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**Kakovost vode – Preskus zaviranja rasti morskih alg s *Skeletonema costatum*  
in *Phaeodactylum tricomutum***

Water Quality - Marine algal growth inhibition test with *Skeletonema costatum* and  
*Phaeodactylum tricomutum*

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Will supersede EN ISO 10253:1998

English version

## Water Quality - Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricomutum*

Qualité de l'eau - Essai d'inhibition de la croissance des  
algues marines avec *Skeletonema costatum* et  
*Phaeodactylum tricomutum*

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EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: rue de Stassart, 36 B-1050 Brussels

## **prEN ISO 10253:2004 (E)**

### **Foreword**

This document (prEN ISO 10253:2004) 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 document is currently submitted to the parallel Enquiry.

This document will supersede EN ISO 10253:1998.

### **Endorsement notice**

The text of ISO/DIS 10253:2004 has been approved by CEN as prEN ISO 10253:2004 without any modifications.

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## DRAFT INTERNATIONAL STANDARD ISO/DIS 10253

ISO/TC 147/SC 5

Secretariat: **AFNOR**

Voting begins on:  
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### **Water quality — Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum***

*Qualité de l'eau — Essai d'inhibition de la croissance des algues marines avec *Skeletonema costatum* et *Phaeodactylum tricornutum**

[Revision of first edition (ISO 10253:1995)]

ICS 13.060.70

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The CEN Secretary-General has advised the ISO Secretary-General that this ISO/DIS covers a subject of interest to European standardization. **In accordance with the ISO-lead mode of collaboration as defined in the Vienna Agreement, consultation on this ISO/DIS has the same effect for CEN members as would a CEN enquiry on a draft European Standard.** Should this draft be accepted, a final draft, established on the basis of comments received, will be submitted to a parallel two-month FDIS vote in ISO and formal vote in CEN.

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## Foreword

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ISO 10253 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

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# Water quality — Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricorutum*

**WARNING** — Persons using this International Standard should be familiar with normal laboratory practice. This International Standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

## 1 Scope

This International Standard specifies a method for the determination of the inhibition of growth of unicellular marine algae by substances and mixtures contained in seawater.

The method can be used for testing substances that are readily soluble in water and are not significantly degraded or eliminated from the test.

**NOTE** With modifications as described in ISO/DIS 14442 and ISO 5667-16, the inhibitory effects of poorly soluble organic and inorganic materials, volatile compounds, metals, effluents, marine water samples and elutriates of sediments can be tested.

## 2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5667-16, *Water quality — Sampling — Part 16: Guidance on biotesting of samples*

ISO/DIS 14442<sup>1)</sup>, *Water quality — Guidelines for algal growth inhibition tests with poorly soluble materials, volatile compounds, metals and waste water*

ISO/TS 20281<sup>1)</sup>, *Water quality — Guidance on statistical interpretation of ecotoxicity data*

## 3 Terms and definitions

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

### 3.1

#### cell density

number of cells per unit volume of medium ( $x$  cells/ml)

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1) Under preparation.

**3.2  
specific growth rate**

proportional rate of increase in cell density per unit of time :  $\frac{1}{x} \times \frac{dx}{dt}$  (1/day)

**3.3  
growth medium**

mixture of seawater and nutrients which is used for pre-cultures and controls

**3.4  
test medium**

mixture of seawater, nutrients (growth medium 3.3) and test material in which algal cells are incubated

**3.5  
test batch**

mixture of seawater, nutrients and test material (test medium 3.4) inoculated with algae

**3.6  
control**

mixture of seawater, nutrients (growth medium 3.3) without test material, inoculated with algae

**3.7  
effective concentration, E<sub>r</sub>C<sub>x</sub>**

concentration of test substance which results in a x % reduction in specific growth rate relative to the controls

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## 4 Principle

Mono-specific algal strains are cultured for several generations in a defined medium containing a range of concentrations of the test substance, prepared by mixing appropriate quantities of nutrient concentrate, seawater, stock solutions of the test substance, and an inoculum of exponentially growing algal cells. The test solutions are incubated for a period of 72 h ± 2 h, during which the cell density in each is measured at intervals of at least every 24 h ± 2 h. Inhibition is measured as a reduction in specific growth rate, relative to control cultures grown under identical conditions.

## 5 Materials

### 5.1 Test organisms

Use either of the following marine algae :

- a) *Skeletonema costatum* (Greville) Cleve (CCAP 1077/1C, NIVA BAC 1, ISTPM P4) ; or
- b) *Phaeodactylum tricornutum* Bohlin (CCAP 1052/1A, SAG 1090-1a , NIVA BAC 2, ISTPM P1).

These algae are important and widely distributed phytoplankton species (phylum *Bacillariophyta*) in estuarine and coastal areas.

The strains recommended are available in unialgal, non-axenic cultures from the following sources.

NIVA            Norwegian Institute for Water Research  
                  P.O Box 173 Kjelsås  
                  N-0411 Oslo  
                  Norway

ISTPM	INERIS 9, rue de Rocroy 75010 Paris France
CCAP	Dunstaffnage Marine Laboratory P O Box 3 Oban Argyll PA37 1QA United-Kingdom
SAG	Collection of Algal Cultures University of Göttingen Albrecht-von-Haller Institute for Plant Science Untere Karspüle 2 37073 Göttingen Germany

NOTE 1 Stock cultures may be maintained in the medium described in 7.1. Regular subculturing is necessary. Weekly intervals may be necessary for *Skeletonema*, every two or three weeks may be sufficient for *Phaeodactylum*. The stock cultures may also be maintained for extended periods on richer algal media such as those recommended by the culture collection. It is recommended to keep the stock culture in the medium described in 7.1 and in an exponential growth phase immediately before preparing the pre-culture for testing as described in 7.2.

NOTE 2 Like many freshwater algae, the diatom *Phaeodactylum tricornutum* can also be stored for several months in alginate beads, without losing its viability [1]. The algae can be liberated from the algal beads when needed to perform the toxicity tests<sup>2)</sup>.

## 5.2 Water

All water used in the preparation of the synthetic seawater, growth medium and test substance solutions shall be deionized or of equivalent purity. Take special care to avoid contamination of the water by inorganic or organic substances during preparation and storage. Equipment made of copper shall not be used.

## 5.3 Seawater

For culturing and testing *Phaeodactylum*, the growth medium (7.1) is made up by adding nutrients to either natural or synthetic seawater of salinity  $30 \pm 5$  g/kg. For *Skeletonema*, the use of natural seawater may be necessary for the long-term maintenance of cultures, and may also be necessary for the test medium because a synthetic seawater medium may not always support sufficient growth to meet the test quality criteria. If natural seawater is used, care shall be taken to ensure that it is not polluted.

Prepare synthetic seawater with the composition given in Table 1 (approximate salinity = 33 g/kg). All the chemicals used shall be of analytical grade.

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2) The algae beads supplied by MICROBIOTESTS Inc., Venecoweg 19, 9810 Nazareth, Belgium, Tel (32) 9 380 8545, Fax (32) 9 380 8546, Email [microbiotests@skynet.be](mailto:microbiotests@skynet.be), are an example of a suitable commercially available product. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.