

Water quality — Sampling —

Part 16: Guidance on biotesting of samples

The European Standard EN ISO 5667-16:1998 has the status of a
British Standard

ICS 13.060.01

Confirmed July 2008

National foreword

This British Standard is the English language version of EN ISO 5667-16:1998. It is identical with ISO 5667-16:1998.

The UK participation in its preparation was entrusted by Technical Committee EH/3, Water quality, to Subcommittee EH/3/6, Sampling, which has the responsibility to:

- aid enquirers to understand the text;
- present to the responsible international/European committee any enquiries on the interpretation, or proposals for change, and keep the UK interests informed;
- monitor related international and European developments and promulgate them in the UK.

A list of organizations represented on this subcommittee can be obtained on request to its secretary.

BS EN ISO 5667-16 is one a series of standards on water quality, others of which have been, or will be, published as Sections of BS 6068. This standard has therefore been given the secondary identifier BS 6068-6.16. The various Sections of BS 6068 are comprised within Parts 1 to 7, which, together with Part 0, are listed below.

- *Part 0: Introduction;*
- *Part 1: Glossary;*
- *Part 2: Physical, chemical and biochemical methods;*
- *Part 3: Radiological methods;*
- *Part 4: Microbiological methods;*
- *Part 5: Biological methods;*
- *Part 6: Sampling;*
- *Part 7: Precision and accuracy.*

Cross-references

Attention is drawn to the fact that CEN and CENELEC Standards normally include an annex which lists normative references to international publications with their corresponding European publications. The British Standards which implement these international or European publications may be found in the BSI Standards Catalogue under the section entitled “International Standards Correspondence Index”, or by using the “Find” facility of the BSI Standards Electronic Catalogue.

A British Standard does not purport to include all the necessary provisions of a contract. Users of British Standards are responsible for their correct application.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, the EN ISO title page, page 2, the ISO title page, pages ii to iv, pages 1 to 26, an inside back cover and a back cover.

The BSI copyright notice displayed in this document indicates when the document was last issued.

This British Standard, having been prepared under the direction of the Health and Environment Sector Committee, was published under the authority of the Standards Committee and comes into effect on 15 December 1998

© BSI 01-2000

ISBN 0 580 30637 2

Amendments issued since publication

Amd. No.	Date	Comments
10703	January 2000	Addition of annex ZA

Contents

	Page
National foreword	ii
EN ISO Foreword	2
ISO Foreword	iii
Text of ISO 5667-16	1

English version

Water quality — Sampling — Part 16: Guidance on biotesting of samples

(ISO 5667-16:1998)

Qualité de l'eau — Echantillonnage —
Partie 16: Lignes directrices pour les essais
biologiques des échantillons
(ISO 5667-16:1998)

This European Standard was approved by CEN on 1 October 1998.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Central Secretariat: rue de Stassart 36, B-1050 Brussels

Foreword

The text of the International Standard ISO 5667-16:1998 has been prepared by Technical Committee ISO/TC 147, Water quality, in collaboration with Technical Committee CEN/TC 230, Water analysis, the Secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by April 1999, and conflicting national standards shall be withdrawn at the latest by April 1999.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of the International Standard ISO 5667-16:1998 was approved by CEN as a European Standard without any modification.

NOTE Normative references to International Standards are listed in Annex ZA (normative).

INTERNATIONAL STANDARD

ISO
5667-16

First edition
1998-10-01

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



Contents

	Page
Foreword	iii
Introduction	1
1 Scope	1
2 Normative references	2
3 Sampling	2
4 Transport	3
5 Preservation and storage	3
6 Apparatus and equipment	3
7 Pretreatment and preparation of samples	4
8 Treatment of samples during the test	9
9 General guidance regarding test design	10
10 Special guidance regarding test performance	11
11 Special biological assays	13
12 Evaluation	16
13 Presentation of results	19
14 Test report	20
15 Basic principles of quality assurance for biotesting	20
Annex A (informative) Lowest Ineffective Dilution (LID)	23
Annex B (informative) Bibliography	23
Annex ZA (normative) Normative references to international publications with their relevant European publications	26

Descriptors: Water, quality, water pollution, biological tests, bioassay, sampling, samples, sampling equipment, rules (instructions).

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.

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.

International Standard ISO 5667-16 was prepared by Technical Committee ISO/TC 147, Water quality, Subcommittee SC 6, Sampling.

ISO 5667 consists of the following parts, under the general title *Water quality — Sampling*:

- *Part 1: Guidance on the design of sampling programmes;*
- *Part 2: Guidance on sampling techniques;*
- *Part 3: Guidance on the preservation and handling of samples;*
- *Part 4: Guidance on sampling from lakes, natural and man-made;*
- *Part 5: Guidance on sampling of drinking water and water used for food and beverage processing;*
- *Part 6: Guidance on sampling of rivers and streams;*
- *Part 7: Guidance on sampling of water and steam in boiler plants;*
- *Part 8: Guidance on the sampling of wet deposition;*
- *Part 9: Guidance on sampling from marine waters;*
- *Part 10: Guidance on sampling of waste waters;*
- *Part 11: Guidance on sampling of groundwaters;*
- *Part 12: Guidance on sampling of bottom sediments;*
- *Part 13: Guidance on sampling of water, wastewater and related sludges;*
- *Part 14: Guidance on quality assurance of environmental water sampling and handling;*
- *Part 15: Guidance on preservation and handling of sludge and sediment samples;*
- *Part 16: Guidance on biotesting of samples.*

Annex A and Annex B of this part of ISO 5667 are for information only.

