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

**Part 3:  
Mussels**



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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](http://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](http://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](http://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](http://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](#).

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# **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](#).