PD ISO/TS 20787:2017

BSI Standards Publication

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

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National foreword

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The UK participation in its preparation was entrusted to Technical Committee NTI/1, Nanotechnologies.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Published by BSI Standards Limited 2018

ISBN 978 0 580 92430 9

ICS 07.120

Compliance with a British Standard cannot confer immunity from legal obligations.

This Published Document was published under the authority of the Standards Policy and Strategy Committee on 31 January 2018.

Amendments/corrigenda issued since publication

Date	Text affected

PD ISO/TS 20787:2017

TECHNICAL SPECIFICATION

ISO/TS 20787

First edition
2017-11-23

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

Reference number
ISO/TS 20787:2017(E)

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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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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.

TECHNICAL SPECIFICATION **ISO/TS 20787:2017(E)**

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*

