

ISO/FDIS 17244:2025(E)

ISO/TC 147/SC 5/WG 2

Secretariat: DIN

Date: 2025-03-24

Water quality — Determination of the toxicity of water samples on the embryo-larval development of Japanese oyster (*Magallana gigas*) and **blue** mussel (*Mytilus edulis* or *M. galloprovincialis*)

Qualité de l'eau — Détermination de la toxicité d'échantillons aqueux sur le développement embryonnaire d'huître creuse (*Magallana gigas*) et moule (*Mytilus edulis* ou *galloprovincialis*)

iTeh Standards
(<https://standards.iteh.ai>)
Document Preview

ISO/FDIS 17244
<https://standards.iteh.ai/catalog/standards/iso/e3ec1652-fa9e-42f9-b13d-ed80ecb210bd/iso-fdis-17244>

FDIS stage

Warning for WDs and CDs

This document is not an ISO International Standard. It is distributed for review and comment. It is subject to change without notice and may not be referred to as an International Standard.

Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

Edited DIS -
MUST BE USED
FOR FINAL
DRAFT



~~COPYRIGHT PROTECTED DOCUMENT~~

© ISO 2024

iTeh Standards (<https://standards.itih.ai>) Document Preview

ISO/FDIS 17244

<https://standards.itih.ai/catalog/standards/iso/e3ec1652-fa9e-42f9-b13d-ed80ecb210bd/iso-fdis-17244>

ISO/FDIS 17244:2025(en)

© ISO 2025

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office

~~CP 401~~ • Ch. de Blandonnet 8 • ~~CP 401~~

CH-1214 Vernier, Geneva, ~~Switzerland~~

~~Tel-Phone:~~ + 41 22 749 01 11

~~Fax + 41 22 749 09 47~~

~~E-mail:~~ copyright@iso.org

~~www.iso.org~~

~~Website:~~ www.iso.org

Published in Switzerland.

iTeh Standards (<https://standards.iteh.ai>) Document Preview

ISO/FDIS 17244

<https://standards.iteh.ai/catalog/standards/iso/e3ec1652-fa9e-42f9-b13d-ed80ecb210bd/iso-fdis-17244>

Contents

Foreword.....	v
Introduction.....	vi
1 Scope.....	1
2 Normative references.....	1
3 Terms and definitions.....	1
4 Principle.....	2
5 Test organisms and seawater.....	3
5.1 Spawning stock or mature bivalves	3
5.2 Reference seawater.....	4
6 Equipment.....	5
7 Reference substance.....	6
8 Test procedure.....	6
8.1 Collection, preparation and preservation of aqueous samples	6
8.2 Preparation of test samples	6
8.3 Selection of concentration range	7
8.4 Collecting gametes.....	7
8.5 Measurement of egg density	10
8.6 Fertilization and inoculation of fertilized eggs.....	10
8.7 Incubation.....	11
8.8 Observation.....	11
8.9 Analytical measurements	12
8.10 Disposal of adults.....	12
9 Expression of results	12
10 Validity criteria.....	15
11 Test report.....	15
Annex A (informative) Overview of the test applied to the Japanese oyster <i>Magallana gigas</i>	16
Annex B (informative) Performance data.....	18
Annex C (informative) Protocol to perform the test in direct contact with sediment.....	27
Bibliography	30

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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document 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 17244:2015), which has been technically revised.

The main changes are as follows:

- ~~Annex C~~ addition of Annex C, which gives a protocol to perform the test in direct contact with sediment, has been added;
- for Japanese oyster, the ~~use of~~ revised nomenclature, *Magallana gigas*, has been used;
- the possibility to extend the test duration to ~~48 h~~ 48 h has been included.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Traditionally, the level of pollution affecting a marine environment is shown in terms of the concentration levels of the contaminants present in the environment of interest. However, these measurements do not provide an estimation of the harmful effects on organisms and have to be complemented with the biological responses obtained through bioassays (see Reference [5]).

Among the marine organisms used to assess the potential impact of chemicals or discharges into the environment, bivalve embryos and larvae are, together with sea urchins, among the organisms which are most frequently used in bioassays (see References [18] and [21]). The embryos and larvae are more sensitive to pollutants than the adults of the same species. They therefore represent the critical stages for the toxicity tests (see References [19] and [30]). Since 1972, it is recommended to use the Pacific oyster, *Magallana gigas*, to assess the quality of seawater (see Reference [35]). Furthermore, their worldwide distribution in coastal waters, as well as their commercial importance (see Reference [10]), make bivalves the species of choice for the undertaking of bioassays.

