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

**Part 1:
General requirements**

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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.

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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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 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 to maintaining the fuel consumption efficiency of ships, minimizing the release of greenhouse gases and other hazardous air pollutants, and also for minimizing the 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 of 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 anti-fouling products are in service is routinely undertaken instead (see Reference [6]).

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.

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

Part 1: General requirements

1 Scope

This document specifies general requirements and common specifications for preparing and aging panels coated with anti-fouling paint to perform laboratory bioassay screening tests against specified organisms. Such tests are given in the other parts of the ISO 21716 series, with which this document is intended to be used.

This document is applicable to all anti-fouling paints that prevent or deter the attachment and growth of sessile organisms on a surface through chemical or biological means.

It is not applicable to the following:

- coatings that deter or prevent fouling solely by physical means such as biocide-free foul release paints;
- anti-fouling methods used for controlling harmful marine organisms and pathogenic organisms in ships' ballast water and sediments according to IMO International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004^[2].

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 2808, *Paints and varnishes — Determination of film thickness*

ISO 1513, *Paints and varnishes — Examination and preparation of test samples*

ISO 21716-2, *Ships and marine technology — Bioassay methods for screening anti-fouling paints — Part 2: Barnacles*

ISO 21716-3, *Ships and marine technology — Bioassay methods for screening anti-fouling paints — Part 3: Mussels*

3 Terms and definitions

For the purposes of this document, the following terms and definitions 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
aging**

providing the *test group* (3.9) with a surface condition that is more representative of the expected in-service surface condition of an *anti-fouling paint* (3.2) than a freshly applied paint surface by exposure to dynamic and/or static immersion in *natural seawater* (3.8) or *artificial seawater* (3.3)

**3.2
anti-fouling paint**

paint containing *biocides* (3.4) specifically designed to prevent or deter the attachment and growth of sessile organisms on a hull surface through chemical or biological means

**3.3
artificial seawater**

mixture of water and salts to mimic the properties of natural seawater

Note 1 to entry: Water prepared from the mixtures of salts described in ISO 15181-1, ISO 10253, ISO/TS 20787, or ASTM D 1141-98, and water prepared from commercially available proprietary artificial seawater salt mixtures have been found to be generally available.

**3.4
biocide**

substance having general or specific action such as mortality, growth inhibition, or repellancy, on sessile organisms, used in *anti-fouling paints* (3.2), for the prevention of attachment of sessile organisms

Note 1 to entry: Also called biocidally active substance, active ingredient or anti-foulant.

**3.5
biofilm**

layer of microscopic organisms such as bacteria, diatoms and the slimy extracellular polymeric substances they produce on the *test surface* (3.12)

**3.6
control group**

replicate *control panels* (3.7) prepared with the same specification and under the same conditions for confirmation of reproducibility of the test

**3.7
control panel**

panel that has no intended or expected ability to prevent or deter the attachment and growth of sessile organisms on its surface by chemical, biological or physical means

Note 1 to entry: An uncoated polyvinyl chloride or other inert plastic plate, or a panel coated only with anti-corrosive paint(s) has been found to be generally suitable.

**3.8
natural seawater**

water taken from the sea of sufficient quality to rear *test organisms* (3.10)

**3.9
test group**

replicate *test panels* (3.11) prepared with the same specifications and under the same conditions for confirmation of reproducibility of the test

**3.10
test organism**

marine organism used in the bioassay

**3.11
test panel**

panel coated with the *anti-fouling paint* (3.2) to be tested in the bioassay

3.12 test surface

surface of a *test panel* (3.11) or *control panel* (3.7) to be investigated in the test

4 Preparation of the test panels

4.1 Test procedure — General

The test procedure consists of the following steps, as shown in [Figure 1](#):

- selecting a material for substrates;
- applying the anti-fouling paint to replicate test panels;
- aging the panels in test seawater, as specified in each part of the ISO 21716 series, and;
- pre-treating the panels prior to bioassay testing.

