TECHNICAL REPORT

ISO/TR 19057

First edition
2017-10

Nanotechnologies — Use and application of acellular in vitro tests and methodologies to assess nanomaterial biodurability

Nanotechnologies — Utilisation et application des tests in vitro sur cellules et méthodes pour évaluer la biodurabilité des nanomatériaux

Reference number
ISO/TR 19057:2017(E)

© ISO 2017



COPYRIGHT PROTECTED DOCUMENT

© ISO 2017, Published in Switzerland

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

Foreword ... **v**

1 Scope ... **1**

2 Normative references ... **1**

3 Terms and definitions ... **1**

4 Symbols and abbreviated terms ... **2**

5 Background including need for assessing the biodurability of particles ... **4**

6 Aims and objectives ... **6**

7 Approaches for assessment of micrometre mineral particle and fibre biodurability ... **6**
7.1 General ... 6
7.2 Dissolution of nanomaterials versus their dispersion and biodegradation ... 7

8 Need for the assessment of nanomaterial biodurability ... **7**

9 Influence of different types of ligands and coatings on nanomaterial biodurability ... **8**

10 Review of methodologies to assess micrometre mineral particle and fibre biodurability ... **8**
10.1 General ... 8
10.2 ***In vitro*** acellular methods ... 8
10.3 Description of different simulated physiological media ... 9
10.3.1 General ... 9
10.3.2 Simulated lung airway lining fluids ... 9
10.3.3 Simulated lung macrophage phagolysosomal fluid ... 10
10.3.4 Digestive system (saliva, gastric and intestinal fluids) ... 10
10.3.5 Simulated sweat (SSW) ... 11
10.4 Description of different simulated environmental media ... 11
10.4.1 General ... 11
10.4.2 Simulated natural freshwaters ... 11
10.4.3 Simulated seawater ... 11
10.4.4 Simulated estuarine waters ... 11
10.5 Description of different test systems to assess dissolution of particles and fibres ... 11
10.5.1 General ... 11
10.5.2 Static dissolution system ... 12
10.5.3 Continuous flow system (CFS) ... 12
10.5.4 Batch and batch filter systems ... 12
10.5.5 Tangential flow filtration system ... 12
10.6 Assessment of dissolved mass concentration post dissolution experiment ... 13
10.6.1 General ... 13
10.6.2 Techniques based on physical principles ... 13
10.6.3 Techniques based on mechanical concepts ... 14
10.6.4 Techniques based on chemical principles ... 15
10.6.5 Ultraviolet-visible (UV-Vis) spectroscopy ... 16
10.6.6 One-dimensional mathematical models ... 16
10.6.7 Single particle inductively coupled plasma-mass spectrometry (spICP-MS) ... 16

11 Calculation of micrometre mineral particle biodurability ... **17**
11.1 General ... 17
11.2 Dissolution kinetics, dissolution rates, and dissolution rate constants ... 18
11.3 Dissolution kinetics and dissolution rate of larger particles and fibres ... 18
11.4 Dissolution kinetics and dissolution rate of nanoparticles ... 20
11.5 Assessment of halftime estimates of particles and fibres ... 20
11.6 Assessment of lifetime estimates for particles and fibres ... 21
11.6.1 General ... 21
11.6.2 Shrinking sphere theory ... 21
11.6.3 Shrinking fibre theory ... 22