The results of these bioassays demonstrate the necessity to determine the potential toxicity thresholds of chemicals which can enter the marine environment either accidentally or chronically, as well as the “biological quality” of an environment or the potential toxicity of river water or a discharge that reach the sea. Potential toxicity has been defined based on teratological effects (see References [11], [27] and [26]).

This document specifies a method based on the embryo-larval development of bivalves (oyster or mussel). It can be routinely used to assess development abnormalities caused by the possible presence of chemicals and mixtures in seawater. It also allows to assess the toxicity of aqueous samples like seawater, surface water, effluents (urban, agricultural, industrial effluents, etc.), aqueous extracts from sediments, and petroleum products that can be leached in the water column at the time of their resuspension or discharge and presence in the sea.

This test can be performed throughout the year with embryos and larvae of mature bivalves sampled from the natural environment during their reproduction periods or mature bivalves which come from a hatchery where they have been conditioned.

This bioassay, recommended by the International Council for the Exploration of the Sea (ICES) [14], (see Reference [14]), has been the subject of the first European inter-calibration test performed in 1991 [31] (see Reference [31]). The protocol described in this document corresponds to a modification and simplification of the ASTM standard method (1994) (see Reference [3]).

The toxicity assessment of metals performed on *M. gigas* and *Mytilus edulis* demonstrated that both organisms had a similar level of sensitivity (see References [19] and [15]). Two other studies performed on urban effluents showed similar findings for both species (see References [16] and [28]). These observations have been confirmed by the work carried out on mercury (see Reference [44]), which compared the findings of four embryo-larval tests: *M. edulis*, *M. galloprovincialis*, *M. gigas* and *C. virginica*. Another study showed that the embryos of *M. gigas* are more sensitive to metals and hydrocarbons than the other marine organisms which are commonly used, for example, polychaete, amphipods, fish and crustaceans (see Reference [8]).

The sensitivity of the bivalve embryo-larval development confirms the suitability of this test to assess the toxicity of chemicals and aqueous samples. The pH, salinity and temperature range acceptable to bivalves make them easy to use in ecotoxicity studies, particularly when assessing the quality of coastal and estuarine environments (see Reference [11]).

Water quality — Determination of the toxicity of water samples on the embryo-larval development of Japanese oyster (*Magallana gigas*) and **blue** mussel (*Mytilus edulis* or *M. galloprovincialis*)

WARNING — Persons using this document should be familiar with normal laboratory practice. This document 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 in accordance with this document be carried out by suitably qualified staff.

1 Scope

This document specifies a method for assessing the effects of chemical and aqueous samples on the embryo-larval development of marine bivalves. This method allows the determination of the concentration levels that result in an abnormality in embryo-larval development. This test is suitable for salinity ranges ~~between 20 PSU (Practical Salinity Unit) and 40 PSU for mussels and between 25 PSU and 35 PSU for oysters.~~

~~— between 20 PSU (practical salinity unit) and 40 PSU for mussels, and~~

~~— between 25 PSU and 35 PSU for oysters.~~

This method in this document applies to:

- ~~— chemical substances and preparations, and~~
- ~~— marine and brackish waters, and~~
- ~~— streams and aqueous effluents (urban, agricultural, industrial effluents, etc.) as long as the salinity is adjusted or dilution is limited so that the aforementioned salinity ranges are respected, and~~
- ~~— aqueous extracts (pore water, elutriates, eluates, and leachates) from sediments and petroleum products, and~~
- ~~— samples of contaminated sediment or dredged material (see [Annex C](#)).~~

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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*

3 Terms and definitions

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

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ~~—~~ ISO Online browsing platform: available at <https://www.iso.org/obp>

— IEC Electropedia: available at <https://www.electropedia.org/>

~~3.1~~

~~3.1~~

~~effect concentration~~

EC_x

concentration at which a specific effect is detected; *x* is the percentage (10, 25, 50) of this effect, e.g. the development abnormality

EXAMPLE EC₅₀ means the concentration estimated to observed 50 % of development abnormality at the end of the test compared to the control.

[SOURCE: ISO 15952:2018, 3.6, modified — "growth inhibition" has been replaced with "development abnormality"; in the definition and the EXAMPLE has been replaced.]

~~3.13.2~~

~~lowest observed effect concentration~~

LOEC

lowest tested concentration at which the test substance or dilution (of an aqueous sample, in %, or of a sediment in g/l) is observed to have a statistically significant effect ($p < 0,05$) when compared with the control

Note 1 to entry: All test concentrations above the LOEC have a harmful effect equal to or greater than those observed at the LOEC. When these two conditions cannot be satisfied, a full explanation should be given for how the LOEC [and hence the no observed effect concentration (3.3(3.3))] has been selected.

