TECHNICAL SPECIFICATION

ISO/TS 20787

First edition
2017-11

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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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*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 10993-12, ISO/TS 11931, ISO/TS 12805, ISO 15088, ISO/TS 16195, ISO/TS 17200, ISO 26824, ISO/TS 80004-1, ISO/TS 80004-2 and ISO/TS 80004-4 and the following apply.

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

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

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

3.1
agglomerate
Note 1 to entry: collection of weakly or medium strongly bound particles where the resulting external surface area is similar to the sum of the surface areas of the individual components

Note 2 to entry: The forces holding agglomerates together are weak forces, for example van der Waals forces, or simple physical entanglement.

Note 3 to entry: Agglomerates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: ISO/TS 80004-2:2015, 3.4]

3.2
aggregate
particle comprising strongly bonded or fused particles where the resulting external surface area is significantly smaller than the sum of surface areas of the individual components

Note 1 to entry: The forces holding an aggregate together are strong forces, for example covalent or ionic bonds, or those resulting from sintering or complex physical entanglement, or otherwise combined former primary particles.

Note 2 to entry: Aggregates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: ISO/TS 80004-2:2015, 3.5]

3.3
hatching vessel
vessel appropriate for *Artemia sp*. cyst hatching

Note 1 to entry: Cone should be transparent or semi-translucent (preferably colourless) for ease of harvesting and light transmission.

Note 2 to entry: As shown in Figure 2, constant aeration from the bottom of the hatching vessel should be used to keep cysts in suspension, and to provide sufficient oxygen levels for the cysts to hatch.

Note 3 to entry: Hatching vessels include glass or plastic cone or “V”–bottomed container as shown in Figure 1.

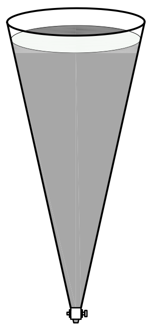

Figure 1 — Schematic of appropriate hatching vessel for *Artemia sp*

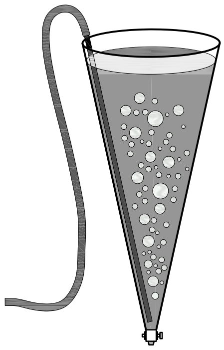

Figure 2 — Schematic of aeration from the bottom of a hatching vessel for *Artemia sp*

3.4
test vessel
vessel appropriate for *Artemia sp*. culture

Note 1 to entry: Test vessels and other apparatus that will come into contact with the test solutions should be made entirely of glass or other chemically inert material.

Note 2 to entry: Test vessels include flasks or beakers.

3.5
positive control
well-characterized material and/or substance, which, when evaluated by a specific test method, demonstrates the suitability of the test system to yield a reproducible, appropriately positive or reactive response in the test system

Note 1 to entry: Potassium dichromate ($K_2Cr_2O_7$) is suggested as a suitable treatment for the positive control in *Artemia sp*. toxicity test.

3.6
test nanomaterial
manufactured nanomaterial in a dispersion that is subjected to biological or chemical testing or evaluation

3.7
stock suspension
concentrated suspension that will be diluted to some lower concentration for actual use

3.8
nanoscale
length range approximately from 1 nm to 100 nm

Note 1 to entry: Properties that are not extrapolations from larger sizes are predominantly exhibited in this length range.

[SOURCE: ISO/TS 80004-1:2015, 2.1]

3.9
nano-object
discrete piece of material with one, two or three external dimensions in the nanoscale

Note 1 to entry: The second and third external dimensions are orthogonal to the first dimension and to each other.

[SOURCE: ISO/TS 80004-1:2015, 2.5]

3.10
nanoparticle
nano-object with all external dimensions in the nanoscale where the lengths of the longest and the shortest axes of the nano-object do not differ significantly

Note 1 to entry: If the dimensions differ significantly (typically by more than three times), terms such as nanofibre or nanoplate may be preferred to the term nanoparticle.

[SOURCE: ISO/TS 80004-2:2015, 4.4]

3.11
particle
minute piece of matter with defined physical boundaries

Note 1 to entry: A physical boundary can also be described as an interface.

Note 2 to entry: A particle can move as a unit.

Note 3 to entry: This general definition of particle applies to nano-objects.

3.12
nanofibre
nano-object with two external dimensions in the nanoscale and the third dimension significantly larger

Note 1 to entry: The largest external dimension is not necessarily in the nanoscale.

Note 2 to entry: The terms nanofibril and nanofilament can also be used.

Note 3 to entry: See nanoparticle, note 1 to entry.

[SOURCE: ISO/TS 80004-2:2015, 4.5]

3.13
nanoplate
nano-object with one external dimension in the nanoscale and the other two external dimensions significantly larger

Note 1 to entry: The larger external dimensions are not necessarily in the nanoscale.

Note 2 to entry: See nanoparticle, note 1 to entry.

[SOURCE: ISO/TS 80004-2:2015, 4.6]

3.14
Artemia sp
species of the genus of aquatic crustaceans known as brine shrimp (*Artemia*)

3.15
nauplii
newly hatched brine shrimp larvae

Note 1 to entry: The nauplius larvae of *Artemia sp*. are less than 0,4 mm in length when they first hatch.

3.16
cyst
dormant *Artemia sp*. eggs

Note 1 to entry: The cysts may be stored for long periods and hatched on demand.

3.17
hatching
process of converting cysts to nauplii under appropriate environmental conditions

3.18
control solution
test medium without sample under test

3.19
immobilization
inability of the nauplii to swim during the 15 second following gentle agitation of the test and control solutions, even if the nauplii can still move their appendages

[SOURCE: ISO 6341:2012, 3.3, modified — "organisms" has been replaced by "nauplii", and "antennae" has been replaced by "appendages".]

3.20
EC_{50}
concentration at which there is an effect on 50 % of the organisms in line with the test criterion

[SOURCE: ISO 15088:2007, 3.3]

4 Materials

4.1 Test organism

Different species of *Artemia sp.* can be used, but *Artemia salina* and *Artemia franciscana* are the preferred test species. There are many commercial sources of brine shrimp cysts. *Artemia sp*. nauplii (newborn brine shrimp) should be produced by hatching high quality cysts in the laboratory.

4.2 Chemicals

4.2.1 Artificial seawater.

4.2.2 Potassium dichromate.

4.2.3 Lugol's solution (Lugol's iodine).

4.2.4 Sodium hypochlorite (5,25 % NaOCl).

4.2.5 Sodium hydroxide solution (400 g/L NaOH).

5 Technical equipment

5.1 Adequate apparatus for temperature control.

5.2 Microscope.

5.3 Binocular stereoscope.

5.4 Centrifuge.

5.5 Air pump.

5.6 Single channel pipettes.

5.7 Laboratory balance.