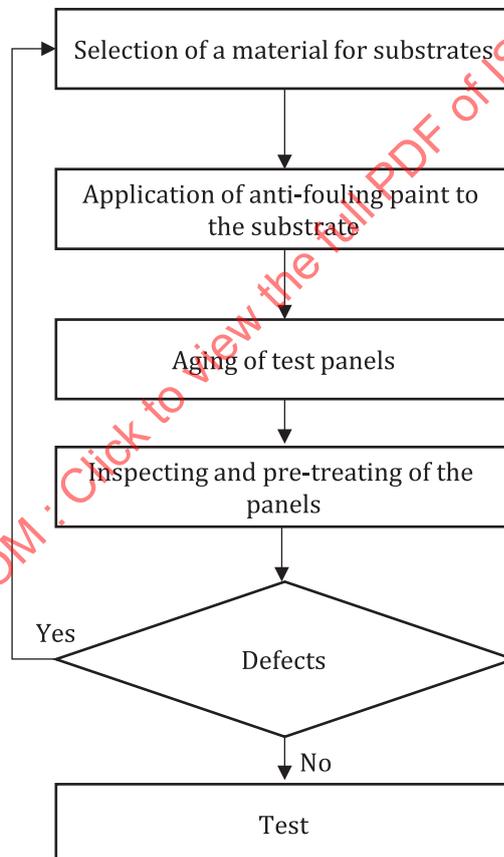


Figure 1 — Test procedure for preparing the test panels

4.2 Selection of a material for substrates

The material for the substrates of the test panels shall be non-conductive and suitable for immersion in seawater. The size of the test surface shall meet the requirements specified in the relevant part of the ISO 21716 series.

4.3 Application of an anti-fouling paint

A test group of panels shall be prepared by applying the anti-fouling paint to the test panels in accordance with the manufacturer's recommendations for surface preparation, painting and drying.

Prior to application, the anti-fouling paint shall be assessed and prepared in accordance with ISO 1513.

The number of test panels in the test group shall meet the requirements specified in the relevant part of the ISO 21716 series. The intended dry film thickness shall be selected to be representative of the dry film thickness that would be expected to be typically specified for commercial use of the paint. At a minimum, the thickness and drying condition should be as recommended on the product data sheet or other material provided by the manufacturer. The dry film thickness of the coating on each test panel shall be measured in accordance with ISO 2808.

NOTE Typical application methods include brush, roller, air-assisted or airless spray.

4.4 Aging of the test panels

The purpose of aging is to provide a test group with a surface condition that is more representative of the expected in-service surface condition of an anti-fouling paint than a freshly applied paint surface. The test group shall be aged by exposure to seawater while the control group consisting of control panels does not go through aging.

The user shall choose the condition and duration of aging that is appropriate for the screening test. For example, the test group may be aged by exposure to dynamic and/or static immersion in natural seawater or artificial seawater for 21 days or longer to reflect different operational patterns of a vessel. Dynamic or combined dynamic and static aging conditions are recommended as they generally provide test panels with a surface condition that is more representative of the expected in-service surface more quickly than aging under solely static conditions. Either artificial seawater or natural seawater may be used for aging the test panels; temperature, pH and salinity of the seawater in the aging tank and other factors that can affect the aging should be controlled during aging.

[Annex A](#) provides examples of suitable aging conditions. National standards and any other documents can be referred to.

4.5 Inspecting and pre-treating of the panels

The surface of the test panels shall be visually inspected after aging to confirm that no defects of the coated films (e.g. crack, holiday, delamination, insufficient coating film thickness and swelling) are present. Any test panels where defects are found on the coated film shall not be used for the test. Any biofilm observed to be present on any panel after aging should be carefully removed from the surface test panel by gently wiping with a cotton swab, so as not to apply pressure to the surface of the test panels. Any test panels that exhibit any damage resulting from wiping of coating (e.g. abrasion) shall not be used for the test. The bioassay screening test shall be started as soon as practicable after completion of the aging phase. Where immediate testing is not possible, care should be taken to prevent test panels from drying out.