[SOURCE: ISO 15952:2018, 3.8, modified — "substance or dilution (of an aqueous sample, in %, or of a sediment in g/l)" has been added to the unit; definition.]

~~3.23.3~~

~~no observed effect concentration~~

NOEC

test concentration immediately below the lowest observed effect concentration (3.2(3.2)), which, when compared with the control, has no statistically significant effect ($p > 0,05$) within a given exposure time

Note 1 to entry: The NOEC is the concentration just below the lowest observed effect concentration.

[SOURCE: ISO 15952:2018, 3.9, modified — Note 2 to entry has been deleted.]

~~3.33.4~~

~~reference seawater~~

natural or artificial seawater used to induce gamete production and prepare the solutions to be tested

~~3.43.5~~

~~D-shaped stage larvae~~

larvae stage so named due to their characteristic D-shaped under microscopic examination

Note 1 to entry: The normal D-shaped larvae obtained after incubation have fully developed and symmetrical shells with a straight hinge. The larvae size is regular and around 70 µm. After fixation, the mantle nearly fills the interior of the larvae, but is totally included within the two closed shells.

4 Principle

This bioassay assesses the effects of chemicals and aqueous environmental samples on the embryo-larval development of marine bivalves under static conditions.

The exposure is performed from fertilized eggs to D-shaped larvae. This static test aims to determine the concentration level (EC_x) which results in abnormalities for x % of exposed larvae in 24 h (optionally 48 h) for the Japanese oyster, also named Pacific oyster (*Magallana gigas*, previously *Crassostrea gigas*), and in 48 h for the Blue and Mediterranean mussel (*Mytilus edulis* or *Mytilus galloprovincialis*). Several parameters can be assessed in the abnormal larvae: alteration of the shell (hinge is not straight, unequal, or incomplete valves), hypertrophy of the mantle, delayed or stopped embryonic development, and finally, death. The results are expressed as EC_x (EC_{20} or EC_{50}). The lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC) can also be determined.

This method can be applied to other species of bivalves (e.g. *C. virginica*). Nevertheless, the test conditions have to be defined to reach the validity criteria of ~~the standard~~ **this document**.

Some regulations can extend the oyster exposure period to 48 h. In this case, the sensitivity test shall be performed in the same conditions and the test duration is mentioned clearly in the test report.

5 Test organisms and seawater

5.1 Spawning stock or mature bivalves

The mature bivalves used for gamete production can be obtained from the natural environment during reproductive periods as long as the sampling area is subject to little or no sources of contamination and free from epizootic diseases. The reproduction period along the European and African coasts depends on the site. In some places, it can occur all year.

For oysters, it is also possible to use mature animals from hatcheries where they have previously undergone a conditioning cycle so that they are ready for spawning as soon as they arrive in the laboratory. This enables ~~to perform~~ **conducting** tests throughout the year.

For transportation or storage purpose, mature oyster can stay out of water up to three days as long as they are kept in cool and damp conditions.

If the bivalves are received in the laboratory within three days after sampling, it is recommended to keep them dry at $15\text{ °C} \pm 2\text{ °C}$ until the collecting gametes or in seawater at a temperature close to their conditioning or rearing temperature (usually, 20 °C for hatchery oysters).

If the mature bivalves have to be kept for more than three days after sampling and/or dispatching, they shall be stored in water (see 5.2.2) at a temperature close to the original location and shall be provided with rich and appropriate feeding (see Reference [9]). In this case, the spawning stock is placed in tanks (15 individuals for 30 l of seawater). The water in the tanks is continuously aerated. On a daily basis, one third of the volume has to be discarded and replaced by the same volume of a single-species Prasinophycean culture (*Tetraselmis suecica*) at an average concentration of 1×10^6 cells per ml or diatom *Skeletonema costatum* at an average concentration of $2,7 \times 10^6$ cells per ml.

The pH of seawater should be between 7,0 and 8,5 for mussels and oysters.

Mussel *Mytilus edulis* and *M. galloprovincialis* basic characterization-identification should be done after organism sampling (e.g. biometry measurements or nuclear marker heterozygosity analyses).

NOTE Other species can be used, such as, *Phaeodactylum tricornutum*. Nevertheless, no information can be given regarding the suitable concentration of algae.

5.2 Reference seawater

5.2.1 General

This test requires good quality reference seawater. This water is used to prepare the controls and dilutions of the samples and/or chemicals to be tested. This seawater may be either natural or artificial.

5.2.2 Natural seawater

Natural seawater shall allow a good bivalve embryo-larval development and enable at least 80 % of the normal D-shaped larvae to be free of any abnormality. The test is sensitive to high concentrations of ammonia (NH₃). Consequently, the ammonium concentration of seawater used shall not exceed 100 µmol/l (= 1,8 mg/l).

