2015, 4.5.3][9]

3.10
growth rate
proportional rate of increase in biomass per unit of time: (1/day)

[SOURCE: ISO 10253:2016, 3.2, modified — "specific grow rate" replaced by "growth rate"; formula and Note 1 to entry not included][6]

3.11
lowest ineffective dilution
dilution factor
LID
lowest ineffective dilution tested, expressed as *dilution level D* (3.6), at which no inhibition, or only effects not exceeding the test-specific variability, are observed

[SOURCE: ISO 15088:2007, 3.5][13]

3.12
nutrient medium
solution of nutrients and micronutrients in water which are essential for the growth of the test organism

[SOURCE: ISO 20079:2005, 3.17, modified — "duckweed" replaced by "the test organism"][18]

3.13
positive control
well-characterized *reference substance* (3.14) that, when evaluated by a specific test method, demonstrates the suitability of the test system to yield a reproducible, appropriately positive or reactive response in the test system

[SOURCE: ISO 10993-12:2010, 3.14, modified — "any" removed before "well-characterized"; "material and/or substance, which" replaced by "reference substance that"][8]

3.14
reference substance
known substance to verify the sensitivity of the method

3.15
reference batch
mixture of dilution water, test specific additives and reference substance, including test organisms

3.16
replicate
one of a selected number of identical *test batches* (3.24) or identical *reference batches* (3.15)

3.17
sample
portion of material selected from a large quantity of material

Note 1 to entry: The method of sample selection can be described in the sampling plan.

Note 2 to entry: The material is from the environment (e.g. waste water, sediment or an eluate), a chemical substance or preparation or related material.

[SOURCE: ISO 16133:2004, 2.11, modified — Notes 1 and 2 to entry have been added][15]

3.18
sample pre-treatment
collective noun for all procedures used for conditioning a sample to a defined state which allows subsequent examination

Note 1 to entry: Depending on the requirements of the method sample, pre-treatment includes for example preservation and storage, centrifugation, filtration, homogenization, preconcentration and pH adjustment.

3.19
sample storage
process, and the result, of keeping a sample available under predefined conditions for a (usually) specified time interval between collection and further treatment of a sample

Note 1 to entry: Specified time is the maximum time interval.

[SOURCE: ISO 5667-3:2012, 3.3][2]

3.20
stock culture
culture of a single species to conserve the original defined species in the laboratory

[SOURCE: ISO 20079:2005, 3.21, modified — deleted "duckweed", "*Lemna*" "and to provide inoculum for the pre-culture"][18]

3.21
stock solution
solution with accurately known analyte concentration(s), prepared from chemicals with an appropriate purity

[SOURCE: ISO 11885:2007, 3.23][10]

3.22
storage time
period of time between filling of the sample container and further treatment of the sample in the laboratory, if stored under predefined conditions

Note 1 to entry: Sampling finishes as soon as the sample container has been filled with the sample. Storage time ends when the sample is taken by the analyst to start sample preparation prior to analysis.

[SOURCE: ISO 5667-3:2012, 3.4, modified — Note 2 to entry not included][2]

3.23
sub-sample
representative portion removed from a sample

[SOURCE: ISO 5667-19:2004, 3.7][5]

3.24
test batch
test medium including organisms used for testing

[SOURCE: ISO 20079:2005, 3.22][18]

3.25
test medium
mixture of test sample or test substance, dilution water and nutrients (without test organisms)

[SOURCE: ISO 20079:2005, 3.23, modified "combination" replaced by "mixture", after test sample added "or test substance", deleted "/or", "nutrient medium used in the test" replaced by "nutrients (without test organisms)"][18]

3.26
test sample
sample to be tested, after finishing all preparations

EXAMPLE Preparations include centrifugation, filtration, homogenization, pH adjustment and measurement of conductivity.

[SOURCE: ISO 13829:2000, 3.7][11]