SLOVENSKI STANDARD
SIST EN ISO 5667-16:2000

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Kakovost vode – Vzorčenje – 16. del: Navodilo za biološke preskuse vzorcev (ISO 5667-16:1998)

Water quality - Sampling - Part 16: Guidance on biotesting of samples (ISO 5667-16:1998)

Wasserbeschaffenheit - Probenahme - Teil 16: Anleitung zur Probenahme und Durchführung biologischer Testverfahren (ISO 5667-16:1998)

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

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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.

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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.

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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of an identical text or by endorsement, at the latest by April 1999, and conflicting national standards shall be withdrawn at the latest by April 1999.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.

NOTE FROM CEN/CS: The foreword is susceptible to be amended on reception of the German language version. The confirmed or amended foreword, and when appropriate, the normative annex ZA for the references to international publications with their relevant European publications will be circulated with the German version.

Endorsement notice

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

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

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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.

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.*

Annexes A and 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.

Water quality — Sampling —

Part 16:
Guidance on biotesting of samples

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 is 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 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);

— impairment of homogenization by shaking or stirring the total volume.

Sample containers, when freezing is envisaged for preservation, should not be filled completely in order to allow expansion of volume.

4 Transport

The samples collected should be protected from breakage, temperature increase and external contamination. Misidentification of samples transported in melting ice should be avoided by using waterproof markers and/or labels.

5 Preservation and storage

As stated in ISO 5667-3, it is impossible to give absolute rules for preservation, e.g. the duration of possible storage and efficiency of various modes, because it depends primarily on the nature of the sample, especially its biological activity.

Potable waters and ground waters are generally less susceptible to biological and chemical reactions than surface waters, treated or raw waste waters. If the chemical composition can be approximately anticipated, reference should be made to ISO 5667-3 for the purposes of biotesting. Some additional precautions, however, should be considered as follows.

Samples for biotesting should be processed preferably without delay after collection to avoid changes in the original composition as a result of physical and chemical reactions and/or biological processes. The maximum duration of storage should not exceed 12 h at ambient temperature (maximum 25 °C). The samples should be kept in the dark to prevent algal growth.

If testing almost immediately after sampling (or sample preparation) is not possible, e.g. when preparing composite samples, cooling or freezing is recommended.

The most common and recommended way of preserving waste water samples is to cool to between 0 °C and 5 °C. When cooled to this range and stored in the dark, most samples are normally stable for up to 24 h (see ISO 5667-10). Cooling should commence as soon as possible after sampling, either in the field, for instance in cool boxes with melting ice, or in a refrigerator in the transport vehicle.

Deep freezing below −18 °C in accordance with ISO 5667-10 allows in general an increase in conservation. A few weeks up to 2 months, depending on the stability of samples, are generally the maximum storage periods.

Experience has shown that the quality of waste water can be affected during both freezing and thawing.

The use of biocidal preservatives should be excluded for the purpose of biotesting. The addition of highly concentrated acids or bases to stabilize the samples, e.g. HCl or NaOH, is not recommended either.

It should be stressed that, if there is any doubt, the chemical analyst and the biotester should consult each other before deciding on the method of handling and preserving the samples. If preservation techniques for the chemical analysis and for biotesting are not compatible, separate subsamples should be provided for the different purposes.

6 Apparatus and equipment

6.1 Selection of apparatus

Type, shape and material of the technical equipment are dependent on the test and nature of the sample. All materials which come into contact with the test sample should be such that interferences caused by sorption or diffusion of the test material, by elution of foreign matter (e.g. plasticizers) or by growth of organisms, are kept to a minimum. Inert materials are suitable, e.g. glass, PTFE. Tubing connections should be as short as possible and replaced from time to time. Contamination of the test material, e.g. by grinding grease from stoppers or fittings, should be avoided. Pipes made from copper, copper alloy or non-inert plastics are not suitable.

6.2 Silanization

In order to minimize adsorption of test material on containers, pipes, tubings, glassware or plasticsware can be silanized (siliconized) by soaking or rinsing in a 5 % mass fraction solution of dichlorodimethylsilane in chloroform or heptane. As the organic solvent evaporates, the silane is deposited on the surface, which should be rinsed many times with water or heated at 180 °C for 2 h before use. Silanization should only be used if highly adsorbable substances or water ingredients are to be tested and suitable inert material (e.g. PTFE) is not available.