As soon as the water is collected, it ~~is recommended that it is~~ should be checked to ensure that the water is not contaminated by any known human activity. The water should be pre-filtered with a 1 µm to 5 µm membrane. The seawater shall then be stored in the dark in controlled conditions between 2 °C and 15 °C and be used within two weeks from collection. Under no circumstance shall this seawater be frozen or autoclaved.

Just before use, adjust the seawater salinity, if necessary, by adding ultra-pure water (for dilution) or hypersaline brine (see 5.2.3.2.3) to reach the salinity range adapted to the selected species: i.e. from 20 PSU to 40 PSU for mussels and 25 PSU to 35 PSU for oysters. The salinity should be close to the original location. Then, filter it through a 0,45 µm membrane. Salinity shall then be checked with a suitable probe (see 6.6.6.6).

The direct addition of sea salts to the sample can be a source of toxicity and should be avoided (see Reference 17(17)).

5.2.3 Artificial seawater

Alternatively, artificial seawater prepared in ~~compliance~~ accordance with Table 1 Table 1 may be used. The composition of this seawater is similar to that suggested in Reference 36[36] without EDTA in order not to reduce the bio-availability of divalent metal ions, thus, resulting in a decrease in the apparent toxicity of these ions (see Reference 25[25]). Artificial seawater is prepared by adding reagent grade chemicals to ultrapure water (distilled or demineralized water) in the order specified in Table 1 Table 1. Prepare a minimum of 5 l of artificial seawater. Mix after each addition of salt to ensure a good dissolution.

Once ready, the artificial seawater is filtered through a 0,45 µm membrane (see 5.2.1 5.2.1).

Table 1 — Composition of artificial seawater for one litre of ultra-pure water

Chemical composition	Concentration in ultrapure water g/l
NaF	0,003
SrCl ₂ ·6H ₂ O	0,02
H ₃ BO ₃	0,03
KBr	0,1
KCl	0,7
CaCl ₂ ·2H ₂ O	1,47
Na ₂ SO ₄	4,0
MgCl ₂ ·6H ₂ O	10,78
NaCl	23,5

ISO/FDIS 17244:2025(en)

Chemical composition	Concentration in ultrapure water g/l
$\text{Na}_2\text{SiO}_3 \cdot \text{H}_2\text{O}$ ^a	0,2
NaHCO_3	0,2
^a Silicate is not needed when the water is prepared in a glass vial.	

Artificial seawater which only contains mineral salts can be kept for up to one year in a watertight container that is kept out of the light in a clean, dry and odourless place between 2 °C and 15 °C.

5.2.4 Hypersaline brine

Hypersaline brine (HSB) can be made by concentrating ~~of~~ natural seawater by freezing or evaporation. The maximum salinity of brine prepared this way is around 100 % (~~USEPA~~^a method ~~is provided in~~ Reference [33]~~[33]~~).

Hypersaline brine can also be prepared following the Zarogian's formula concentrated up to 5× maximum.

Commercial sea salts may also be used to prepare HSB, but a test with the reference substance shall be conducted to assess the absence of complexing agents.

A control test with the HSB diluted to an acceptable salinity for the embryo development has to be realized to check the lack of effect of this preparation.

6 Equipment

Usual laboratory equipment and in particular, the following.

6.1 ~~6.1~~ Thermoregulated room or enclosure for the incubations.

6.2 ~~6.2~~ Microscope, at least 200×, but preferably 400×.

If possible, use an inverse light microscope so that observations can be made directly in the small experiment vials (e.g. microplate wells).

6.3 ~~6.3~~ Culture flasks, capacity from a fraction of millilitres to several litres.

Experiments are usually performed using a volume of test solution of 50 ml. Therefore, culture flasks with the capacity of 100 ml to 200 ml are preferred. Culture flasks may be made of glass (systematically washed and sterilized) or single-use crystal polystyrene such as multi-well plates or medical sampling containers.

6.4 ~~6.4~~ Cartridge or membrane-based filtering device, equipped with filters and pre-filters suitable for the preparation of the test media.

6.5 ~~6.5~~ Oven or autoclave, for sterilizing the equipment and glassware for the bioassay.

6.6 ~~6.6~~ Equipment for measuring temperature, salinity, pH₇ and dissolved oxygen in water.

6.7 ~~6.7~~ Sieves, with mesh size of 32 µm and 100 µm for the filtering of male and female gametes, respectively.

The gametes which pass through the appropriate sieves are collected for the fertilization step.

6.8 ~~6.8~~ Pipettes, single-use polyethylene transfer pipettes (1 ml to 3 ml).