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Ships and marine technology — Bioassay methods for screening anti-fouling paints —

Part 3: Mussels

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Foreword

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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 documents 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).

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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 8, *Ships and marine technology*, Subcommittee SC 2, *Marine environment protection*, in collaboration with Technical Committee ISO/TC 35, *Paints and varnishes*, Subcommittee SC 9, *General test methods for paints and varnishes*.

A list of all parts in the ISO 21716 series can be found on the ISO website.

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

Anti-fouling paints that contain biocides are widely used to prevent fouling of ship hulls by marine organisms. Effective anti-fouling technologies are critical for maintaining fuel consumption efficiency of ships and also for minimizing possible translocation of aquatic species through maritime trade. The evaluation of anti-fouling paints is generally undertaken by adopting a tiered approach whereby paint manufacturers use a battery of laboratory, raft, patch tests and full vessel trials. Raft, patch tests and full vessel trials are generally conducted over extended periods of time and are predominantly relied upon for the prediction of coating performance when used commercially on in-service ships.

The results of raft, patch test and full vessel trials (field testing) can be used as part of the regulatory process for pesticidal or biocidal products in certain countries in order to demonstrate the efficacy of an anti-fouling paint. Laboratory testing alone is recognized as being unable to predict in-service performance or efficacy. For example, guidance published by the European Chemicals Agency (ECHA) on the assessment and evaluation of efficacy for anti-fouling products states clearly that laboratory testing of individual anti-fouling paints is not undertaken as it is not considered to be a realistic evaluation of the product; field testing, which permits anti-fouling products to be tested under similar operating conditions and stresses as those encountered when the antifouling products are in service is routinely undertaken instead (see Reference [35]).

Whilst laboratory tests are unable to reliably predict in-service coating performance, they have merit in the screening of experimental coatings for further evaluation during the research and development process.

Reproducible objective data obtained by following standardized screening methods, independent of the test location or the season, can be a useful tool to support the selection of anti-fouling paints for higher tier testing, e.g., raft or ship tests. ISO 21716 provides a compilation and description of *in vitro* bioassay methods intended to aid the process of screening anti-fouling paints prior to higher tier raft or ship tests. Toxicological screening methods included in each part of ISO 21716 can be used for such purposes as early decision-making in research and product development, rapid feedback on potential toxicological concerns, or for the preliminary assessment of anti-fouling paints. For instance, ISO 21716 provides information on methods that can be used to screen anti-fouling paints in order to determine whether to continue development of an experimental paint and/or a product that contains a particular ingredient, or to determine whether to take on the cost of performing the remaining tiers within a complete tiered-testing strategy.

ISO 21716 provides screening bioassays related to certain common genera of fouling organisms, namely barnacles, mussels and algae. These screening tests are relatively simple and rapid laboratory tests that can be performed to provide an indication of the toxicity of a painted surface towards selected test organisms. The screening tests described in each part of ISO 21716 can be used as part of a tiered approach to predict the ability of an anti-fouling paint to prevent fouling on ships. Alternatively, to prevent the translocation of invasive marine species by progressively involving subsequent semi-field (e.g. raft panels) and field testing (e.g. ship trials). On their own, the screening tests described in each part of ISO 21716 do not reliably predict the ability of an anti-fouling paint to prevent fouling on ships or the translocation of invasive marine species.

ISO 21716 is not intended to provide a list of validated tests for testing the efficacy of anti-fouling paints; this can be covered in regulations. It is not intended to provide a list of validated tests for this purpose, nor for predicting the ability of a fouling control paint to prevent fouling on ships or to prevent the translocation of invasive marine species.

Mussels are typical marine sessile organisms regarded as harmful fouling organisms because of the impact on fuel consumption and the potential for translocation of non-indigenous species if they become attached to ship hulls.

This test method utilizes young mussels to assess settling behaviour in the presence of treated panels. Young mussels are used because they have higher byssus threads production activity as compared to the adults. More information is provided in Annex B.

Ships and marine technology — Bioassay methods for screening anti-fouling paints —

Part 3: Mussels

1 Scope

This document specifies a laboratory test method for screening anti-fouling paints in a flow-through system using mussels as the test organism. It is intended to be used in conjunction with ISO 21716-1, which specifies the general requirements. The purpose of the test is to determine if there is a difference in mussel settlement on painted test panels compared with mussel settlement on inert non-toxic control panels under the conditions of the test. Examples of statistical analysis to determine if the difference in mussel settlement is statistically significant are given in Annex A.

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 21716-1:2020, *Ships and marine technology — Bioassay methods for screening anti-fouling paints — Part 1: General requirements*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 21716-1 and the following apply.