5 Experimental system

5.1 General

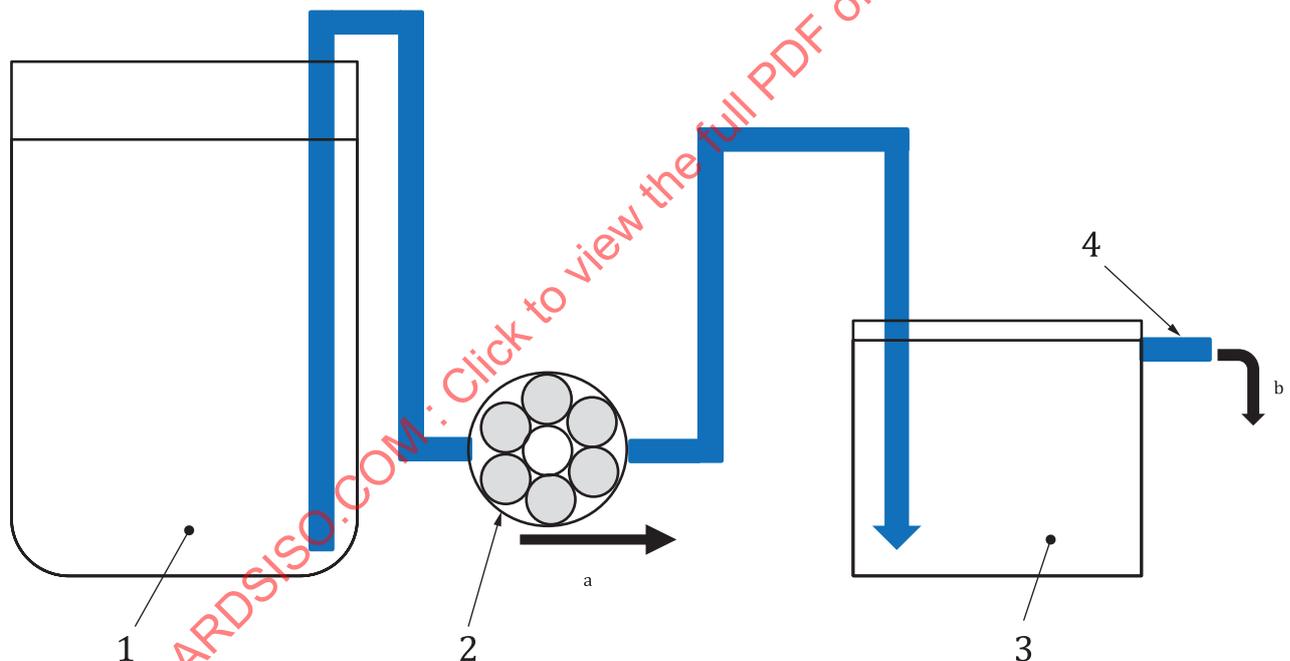
A number of bioassays using marine organisms have been reported in the scientific literature, which mostly employ testing under closed or isolated experimental conditions. When testing anti-fouling paints, biocides leach out from the painted surface into the surrounding water under closed or isolated experimental conditions where the leached biocides accumulate in the test seawater of the system. As a result, the concentration of the biocides may increase to a level where the biocides, or their degradates or metabolites, affect the behaviour of the test organism, thereby making it difficult to appropriately assess the efficacy of the coating surface. In order to avoid the possible effects of an

excessive concentration of biocides in test seawater, the experimental system shall be equipped with a water flow-through system to continuously replenish the test seawater in the test seawater tank.

5.2 Water flow-through equipment

A typical continuous flow-through system is schematically drawn in [Figure 2](#). The water flow-through system shall meet the requirements of the relevant parts of the ISO 21716 series, and should be designed to mitigate the risk that the concentration of biocide in the water exceeds the level that would be expected to have an observable biological effect on the test organism. In addition, the system shall be constructed of materials that ensure there is no significant effect on the nominal concentration of active ingredients in the water. The size of the test seawater tank shall be large enough to completely submerge the test surface. The system shall consist of the following components:

- a seawater storage tank for storing abundant test seawater;
- test seawater tanks made of inert material, e.g. polycarbonate, glass;
- a pump, typically a constant volume pump, for providing a water flow; and
- a means for maintaining a constant water level in the test seawater tank, e.g. a siphon, a peristaltic pump or spout.



Key

- 1 seawater storage tank
- 2 peristaltic pump
- 3 test seawater tank
- 4 siphon or spout
- a Direction of water through the pump.
- b Direction of water flow.

Figure 2 — Typical continuous water flow-through system

Annex A (informative)

Aging methods — Examples

A.1 Introduction

The properties of the coating surface of anti-fouling paints often change significantly after application to ship hulls, particularly at the initial stage following entry of the ship into service.

