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Nanotechnologies — Extraction method of nanomaterials from lung tissue by proteinase K digestion

Nanotechnologies — Méthode d'extraction de nanomatériaux d'organes par digestion par protéinase K

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Foreword

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This document was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

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Introduction

Quantification of nanomaterials deposited in organs is important to evaluate the lung burden in an inhalation toxicity study and organ distribution in toxicokinetics studies[3][4][5]. Owing to the long retention period of nanomaterials deposited in the alveoli in an inhalation setting, the OECD revised the subacute and subchronic inhalation test guidelines, TG 412 and TG 413 respectively, to include lung burden analysis when testing poorly soluble nanomaterials[3][4]. However, the lung burden analysis method varies depending on the nanomaterials, thus the development of standard methods is highly needed. In addition, a new or revised OECD toxicokinetics test guideline (i.e. TG417) is needed to accommodate nanomaterials[6][7]. Furthermore, OECD launched a new project (1.10) for developing a guidance document on the determination of concentrations of nanoparticles in biological samples for (eco)toxicity studies[8]. ISO/TR 22019 addresses considerations for performing toxicokinetic studies with nanomaterials. However, standard methods to measure concentrations of nanomaterials deposited in organs are needed to complement TG 412, TG 413 and ISO/TR 22019.

Quantification of nanomaterials in organs can be divided into two steps:

a) collection of nanomaterials from organs;

b) quantification of nanomaterials using instrumental analysis.

To collect nanomaterials deposited in organs, chemical or enzymatic digestion methods can be used. The ultimate goal of step a) is to collect the particle in particle-form (i.e. the same material that animals were exposed to) rather than the ionic counterparts. However, many of the chemicals used for digestion such as hydrogen chloride, nitric acid, and hydrofluoric acid can ionize some nanomaterials or damage their structure[9][10]. Among nanomaterials, metals or metal oxides can be dissolved by chemicals for digesting organs. Thus, the measured amount of metal ions in organs treated with these digestion chemicals would not be the amount of nanomaterials inhaled. It could be the ionic counterparts of these nanomaterials as well as the same metal present as endogenous ions in the organ[11][12]. Although carbon-based nanomaterials such as carbon nanotubes (CNTs), graphene, and nanodiamonds are not dissolved by chemical digestion, the structure of the carbon-based nanomaterials can undergo alterations including defects and oxidation[9]. The second step is the quantification of nanomaterials by instrumental analysis including methods such as inductively coupled plasma mass spectrometry (ICP-MS), fluorometry, and optical absorbance spectrometry. Because the instrumental analysis is diverse and needs to correspond to the physicochemical properties of the nanomaterial analysed, this document focuses on the method of extracting nanomaterials from organs.

In contrast, the enzymatic digestion of the mixture of powderised lung tissue and nanomaterials in vitro, and lung tissue instilled nanomaterials in vivo using proteinase K (PK) can successfully dissolve tissues with less alterations of the structure of carbon-based nanomaterials and many metal oxides compared to the chemical digestion method[13]. This method allows to collect nanomaterial particles separately from their ionic counterparts dissolved in supernatants. In a previous study, the PK digestion successfully digested lung tissues, and it was possible to separately collect carbon-based nanomaterials including carbon black, carbon nanotube, carbon nanofibre, graphene, and nanodiamond[13]. Other studies have also demonstrated the use of this method to successfully collect and quantify single-walled carbon nanotubes (SWCNTs) instilled into mouse lung, CNTs spiked into rat lung tissue, and microplastics in marine invertebrates species[9][14][15]. However, misleading or inaccurate results may occur if nanomaterials are dissociated or dissolved during the process of enzymatic digestion. The examples of nanomaterials for which the PK digestion method is applicable or not applicable are listed in Annex A. Although this document focuses on the lung tissue digestion, it can be further applicable to other tissues. However, organs besides the lung should be tested for their validity based on this document because the efficacy of PK for tissue digestion varies by the organ-specific nature. Therefore, an optimized procedure to extract nanomaterials from lung tissue is highly needed as a part of recommendations and guidelines on how to conduct lung burden analysis or toxicokinetic studies.

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Nanotechnologies — Extraction method of nanomaterials from lung tissue by proteinase K digestion

1 Scope

This document provides an extraction method using the proteinase K (PK) for nanomaterials deposited in the lung. This document specifies the advantages of the PK digestion method and examples of nanomaterials to which it can be applied. This document focuses on extracting nanomaterials from lung tissue and separating nanoparticles from their ionic counterparts. This method is potentially (or theoretically) applicable to any particles that are insoluble during the PK digestion process.

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 80004-1, *Nanotechnologies — Vocabulary — Part 1: Core vocabulary*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in and ISO 80004-1 and the following apply.

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

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

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

3.1
nanoparticle
nano-object with all external dimensions in the nanoscale

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

[SOURCE: ISO 80004-1:2023, 3.3.4]

3.2
nanotube
hollow nanofibre

[SOURCE: ISO 80004-1:2023, 3.3.8]

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