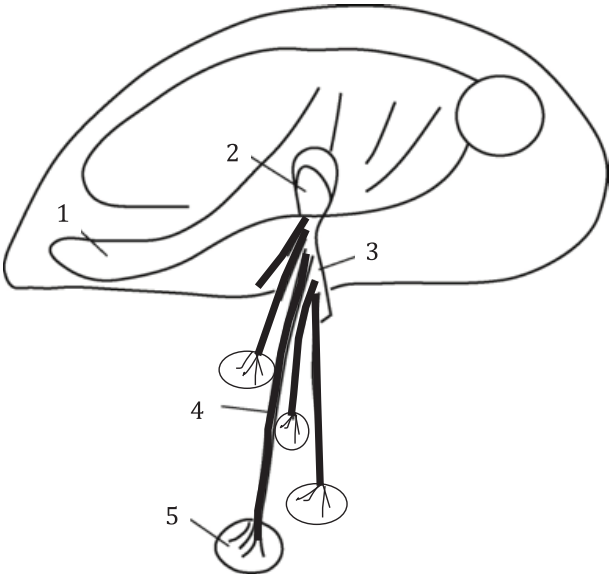
ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp;

— IEC Electropedia: available at http://www.electropedia.org/.

3.1
byssus
attachment organ secreted by a mussel, consisting of stem, byssus threads and adhesive discs

Note 1 to entry: See Figure 1.



Key

1 foot
2 root (in byssus gland)
3 stem
4 byssus thread
5 adhesive disc (or plaque)

Figure 1 — Attachment organ of a mussel

3.2
shell length
longest linear distance between two points on the outside edge of the shell of a mussel

3.3
purified water
water with an electric conductivity of 2 µS/cm or less, prepared by distillation and/or treatment with ion exchange resin(s)

4 Principle

The test procedure consists of the following 5 sequential steps, summarized in Figure 2:

— preparation of the test organism and the test seawater;

— preparation of the test panel and control panel;

— operation of the test;

— validation of the test; and

— data treatment and interpretation of the results.

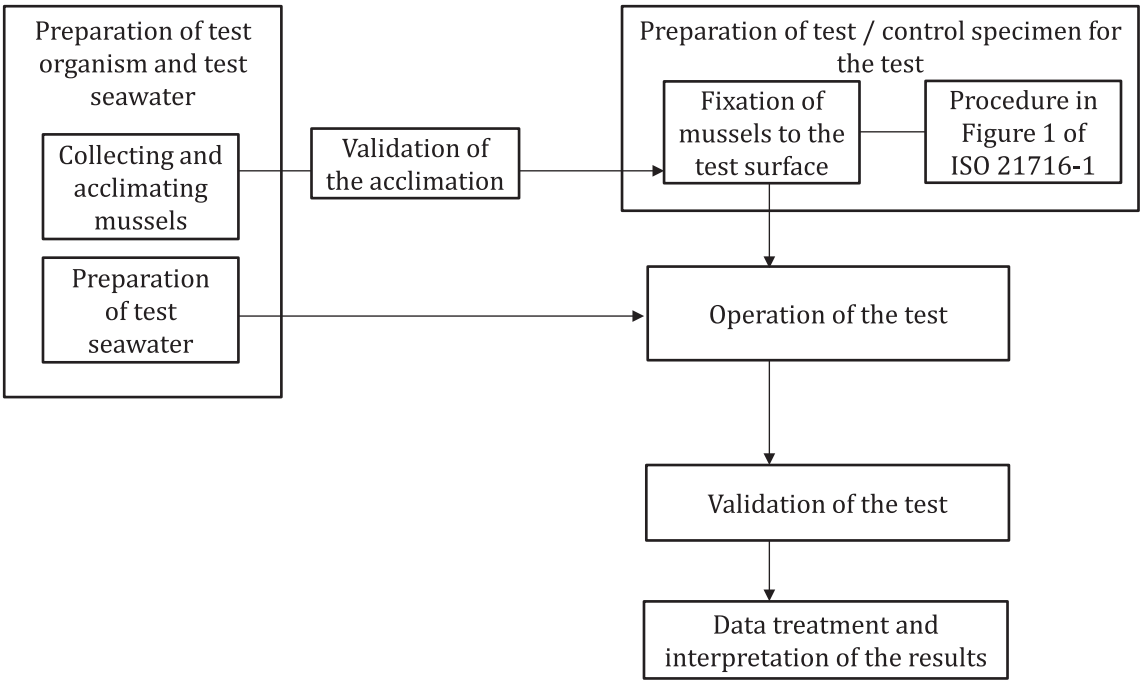


Figure 2 — Schema of the test procedure

Each bioassay shall consist of three runs at a minimum. Each run shall consist of a test group of three or more test panels and a control group of three or more control panels. Provided that the validation criteria are met, then the number of byssus threads for the test and control groups can be compared.

5 Material and apparatus

The items listed in Tables 1 and 2 shall be used for the test.

Table 1 — List of material used

Material	Remarks
Adhesive of surgical grade	E.g., cyano-acrylate adhesive
Control panels	50 mm square of PVC is recommended.
Cultured stock of live young mussels	At least 30 live mussels, *Mytilus galloprovincialis sp.*, with a shell length of 8 mm to 10 mm, that are attached to natural or artificial substrates. Other mussel species may be used if *Mytilus galloprovincialis sp.* mussels are not available.
Natural seawater	Defined in ISO 21716-1:2020, 3.8
Purified water	Defined in 3.3
Scissors	
Small piece of paper	Filter paper may be used as spacer.
Test panels	Specified in ISO 21716-1:2020, 4.2. 50 mm square is recommended.
1 µm filters	Used to prepare test seawater.

