TECHNICAL ISO/TS

First edition
2016-03-15

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*

Reference number
ISO/TS 19337:2016(E)

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Foreword

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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.