ICS 07.120
CCS C 04

NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 42470-2023

Nanotechnologies - Assessment of nanomaterial toxicity using dechorionated zebrafish embryo

Issued on: March 17, 2023 **Implemented on: October 1, 2023**

Issued by: State Administration for Market Regulation, China National Standardization Administration

Table of Contents

foreword

This document is in accordance with the provisions of GB/T 1:1-2020 "Guidelines for Standardization Work Part 1: Structure and Drafting Rules for Standardization Documents"

drafting:

This document is modified to adopt ISO /T S22082:2020 "Nanotechnology-based Toxicity Evaluation of Nanomaterials in Zebrafish Embryos", document category

The type is adjusted from the ISO technical specification to the national standard of our country:

The technical differences between this document and ISO /T S22082:2020 and their reasons are as follows:

---The original picture 1 does not conform to the morphological characteristics of the female fish and the male fish are small in the description in 5:1, so the picture of the male and female zebrafish in Figure 1 has been changed

slice (see Figure 1);

--- Added notes in 5:2, citing national standards to explain the quality of purified water (see 5:2);

---In 5:2, the reference to the later part of the stock solution configuration method is added (see 5:2);

---The photoperiod in 6:1:2 does not conform to conventional experiments and domestic practices, so the relevant national standards are quoted and the value of photoperiod is changed

(see 6:1:2);

--- According to domestic practice, "analytical purity" is added in 7:3:2 to define the purity level of chemicals (see 7:3:2);

---The test steps in 7:8:3 need to be carried out sequentially, so the "-" symbol before each step has been changed, and it has been changed to an English letter number (see

7:8:3):

Please note that some contents of this document may refer to patents: The issuing agency of this document assumes no responsibility for identifying patents:

This document was proposed by the Chinese Academy of Sciences:

This document is under the jurisdiction of the National Nanotechnology Standardization Technical Committee (SAC/TC279):

Introduction

Fish test is an important means widely used to evaluate the toxicity of chemicals in water environment: However, using vertebrates including fish

There are animal welfare concerns about testing chemicals: Testing with fully developed juveniles can cause significant discomfort to the animals (e:g: using

OECDTG203), the use of early-life fish embryos rather than adults or juveniles for testing is a more animal welfare alternative

method:

Nanotechnology is playing an active role in many fields, but people still worry about the potential harm of nano products to the environment: economic cooperation

The Organization for Operations and Development's (OECD) test guideline for the assessment of acute toxicity using fish embryos (see OECDTG236) states that some chemicals may

This method is not suitable for testing, such as macromolecular structure substances with molecular weight >= 3kDa and substances that cause incubation delay: fish embryo

The outermost acellular envelope of the fetus, its barrier function will hinder the evaluation of the biological activity of chemicals or nanomaterials, and it is currently impossible to predict which nanomaterials

Rice materials are affected by this barrier: Toxicity assessments using ovariectomized embryos, although not providing direct ecotoxicological information, may

Can help to better identify potentially hazardous nanomaterials: Therefore, researchers from all over the world have developed many methods to remove biological

egg membranes of early stage zebrafish embryos[4][5]: There are two ways to remove the egg membrane of embryos: enzymatic membrane rupture and mechanical membrane rupture: mechanical membrane rupture

Compared with the traditional method, the enzymatic membrane disruption method has the following advantages (see Appendix A): it saves time and labor, avoids mechanical damage to the embryo, and at the same time provides a large

A large number of membrane-ruptured embryos for high-throughput testing: However, one disadvantage of enzymatic membrane disruption is that changes in pronase activity may affect egg membrane removal:

success rate of removal: Many research groups have used egg-thinned embryos to evaluate the toxicity of chemicals and nanomaterials [6]-[9], but the methods have not yet been developed:

Fully normalized [10]-[13]:

Ovulated zebrafish embryos can be used as an alternative system to other vertebrate systems for detecting potential hazards of nanomaterials: with high

With the continuous optimization of animal model toxicity tests, the demand for alternative test methods continues to increase: Zebrafish in early life stages (independent photo

Before feeding, that is, within 120h after fertilization) is a very good surrogate model for in vivo toxicity test[23]-[28]: Compared with other animal models, the zebrafish

The model has many advantages, including relative ease of raising and breeding, strong fecundity (in vitro fertilization, a female fish can produce:200-300 embryos),

Short developmental cycle (approximately 3 months to adulthood), availability of genomic resources (complete zebrafish genome sequence), and genetic correlation with humans

Similarity: Comparison with the human genome shows that about 70% of human genes have at least one homologous gene in zebrafish[14], so zebrafish

Horsefish have been increasingly used in the evaluation of chemical toxicity:

This document provides a method for toxicity assessment using ovariectomized zebrafish embryos and recommends an optimal method for removing the egg membranes of zebrafish embryos:

The procedure was discussed, and the advantages of using oviembranous embryos for fish toxicity assessment were discussed:

Nanotechnology based on zebrafish embryos

Toxicity Evaluation of Nanomaterials

1 Scope

This document specifies a method for rapid assessment of the toxicity of nanomaterials (early life stages of fish, 0HPF~120HPF), including

Detailed protocol for removing egg membranes and a complete protocol for evaluating nanomaterial toxicity using egg membrane-depleted zebrafish embryos: The focus of this document is to test the

Toxicity of nanomaterials:

This document applies to the toxicity testing of commercial and non-commercial nanomaterials:

2 Normative references

The contents of the following documents constitute the essential provisions of this document through normative references in the text: Among them, dated references

For documents, only the version corresponding to the date is applicable to this document; for undated reference documents, the latest version (including all amendments) is applicable to

this document:

GB/T 6682-2008 Analytical laboratory water specifications and test methods

ISO /T S12805 Nanotechnologies-Materialsspecifi-

Note: GB/T 37156-2018 Nanotechnology Material Specification Guidelines for Nano-object Characteristics (ISO /T S12805:2011, IDT)

ISO /T R13014 Guidelines for Characterization of Physicochemical Properties of Nanotechnology Nanomaterials Before Toxicological Evaluation (Nanotechnologies-Guid-