Table 2 — List of apparatus used

Apparatus	Remarks
Light	White fluorescence or LED
Light intensity meter	Accuracy: ±10 lx

Table 2 *(continued)*

Apparatus	Remarks
pH meter	Accuracy: ±0,1
Salinometer	Accuracy: ±0,1
Stereo microscope	Magnification: 5-30X with fiber light
Thermometer	Accuracy: ±0,1 °C
Water flow-through system	Specified in ISO 21716-1:2020, 5.2, with a means of maintaining the test seawater tank at 20 °C ± 1 °C and alternately illuminating the test seawater tank with a light intensity of 3 000 lx [Clause 8, d), light conditions] and with a light intensity of <5 lx [see Clause 8, d), dark conditions].

6 Preparation of the test organism and the test seawater

6.1 General

The cultured stock of live mussels is used to perform the bioassay test in sea water.

6.2 Preparation of the test organism

Live mussels are generally prepared by collecting wild mussels and acclimatizing them in the laboratory prior to testing. Guidance on this process and on storing mussels can be found in Annex B.

Information on the life cycle of mussels can also be found in Annex B, and information on the identification of *M. galloprovincialis sp.* mussels can be found in Annex C.

6.3 Preparation of test seawater

Pass natural seawater through a 1 µm filter unit and adjust to salinity 30,0 ± 0,5 using purified water.

7 Preparation of the test panels and control panels

7.1 General

The same test and control groups shall be used throughout the whole test.

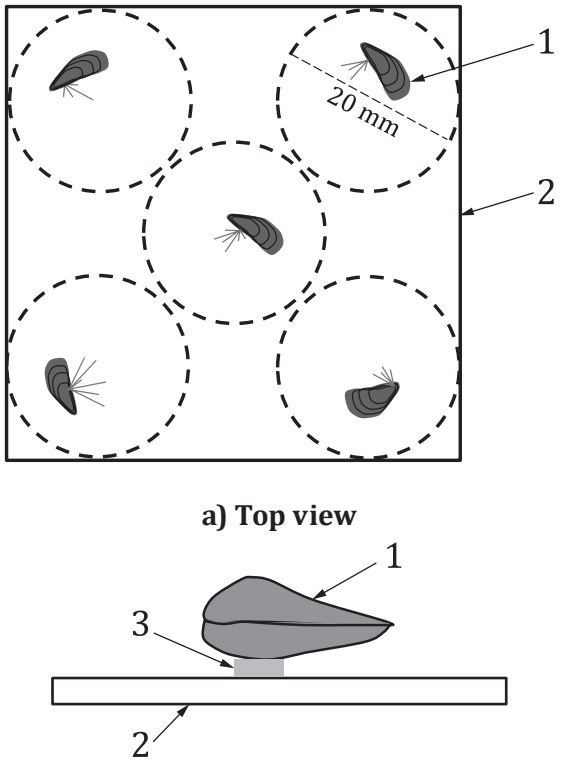
7.2 Preparation of the test panels

Test panels shall be prepared following the specifications described in ISO 21716-1:2020, Clause 4.

7.3 Affixing of mussels to the test panels and control panels

Separate the live mussels from their substrate by cutting the byssus threads with scissors, taking care to avoid damage to other tissue and organs. Affix five mussels to each test with the adhesive, using filter paper as a spacer between the shell of the mussel and the surface of the panel, providing a 20 mm diameter circular separation zone around each mussel [see Figure 3 a)].

NOTE A spacer of adhesive-infiltrated filter paper can be used to prevent excessive spreading of the adhesive and improve adhesion of the mussel to the test and control panels [see Figure 3 b)].



a) Top view

b) Side view

Key

1 mussel

2 test/ control panel

3 spacer of adhesive-infiltrated filter paper

Figure 3 — The mussels on the test or control panel

8 Operation of the test

The bioassay shall be simultaneously performed on the test group and the control group as follows (see Figure 4).

The experimental system specified in ISO 21716-1 shall be used for the test. The system is equipped with the devices that maintain the specified water temperature and light irradiation of the test.

a) Fill the test seawater tank with test seawater and provide a continuous flow of test seawater from the seawater storage tank. Maintain the temperature of the test seawater at 20 °C ± 1 °C for the duration of the test. The flow rate should be set to achieve at least about 0,8 turnover per hour of the water of test seawater tank.

NOTE If the flow rate is too low, the result can be affected by the concentration of biocide in seawater of the test seawater tank.

b) Place the test and control panels in the test seawater tanks, ensuring all the panels are fully immersed in the test seawater.

c) Measure and record the temperature, pH and salinity. Measure and record those parameters again after 24 h from the beginning of the test.

d) Illuminate the test seawater tank for 12 h with a light intensity of 3 000 lx, and then leave the test seawater tank in darkness for 12 h.