TECHNICAL SPECIFICATION

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Nanotechnologies — Characteristics of working suspensions of nano-objects for *in vitro* assays to evaluate inherent nano-object toxicity

Nanotechnologies — Caracteristiques des suspensions de nano-objets utilisées pour les tests in vitro *évaluant la toxicité inherente aux nano-objets*

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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 on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

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

Introduction

Before nano-objects enter into the market, their possible impact on human health and the environment needs to be carefully evaluated.

In vitro toxicity assays using cultured cells are frequently used as a tool in screening hazardous materials. This testing provides essential information for understanding the mechanisms of biological effects induced by the materials. However, nano-objects require specific considerations with respect to the *in vitro* toxicity assays, because their behaviour is distinct from water soluble chemicals. For example, immediately after the introduction of nano-object samples into the culture medium, the nano-objects undergo changes, such as (a) dissolution, which is the dissolving of nano-objects into their ionic counterparts, (b) corona formation, which is the adsorption of the components of culture medium onto the nano-object surface, or (c) changes in aggregation/agglomeration state, leading to alteration in particles size and sedimentation. Therefore, it is critical to consider the aforementioned phenomena in clarifying if the observed effects are related to the tested nano-object itself or from other uncontrolled sources and to avoid false interpretation of assay results.

The rigorous characterization of the working suspension prior and during *in vitro* toxicity assays is essential to exclude the *in vitro* experimental artefacts. For example, the corona formation, metal ion release from the nano-objects and impurities (residual from the nano-object synthesis process) can interfere with some *in vitro* assays,[1] producing inaccurate results. Additionally, the formation of agglomerates and aggregates can alter the toxicity of a suspension. Therefore, it is important to carefully assess and describe the characteristics of the suspension of nano-objects being tested.

This Technical Specification describes the essential characteristics and applicable measurement methods of working suspension containing nano-object samples for *in vitro* toxicity assays. Intention is that reliable test results on nano-object toxicity could be shared and communicated among stakeholders of nano-objects, such as regulators, general public, manufacturers and end users. This Technical Specification does not describe a procedure for validation of working suspension.

Nanotechnologies — Characteristics of working suspensions of nano-objects for *in vitro* assays to evaluate inherent nano-object toxicity

1 Scope

This Technical Specification describes characteristics of working suspensions of nano-objects to be considered when conducting *in vitro* assays to evaluate inherent nano-object toxicity. In addition, this Technical Specification identifies applicable measurement methods for these characteristics.

This Technical Specification is applicable to nano-objects, and their aggregates and agglomerates greater than 100 nm.

NOTE This Technical Specification intends to help clarify whether observed toxic effects come from tested nano-objects themselves or from other uncontrolled sources.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 29701, *Nanotechnologies — Endotoxin test on nanomaterial samples for in vitro systems — Limulus amebocyte lysate (LAL) test*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
culture medium
aqueous solution of nutrients required for cell growth

3.2
secondary particle
complex agglomerate/aggregate of primary particle(s), proteins and other medium components

3.3
stability
properties to remain unchanged over a given time under stated or reasonably expected conditions of storage and use for an *in vitro* toxicity assay

3.4
working suspension
suspension prepared for an *in vitro* assay that includes culture medium and nano-object sample