TECHNICAL SPECIFICATION

ISO/TS 22082

First edition
2020-05

Nanotechnologies — Assessment of nanomaterial toxicity using dechorionated zebrafish embryo

Nanotechnologies — Évaluation de la toxicité des nanomatériaux au moyen d'embryons déchorionés de poisson zèbre

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Reference number
ISO/TS 22082:2020(E)

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Published in Switzerland

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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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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.

The committee responsible for this document is ISO/TC 229, *Nanotechnologies*.

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.

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Introduction

Fish assays are important and widely used tools for evaluating the toxicity of chemicals in the aquatic environment. However, there are animal welfare concerns regarding the use of vertebrate animals for chemical testing, including fish. The use of early life stage embryos, instead of adult or juvenile fish, is considered an alternative assay because there are animal welfare benefits to testing fish embryos as an alternative to the clear distress caused by testing more developed juvenile fish, e.g. by using OECD TG 203.

Nanotechnology is positively affecting many commercial sectors, but there remain concerns regarding the potential adverse environmental effects from nano-enabled products. The OECD test guideline using fish embryos to evaluate acute toxicity (see OECD TG 236) states that some substances having a molecular weight ≥ 3 kDa, a very bulky molecular structure, and substances causing delayed hatching might be inappropriate for testing using that method. The presence of the chorion could also confound assessment of the nanomaterials biological activity. The chorion is the outmost acellular envelope of a fish embryo and it can serve as an exposure barrier for some chemicals or nanomaterials. It is currently not possible to predict which nanomaterials might be blocked by the chorion. Using dechorionated embryos for toxicity assessments may not provide direct ecotoxicological information, but may help to better identify potentially hazardous nanomaterials. Accordingly, many researchers around the world have developed a number of methods for removing a chorion from early life stage zebrafish embryos[1][2]. There are two ways to remove chorion from embryos: by enzymatic or mechanical method. The enzymatic dechorionation method has some advantages over the mechanical dechorionation method (see Annex A), including time and labour efficiency by easy preparation for dechorionation, no mechanical embryonic damage, and the ability to simultaneously prepare a large number of dechorionated embryos for a high throughput-based approaches. On the other hand, there is a disadvantage of variability in pronase activity that could influence the success rate of chorion removal. Numerous groups have used dechorionated embryos for the assessment of chemical and nanomaterial toxicity[3][4][5][6]. However, these methods have not yet been fully standardized[7][8][9][10].

Dechorionated zebrafish embryos toxicity assay can serve as a surrogate system to detect potentially hazardous nanomaterials for other vertebrate systems. As the use of higher organism animal models for toxicity testing is being refined, there is an increasing need for alternative test methods. Early life stage zebrafish (up to independent feeding, e.g. 120 HPF) could be an excellent alternative model of in vivo toxicity[22][23][24][25][26][27]. Compared with other animal models, zebrafish have a number of advantages for assessing toxicity, including the relative ease of rearing and breeding, high fecundity (external fertilization, 200 embryos to 300 embryos from a female), short generation time (approximately 3 months to adulthood), availability of genomic resources (complete zebrafish genome sequence), and genetic similarity to humans. About 70 % of human diseases have at least one zebrafish orthologue and 84 % of the human genes associated with disease have orthologues in zebrafish[11]. Therefore, the use of zebrafish to assess chemical toxicity is increasing.

This document provides an optimized procedure to remove chorions along with recommendations on how to conduct toxicity assays using dechorionated zebrafish embryos. It also discusses the advantages of the fish toxicity assay using dechorionated embryos.

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1 Scope

This document specifies a method for rapidly assessing nanomaterial toxicity (fish early life stage, 0 HPF to 120 HPF). It includes information on the importance of acellular chorion removal, detailed chorion removal procedures, and a complete protocol for the toxicity assessment of nanomaterials using dechorionated zebrafish embryos. The focus of this document is on testing nanomaterial toxicity.

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

ISO/TR 18196, *Nanotechnologies — Measurement technique matrix for the characterization of nano-objects*

ISO 22412, *Particle size analysis — Dynamic light scattering (DLS)*

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

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

OECD. *Test No. 236: Fish Embryo Acute Toxicity (FET) Test*. OECD Guidelines for the Testing of Chemicals. Section 2. OECD Publishing, Paris, 2013

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 12805, ISO/TR 13014, ISO/TS 17200, ISO/TS 80004-1, ISO/TS 80004-2, OECD TG 236 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
solubilizing agent
solvent or dispersant that can disperse and stabilize nanomaterials in solution

3.2
spawning
releasing the eggs into the water for fertilization

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