



BSI Standards Publication

**Nanotechnologies - Aquatic toxicity assessment
of manufactured nanomaterials in saltwater
lakes using *Artemia sp.* Nauplii**

National foreword

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**Nanotechnologies - Aquatic toxicity
assessment of manufactured
nanomaterials in saltwater lakes using
Artemia sp. Nauplii**

*Nanotechnologies - Evaluation de la toxicité des nanomatériaux en
milieu aquatique par des Artemia sp*



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Contents

Page

Foreword	v
Introduction	vi
1 Scope	1
2 Normative references	1
3 Terms and definitions	2
4 Materials	5
4.1 Test organism.....	5
4.2 Chemicals.....	5
5 Technical equipment	5
5.1 Adequate apparatus for temperature control.....	5
5.2 Microscope.....	5
5.3 Binocular stereoscope.....	5
5.4 Centrifuge.....	5
5.5 Air pump.....	5
5.6 Single channel pipettes.....	5
5.7 Laboratory balance.....	5
5.8 Laboratory water purification system.....	6
5.9 Laboratory oven.....	6
5.10 Laboratory autoclave.....	6
5.11 Centrifuge.....	6
5.12 Sonication system.....	6
5.13 Hot plate stirrer.....	6
5.14 Oxygen meter.....	6
5.15 Thermometer.....	6
5.16 pH meter.....	6
5.17 Salt meter (salinity meter).....	6
5.18 Multiparameter photometer.....	6
5.19 Light source.....	6
5.20 Adequate apparatus for the control of the lighting regime and measurement of light intensity (lux meter).....	6
5.21 Equipment for the determination of total organic carbon.....	6
5.22 Equipment for the determination of chemical oxygen demand (COD).....	6
6 Preparation and characterization of dispersion of nanomaterial	6
6.1 Dispersion preparation.....	6
6.2 Dispersion characterization.....	7
6.3 Dispersion stability in stock suspension.....	7
6.4 Dispersion stability in artificial seawater.....	7
6.5 Preparation of exposure media for toxicity tests.....	7
7 Hatching procedure	7
7.1 General.....	7
7.2 Dilution water.....	7
7.3 Storage of cysts.....	8
7.4 Disinfection of <i>Artemia sp.</i> cysts.....	8
7.5 Hatching method of <i>Artemia sp.</i> cysts.....	8
7.6 Harvesting of nauplii.....	8
7.7 Calculation of hatching percentage.....	9
8 Effect of nanomaterial on <i>Artemia sp.</i> nauplii	9
8.1 Test groups and controls.....	9
8.2 Test concentrations.....	9
8.3 Exposure condition.....	10
8.4 Duration.....	10

8.5	Observations	10
8.6	Analytical measurements	10
9	Data analysis	10
10	Test report	10
10.1	Test procedure	10
10.2	Information to include in the report	11
10.2.1	Test nanomaterial	11
10.2.2	Test species	11
10.2.3	Test conditions	11
10.2.4	Bioassay results	11
11	Results validity	11
	Bibliography	13

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).

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For an explanation on 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 the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

Introduction

With the increasing development and use of manufactured nanomaterials (MNMs) in consumer and other products, concern about the possible impact of MNMs on human and environmental health is increasing. Various aquatic organisms (such as fish, daphnia, algae, etc.) are currently used to predict the possible adverse effects of chemicals, including nanomaterials, on the aquatic environment. Brine shrimp (*Artemia sp.*) are found nearly worldwide in saline lakes and pools,^[42] and are one of the most widespread euryhaline organisms that are suitable for ecotoxicity testing. *Artemia sp.* nauplii can be used to assess the effects of nanomaterials in salt water ecosystems, primarily salt lakes. *Artemia sp.* usually live in salt lakes, and are almost never found in an open sea. This species also adapts to a wide range of salinities (5 g/L to 300 g/L) and temperatures (6 °C to 40 °C). In fact, the physiologically optimal levels of salinity for *Artemia sp.* are about 30 g/L to 35 g/L. Due to predators at these salt levels, however, *Artemia sp.* seldom occur in natural habitats at salinities of less than 45 g/L to 80 g/L. Favoured for the absence of predators and food competitors in such places, *Artemia sp.* develop very dense populations.

