
**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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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.

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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.

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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>

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mixture of water and nutrients without test organism