
**Water quality — Guidance for determination
of biodegradability in the marine
environment**

*Qualité de l'eau — Lignes directrices pour la détermination de la
biodégradabilité en milieu marin*



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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 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 16221 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

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Introduction

ISO/TC 147 has established International Standards for testing biodegradability of substances and waste water in the aquatic environment. All these methods, which are summarized in ISO 15462, can only be used for the determination and prediction of biodegradability in fresh water. There are, however, many cases, for example, substances used off-shore, where an urgent need exists for testing biodegradability in the marine environment. This International Standard describes biodegradation testing in marine test systems, and is based on an established OECD Guideline and the experience gained by a working group of the Oslo and Paris Commission (OSPARCOM) which has selected suitable standardized ISO methods, adopted for marine conditions and checked in a ring test.

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Water quality — Guidance for determination of biodegradability in the marine environment

1 Scope

This International Standard specifies five methods for determining the ultimate aerobic biodegradability of organic compounds in the marine environment by aerobic microorganisms in static aqueous test systems. Standard degradation methods developed for testing in fresh water are modified and adapted to marine conditions. These methods are the DOC die-away test (ISO 7827), the closed bottle test (ISO 10707), the two-phase closed bottle test (ISO 10708), the CO₂ evolution test (ISO 9439) and the CO₂ headspace test (ISO 14593).

The methods apply to organic compounds which

- a) are water-soluble under the conditions of the test used;
- b) are poorly water-soluble under the conditions of the test used, in which case special measures may be necessary to achieve good dispersion of the compound (see for example, ISO 10634);
- c) are volatile, provided that an appropriate test with suitable conditions is used;
- d) are not inhibitory to the test microorganisms at the concentration chosen for the tests. The presence of inhibitory effects can be determined as specified in this International Standard.

NOTE The conditions described in this International Standard do not always correspond to the optimal conditions for allowing the maximum degree of biodegradation to occur. For biodegradation methods in fresh water see ISO 14593 and ISO 15462, and for biodegradation at low concentrations see ISO 14592.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 7827, *Water quality — Evaluation in an aqueous medium of the “ultimate” aerobic biodegradability of organic compounds — Method by analysis of dissolved organic carbon (DOC)*.

ISO 9439, *Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium — Carbon dioxide evolution test*.

ISO 10707, *Water quality — Evaluation in an aqueous medium of the “ultimate” aerobic biodegradability of organic compounds — Method by analysis of biochemical oxygen demand (closed bottle test)*.

ISO 10708, *Water quality — Evaluation in an aqueous medium of the “ultimate” aerobic biodegradability of organic compounds — Determination of biochemical oxygen demand in a two-phase closed bottle test*.

ISO 14592-1, *Water quality — Evaluation of the aerobic biodegradability of organic compounds at low concentrations — Part 1: Shake-flask batch test with surface water or surface water/sediment suspensions.*

ISO 14592-2, *Water quality — Evaluation of the aerobic biodegradability of organic compounds at low concentrations — Part 2: Continuous flow river model with attached biomass.*

ISO 14593, *Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium — Method by analysis of inorganic carbon in sealed vessels (CO₂ headspace test).*

3 Terms and definitions

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

3.1

ultimate aerobic biodegradation

breakdown of a chemical compound or organic matter by microorganisms in the presence of oxygen to carbon dioxide, water and mineral salts of any other elements present (mineralization) and normally the production of new biomass

3.2

primary biodegradation

structural change (transformation) of a chemical compound by microorganisms resulting in the loss of a specific property

3.3

total organic carbon

TOC

all that carbon present in organic matter which is dissolved and suspended in the water sample

3.4

dissolved organic carbon

DOC

that part of the organic carbon in the water sample which cannot be removed by specified phase separation

NOTE Examples of specified phase separation are centrifugation at 40 000 ms⁻² for 15 min or membrane filtration using membranes with pores of 0,2 µm to 0,45 µm diameter.

3.5

total inorganic carbon

TIC

all that carbon in the water sample deriving from carbon dioxide and carbonate.

3.6

dissolved inorganic carbon

DIC

that part of the carbon in the water sample which cannot be removed by specified phase separation

NOTE Examples of specified phase separation are centrifugation at 40 000 ms⁻² for 15 min or membrane filtration using membranes with pores of 0,2 µm to 0,45 µm diameter.

3.7

chemical oxygen demand

COD

mass concentration of oxygen equivalent to the amount of a specified oxidant consumed by a chemical compound or organic matter when a water sample is treated with that oxidant under defined conditions

NOTE It is expressed in this case as milligrams oxygen uptake per milligram (or gram) test compound.