Introduction

Biological tests are suitable for determining the effect of chemical and physical parameters on test organisms under specific experimental conditions. In principle, the methods of chemical analysis are not suitable for determining the biological effects. These effects can be enhancing or inhibiting, and can be determined by the reaction of the organisms, e.g. death, growth, proliferation, morphological, physiological and histological changes. Inhibiting effects are triggered by toxic water constituents or by other noxious influences.

Effects can refer to various levels, e.g. proceeding from (sub)cellular structures or enzyme systems, concerning the whole organism, and eventually the supra-organism or community level.

In the context of this part of ISO 5667, toxicity is the ability of a substance to exert a deleterious effect on organisms or biocenoses due to its chemical properties and its concentration.

The deleterious potential of a toxic substance can be counteracted by the protective potential of the biological system, for instance by metabolic detoxification and excretion. The apparent toxicity measurable in the biological test is the result of the interaction between the substance and the biological system.

Apart from the direct toxic effect of one or more water constituents, damaging biological effects can be exerted by the combined action of all noxious substances, e.g. by substances which are not toxic *per se* but affect the chemical or physical properties of the medium and, consequently, the living conditions for the organisms. This applies for instance to oxygen-depleting substances, coloured substances or turbid matter which reduce light exposure. It also includes non-substance-related effects such as impairment or damage due to extreme temperature.

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

The results of the biological tests refer primarily to the organisms used in the test and the conditions stipulated in the test procedure. A harmful effect stated by means of standardized tests can justify concern that aquatic organisms and biocenoses 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 sub-organism systems, as important properties and physiological functions of intact organisms (e.g. protective integuments, repair mechanisms) are removed or deactivated.

In principle there is no organism and no biocenosis which can be used to test all the effects on the ecosystem possible under the various constellations 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, the sample to be tested can also pose experimental problems on biotesting. Waters, in particular waste waters, are complex mixtures and often contain sparingly soluble, volatile, unstable, coloured substances and/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.

This part of ISO 5667 is one of a group of International Standards dealing with the sampling of waters. It should be read in conjunction with the other parts and in particular with ISO 5667-1, ISO 5667-2 and ISO 5667-3.

1 Scope

This part of ISO 5667 gives practical guidance on sampling, pretreatment, performance and evaluation of waters in the context of biotesting. Information is given on how to cope with the problems for biotesting arising from the nature of the water 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 waters.

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

Primarily dealt with are substance-related problems concerning sampling, pretreatment and preparation of water samples for biotesting and treatment of samples during the test, especially when performing tests with waters and waste waters containing unstable or removable ingredients. Basic principles of quality assurance, evaluation of data and presentation of results are outlined.

Special emphasis is laid on ecotoxicological testing with organisms ("single-species biotests"). Some features addressed in this general guidance apply as well to biodegradation and/or bioaccumulation studies as far as sampling and sample preparations are concerned. Preparation of poorly soluble substances and testing beyond the water-solubility limit is also addressed.

This part of ISO 5667 is not applicable to bacteriological examination of water. Appropriate methods are described in other International Standards.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 5667. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 5667 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 5667-3:1994, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of samples*.

ISO 5667-10:1992, *Water quality — Sampling — Part 10: Guidance on sampling of waste waters*.

3 Sampling

3.1 General

The choice of representative sampling points, frequency of sampling, type of samples taken, etc. is dependent on the objective of the study. In general, the sampling approach for chemical analysis is compatible with the purpose of biotesting.

Some tests, however, require the water and waste water to be handled and kept in a particular way.

Depending on the type of investigation (e.g. toxicity or biodegradation tests) and the way the samples are to be processed, it is necessary to divide a sample into different portions which are preserved and/or stored under different conditions and processed in different ways.

If several samples have been taken (e.g. from different locations or at several times) they may be combined to achieve greater representativity. These samples should be thoroughly mixed and, if necessary, divided into subsamples. To obtain subsamples of equal quality, it should be ensured that the bulk sample maintains homogeneity during the subsampling process, e.g. by continuous shaking or stirring. This holds particularly in the case of two-phase mixtures, e.g. waters containing suspended particles, algal suspensions. It is recommended to use cooling sampling apparatus when several samples taken at several times are combined.

3.2 Samplers/vessels/containers

The volume, shape and material of the vessels are dependent on the nature of the sample (e.g. degradability/stability), the number of replicates, the volume required for these tests and the necessity of preserving and storing the samples prior to further processing.

The time required for freezing and thawing should be minimized by reducing the sample volume, i.e. the size of the vessel. In general it is appropriate to use one-litre vessels for freezing. For tests requiring larger volumes, the sample should be divided into vessels holding not more than 10 l.

The total sample volume taken should be sufficient to cover any supplementary or repeated testing. Remaining subsamples stored frozen separately should be saved until the final evaluation has been made.

The material of vessels should be chemically inert, easily cleaned and resistant to heating and freezing. Glassware, polyethene or polytetrafluoroethene (PTFE) vessels are recommended.

3.3 Filling status of containers

It should be decided whether the containers should be filled completely to the brim or only partially, having an air space, by taking into account the type of sample, the preservation mode and the biotest envisaged.

Problems related to partial filling can be:

- enhanced agitation during transport, leading to breakdown of aggregated particles;
- interaction with gas phase, leading to stripping;
- oxidation of substances, leading e.g. to precipitation of compounds of heavy metals.

Problems related to complete filling can be:

- oxygen depletion, with possible decomposition, leading to formation of toxic metabolites (e.g. nitrite, sulfide);