There are several advantages to using *Artemia sp.* as a biological model in salt water aquatic toxicology:

- a) Less concern about animal welfare than for a vertebrate species;
- b) There is good knowledge of *Artemia sp.* biology and ecology;
- c) *Artemia sp.* have a wide geographic distribution in salt water lakes and pools;
- d) Tests performed on *Artemia sp.* nauplii are simple and cost-effective;
- e) Small body size allows accommodation of *Artemia sp.* nauplii in small beakers or plates;
- f) *Artemia sp.* adapt to a wide range of water salinity and temperature;
- g) *Artemia sp.* are simple to maintain in the laboratory;
- h) The life cycle of *Artemia sp.* is short, so it is suitable for growth, reproduction and short-term toxicity tests;
- i) *Artemia sp.* cysts are commercially and readily available so that the tests can be carried out worldwide. The cysts can be stored for years under cool and dry conditions without losing viability. Upon immersion in sea water, the free swimming nauplii will hatch within approximately 24 h;
- j) Hatching from cysts gives organisms of similar age, genotype and physiological condition.

In recent years, several researchers around the world have used *Artemia sp.* as a test organism in aquatic nanotoxicology (see References [1] to [35]). The lack of a standardized protocol for testing *Artemia sp.* for aquatic toxicity means that data from these studies are more likely to be non-repeatable and non-reliable.^[22] The goal of this document is to provide a standard protocol intended to generate reliable aquatic toxicity data by testing *Artemia sp.*, which can be used for ecotoxicity evaluation of MNMs in salt water lake ecosystems.

Nanotechnologies - Aquatic toxicity assessment of manufactured nanomaterials in saltwater lakes using *Artemia sp. Nauplii*

1 Scope

This document specifies a test method, aiming to maximize repeatability and reliability of testing, to determine whether MNMs are toxic to aquatic organisms, specifically *Artemia sp. nauplius*.

This document is intended to be used by ecotoxicological laboratories that are capable in the hatching and culturing of *Artemia sp.* and the evaluation of toxicity of nanomaterials using *Artemia sp. nauplius*.

This method uses *Artemia sp. nauplii* in a simulated environment, artificial seawater, to assess effects of nanomaterials.

This document is applicable to MNMs that consist of nano-objects such as nanoparticles, nanopowders, nanofibres, nanotubes, nanowires, as well as aggregates and agglomerates of such MNMs.

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 10993-12, *Biological evaluation of medical devices — Part 12: Sample preparation and reference materials*

ISO/TS 11931, *Nanotechnologies — Nanoscale calcium carbonate in powder form — Characteristics and measurement*

ISO/TS 12805, *Nanotechnologies — Materials specifications — Guidance on specifying nano-objects*

ISO/TR 13014, *Nanotechnologies — Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment*

ISO 15088, *Water quality — Determination of the acute toxicity of waste water to zebrafish eggs (*Danio rerio*)*

ISO/TS 16195, *Nanotechnologies — Guidance for developing representative test materials consisting of nano-objects in dry powder form*

ISO/TS 17200, *Nanotechnology — Nanoparticles in powder form — Characteristics and measurements*

ISO 26824, *Particle characterization of particulate systems — Vocabulary*

ISO/TS 80004-1, *Nanotechnologies — Vocabulary — Part 1: Core terms*

ISO/TS 80004-2, *Nanotechnologies — Vocabulary — Part 2: Nano-objects*

ISO/TS 80004-4, *Nanotechnologies — Vocabulary — Part 4: Nanostructured materials*