
**Water quality — Guidelines for algal
growth inhibition tests with poorly
soluble materials, volatile compounds,
metals and waste water**

*Qualité de l'eau — Lignes directrices pour essais d'inhibition de la
croissance algale avec des matières peu solubles, des composés
volatils, des métaux et des eaux résiduaires*



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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

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.

ISO 14442 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

This second edition cancels and replaces the first edition (ISO 14442:1999), which has been technically revised.

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Water quality — Guidelines for algal growth inhibition tests with poorly soluble materials, volatile compounds, metals and waste water

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.

IMPORTANT — It is absolutely essential that tests conducted according to this standard be carried out by suitably trained staff.

1 Scope

This International Standard provides procedures, not covered by the methods described in ISO 8692 and ISO 10253, for testing difficult substances for inhibition of algal growth.

The main subjects covered by the guideline are the methods for preparing the test substance for testing and the procedures needed to carry out an appropriate test. The following test substances are covered by this guideline:

- a) poorly soluble pure organic compounds;
- b) poorly soluble mixtures of organic substances;
- c) poorly soluble inorganic materials;
- d) volatile substances;
- e) waste waters and environmental samples containing water and sediments;
- f) coloured and/or turbid samples;
- g) compounds of heavy metals.

The following methods of addition are covered:

- direct;
- dispersion;
- water-soluble and water-accommodated fractions.

Some guidelines related to the analytical procedures and to the interpretation of the results have been included.

References to documents describing the background for the testing of difficult substances are given in the Bibliography.

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 8692, *Water quality — Freshwater algal growth inhibition test with unicellular green algae*

ISO 10253, *Water quality — Marine algal growth inhibition test with Skeletonema costatum and Phaeodactylum tricornutum*

3 Analytical characterization of test materials and confirmation of concentrations and stability

Analytical characterization of test substances and materials and the confirmation of their concentrations and stability in the testing environment is of major concern of regulatory authorities. Such activities are usually not an integral part of this International Standard algal growth inhibition test methods.

However, there may be situations where analysis may assist in defining the appropriate exposure conditions of test materials and chemicals and/or in the interpretation of the results.

The relevant properties of substances and materials can be assessed from basic properties such as solubility in water, partition coefficient ($\lg P_{ow}$), Henry's constant, photochemical and hydrolytic stability and biodegradability.

Analytical confirmation is strongly recommended in order to confirm test substance concentrations and is required for the calculation of effective concentration (EC) values of volatile substances (Clause 7). If losses due to adsorption on the test vessels or during transfer of test solutions and media occur, then analytical confirmation are of particular importance. This aspect is also specified in ISO 5667-16.

Due to the batch test system used for algal growth inhibition tests, loss of substances due to biodegradation (nearly all algal cultures contain bacteria), photodegradation, hydrolysis and/or adsorption cannot always be avoided. A decrease in measured concentrations is difficult to prevent by technical means, and is therefore considered acceptable for algal growth inhibition tests.

The following precautions are suggested for maintaining test substance concentrations in algal growth inhibition tests:

- a) sterilization of media and equipment to reduce the effect of bacterial growth;
- b) change of the light quality to prevent photodegradation of test substances;
- c) avoidance of contact of test substance with water prior to testing to reduce hydrolytic decomposition;
- d) treatment of glassware (e.g. silanization); the effectiveness of such a treatment varies from one chemical to the other;
- e) pre-conditioning of the glassware, before addition of the test media, with the test substance at concentrations to be used in the test.

The effect of such technical measures is, if relevant and if possible, monitored by chemical analysis.

Water, waste water and organic/inorganic solids/liquids may contain components that may modify the composition of the algal growth medium (by precipitation of a limiting nutrient, complexation of essential elements, addition of nutrients), and subsequently may cause effects on algal growth not related to toxic