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

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



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# Foreword

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT), see the following URL: Foreword — Supplementary Information.

The committee responsible for this document is ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.



 

# 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 biotests (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 biotests. This has been the case since the first research undertaken by Lillies (1921) (see Reference [18]) on the sea urchin *Arbacia* and by Prytherch (1924) (see Reference [21]) on the oyster *Crassostrea virginica*. The embryos and larvae are less tolerant 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, Woelke (see Reference [35]) has recommended the use of the Pacific oyster, *Crassostrea gigas*, to assess the quality of seawater. 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 biotests.

The results of these biotests demonstrate the necessity to determine the potential toxicity thresholds of chemicals which could 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. Quiniou et al. (1993, 1997) (see References [27] and [26]) and His *et. al* (1999) (see Reference [11]) defined potential toxicity on the basis of teratological effects.

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

The toxicity assessment of metals performed on *C. 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 by Beiras and His (1994) (see Reference [4]), who compared the findings of four embryo-larval tests: *M. edulis*, *M. galloprovincialis*, *C. gigas,* and *C. virginica.* Another study showed that the embryos of *C. 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 ranges acceptable to bivalves make them easy to use in ecotoxicity studies, particularly when assessing the quality of coastal and estuarine environments (see Reference [11]).



 

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**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 in accordance with this International Standard be carried out by suitably qualified staff.**

## 1 Scope

This International Standard specifies a method for determining the effects of chemical and aqueous samples on the embryo-larval development of marine bivalves. It 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 and 40 for mussels and between 25 and 35 for oysters. This method applies to

— chemical substances and preparations,

— marine and brackish waters,

— streams and aqueous effluents (urban, agricultural, industrial effluents, etc.) as long as the salinity is adjusted and/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.

## 2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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 14442, *Water quality — Guidelines for algal growth inhibition tests with poorly soluble materials, volatile compounds, metals and waste water*

## 3 Terms and definitions

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

**3.1**
**$EC_x$**
calculated concentration (of a substance) or dilution (of an aqueous sample, in %) for which an effect of $x$ % is expected compared to the control



 