The purpose of aging is to provide a test group whose surface condition is more representative of the expected in-service surface condition of an anti-fouling paint applied to a vessel.

A.2 Dynamic aging

Dynamic aging is an aging method in which panels are exposed to natural seawater or artificial seawater under continuous flow conditions. For example, a suitable dynamic aging system consists of a water tank (ca. 230 l) installed with an apparatus to hold the test panels, a thermostat to keep the water temperature constant (typically $20\text{ °C} \pm 3\text{ °C}$), a controller and a primary storage tank (ca. 300 l), a pump (typical flow-rate: ca. 0,7 l/min), as shown in Figure A.1. The water tank for dynamic aging has a concentric cylindrical apparatus inside it. The apparatus is a polyhedral section cylinder (e.g. 14 polygon section) to hold the test panels for laboratory experiments. Test panels are randomly arranged on polyhedral section cylinder to exclude the differences emerged from partial settling of biofilm growth.

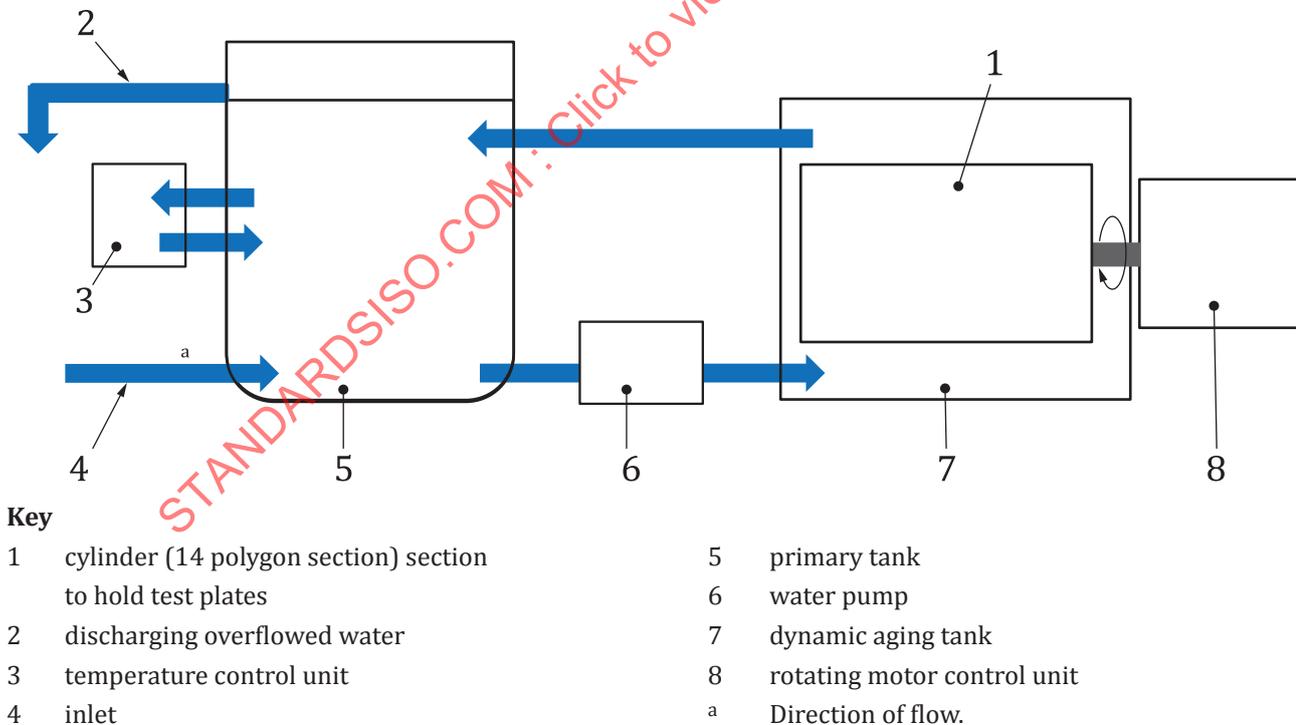


Figure A.1 — Schematic diagram of a dynamic aging system

During the aging process, the cylinder apparatus holding the test panels is rotated by a motor under the conditions that a user wants to test. For example, when the vessel speed and travel distance are expected to be 10 kn (knots) and around 20 000 km respectively, the test panels should be rotated at