© ISO 2017 – All rights reserved

11.7 Assessment of halftime and lifetime estimates 23
12 Examples of micrometer mineral particles and fibres where biodurability was assessed using *in vitro* acellular systems 24
12.1 Glass and asbestos fibres 24
12.2 Silicon dioxide (SiO_2) 24
12.3 Talc 25
12.4 Tungsten oxide 25
12.5 Beryllium 25
13 Examples of nanomaterials where biodurability was assessed using *in vitro* acellular systems 25
13.1 SWCNTs and MWCNTs 25
13.2 Silver nanoparticles (AgNPs) 26
13.3 Titanium dioxide (TiO_2) 26
13.4 Zinc oxide (ZnO) 26
14 Biodurability of ligands 27
14.1 General 27
14.2 Examples of ligands attached to particles where biodurability has been assessed 27
14.3 Methodologies to assess the biodurability of the attached ligands 27
14.3.1 General 27
14.3.2 Gel permeation chromatography (GPC) 27
14.3.3 Matrix-assisted laser desorption ionization mass spectrometer (MALDI-MS) 28
14.3.4 Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) 28
14.3.5 Liquid chromatography coupled with mass spectrometry (LC-MS/MS) 28
15 Relationship with relevant international documents 28
15.1 Simulated sweat 28
15.2 Simulated sebum 29
15.3 Simulated lung fluids 29
15.4 Simulated digestive system fluids 29
16 Assessing the validity of assay/test systems 29
17 Biological relevance of the dissolution assay 30
18 Use of biodurability tests in risk assessment and its limitations 31
Annex A (informative) **Tables of relevant information 32**
Bibliography 36

 © ISO 2017 – All rights reserved

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on 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 the following URL: www.iso.org/iso/foreword.html.

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

© ISO 2017 – All rights reserved

Nanotechnologies — Use and application of acellular in vitro tests and methodologies to assess nanomaterial biodurability

1 Scope

This document reviews the use and application of acellular *in vitro* tests and methodologies implemented in the assessment of the biodurability of nanomaterials and their ligands in simulated biological and environmental media.

This document is intended to focus more on acellular *in vitro* methodologies implemented to assess biodurability and, therefore, excludes the general review of relevant literature on *in vitro* cellular or animal biodurability tests.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions 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 http://www.iso.org/obp

3.1
bioaccumulation
process of accumulation of a substance in organisms or parts

[SOURCE: ISO/TR 13329:2012, 3.3]

3.2
biodegradation
degradation due to the biological environment

Note 1 to entry: Biodegradation might be modelled by *in vitro* tests.

[SOURCE: ISO/TR 13329:2012, 3.4]

3.3
biodurability
ability of a material to resist *dissolution* (3.6) and mechanical disintegration from chemical and physical clearance mechanisms

[SOURCE: ISO/TR 13329:2012, 3.5, modified]

3.4
biopersistence
ability of a material to persist in a tissue in spite of the tissue's physiological clearance mechanisms and environmental conditions

[SOURCE: EN 18748:1999]

© ISO 2017 – All rights reserved

3.5
dispersion
microscopic multi-phase system in which discontinuities of any state (solid, liquid or gas: discontinuous phase) are dispersed in a continuous phase of a different composition or state

[SOURCE: ISO 26824:2013, 16.5]

3.6
dissolution
process of obtaining a solution containing the analyte of interest

Note 1 to entry: Dissolution is the act of dissolving and the resulting species may be molecular or ionic.S

[SOURCE: ISO 17733:2015, 3.4.10, modified]

3.7
ligands
atoms or groups joined to the central atom

[SOURCE: IUPAC Recommendations 1994]

3.8
nanomaterial
material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale

Note 1 to entry: This generic term is inclusive of nano-object and nanostructured material.

Note 2 to entry: See also engineered nanomaterial, manufactured nanomaterial and incidental nanomaterial.

[SOURCE: ISO/TS 80004-1:2015, 2.4]

3.9
nanoparticle
nano-object with all three external dimensions in the nanoscale

Note 1 to entry: If the lengths of the longest to the shortest axes of the nano-object differ significantly (typically by more than three times), the terms nanorod or nanoplate are intended to be used instead of the term nanoparticle.

[SOURCE: ISO/TS 27687:2008, 4.1]

3.10
specific surface area for powders
absolute surface area of the sample divided by sample mass and is therefore expressed in units of m^2/g or kg

[SOURCE: ISO 9277:2010, 3.11, modified]

3.11
suspension
heterogeneous mixture of materials comprising a liquid and a finely dispersed solid material

[SOURCE: ISO/TS 80004-6:2013, 2.13]

4 Symbols and abbreviated terms

AAS	Atomic absorption spectroscopy
AF4	Asymmetrical flow field flow fractionation
AgNPs	Silver nanoparticles

© ISO 2017 – All rights reserved