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Nanotechnologies — Considerations for in vitro studies of airborne nano-objects and their aggregates and agglomerates (NOAA)

Nanotechnologies — Considérations pour les études in vitro des nano-objets en suspension dans l'air et de leurs agrégats et agglomérats (NOAA)

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Foreword

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Inhalation is one of the prominent routes by which humans can come in contact with natural, unintended and engineered nano-objects and their aggregates and agglomerates (NOAA). Due to the physiological, biochemical and anatomical differences between humans and animals, as well as the considerable time, cost and animal numbers required to conduct in vivo toxicity tests, there is much interest in developing in vitro strategies for risk assessment that are based on human cells and mechanisms of toxicity. To enable comparability of the results of in vitro assay and in vivo effects observed after inhalation of NOAA, certain parameters should be considered, including:

a) the choice of cell types;

b) characterization of the NOAA throughout the assay, including life-cycle transformations;

c) the choice of nano-object concentration relevant to human exposures;

d) generation of NOAA form that mimics human exposures;

e) the use of relevant dispersants;

f) the use of appropriate mode of exposure (submerged or air liquid interface) and exposure duration[1].

This document includes descriptions of the aforementioned parameters with regard to using in vitro-based strategies for assessing specific aspects related to the inhalation toxicity of NOAA. For example, for inhalation studies, it is critical to choose the proper equipment for generation, exposure to, and characterization of the nano-objects. This document includes information about available in vitro aerosol exposure chambers and biological models that have been used to assess the inhalation toxicity of NOAA. This document does not include details regarding the techniques for aerosol generation or characterization of specific nanomaterials (NMs), their life cycle transformations or in vivo testing. An overview of the aerosol generation of NMs and in vivo testing is given in ISO/TR 19601[2].

Nanotechnologies — Considerations for in vitro studies of airborne nano-objects and their aggregates and agglomerates (NOAA)

1 Scope

This document collates information regarding the systems available for exposure and assessment of nano-objects and their aggregates and agglomerates (NOAA) for in vitro air exposure studies. It provides an overview of the various exposure systems and in vitro cell systems used to perform in vitro studies that simulate an inhalation toxicology study design.

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:

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

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

3.1
aerosol
system of solid or liquid particles suspended in gas

[SOURCE: ISO 15900:2009, 2.1]

3.2
agglomerate
collection of weakly bound particles or *aggregates* (3.3) or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components

Note 1 to entry: The forces holding an agglomerate together are weak forces, for example van der Waals forces, or simple physical entanglement.

Note 2 to entry: Agglomerates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: ISO/TS 80004-4:2011, 2.8]

3.3
aggregate
particle comprising strongly bonded or fused particles where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components

Note 1 to entry: The forces holding an aggregate together are strong forces, for example covalent bonds, or those resulting from sintering or complex physical entanglement.

Note 2 to entry: Aggregates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: ISO/TS 80004-4:2011, 2.7]

3.4
engineered nanomaterial
nanomaterial designed for specific purpose or function

[SOURCE: ISO/TS 80004-1:2015, 2.8]

3.5
incidental nanomaterial
nanomaterial generated as an unintentional by-product of a process

Note 1 to entry: The process includes manufacturing, bio-technological or other processes.

Note 2 to entry: See "ultrafine particle" in ISO/TR 27628:2007, 2.21.

[SOURCE: ISO/TS 80004-1:2015, 2.10]

3.6
manufactured nanomaterial
nanomaterial intentionally produced to have specific properties or composition

[SOURCE: ISO/TS 80004-1:2015, 2.9, modified — "specific" has replaced "selected".]

3.7
nanoparticle
nano-object with all external dimensions in the nanoscale where the lengths of the longest and the shortest axes of the nano-object do not differ significantly

Note 1 to entry: If the dimensions differ significantly (typically by more than 3 times), terms such as nanofibre or nanoplate may be preferred to the term nanoparticle.

[SOURCE: ISO/TS 80004-2:2015, 4.4]

3.8
particle size distribution
distribution of particles as a function of particle size

Note 1 to entry: Particle size distribution may be expressed as cumulative distribution or a distribution density.

[SOURCE: ISO/TS 80004-6:2013, 3.1.2, modified — "(distribution of the fraction of material in a size class, divided by the width of that class)" has been deleted from the Note 1 to entry.]

4 Abbreviated terms

Ag NPs	silver nanoparticles
ALI	air-liquid interface
AOP	adverse outcome pathway
Au NPs	gold nanoparticles
CCSP	clara cell secretary protein
CD	cluster of differentiation
CFTR	cystic fibrosis transmembrane conductance regulator
CNT	carbon nanotube

CO_2	carbon dioxide
ENM	engineered nanomaterial
IATA	integrated approach to testing an assessment
ICAM-1	intercellular adhesion molecule 1
IL	interleukin
ISDD	in vitro sedimentation, diffusion and dosimetry
ISD3	in vitro sedimentation, diffusion, dissolution and dosimetry
KE	key event
MIE	molecular initiating event
MPPD	multiple-path particle dosimetry
MT	metallothionein
MUC 1	mucin 1
MWCNT	multi-walled carbon nanotubes
NM	nanomaterial
NOAA	nano-objects and their aggregates and agglomerates
OECD	Organisation for Economic Co-operation and Development
QCM	quartz crystal microbalance
ROS	reactive oxygen species
SiO_2	silicon dioxide
SP-A	surfactant protein A
SP-D	surfactant protein D
TEER	transepithelial electrical resistance
TiO_2	titanium dioxide
VCAM-1	vascular cell adhesion molecule 1

5 Considerations for in vitro systems for assessing inhalation exposure to NOAA

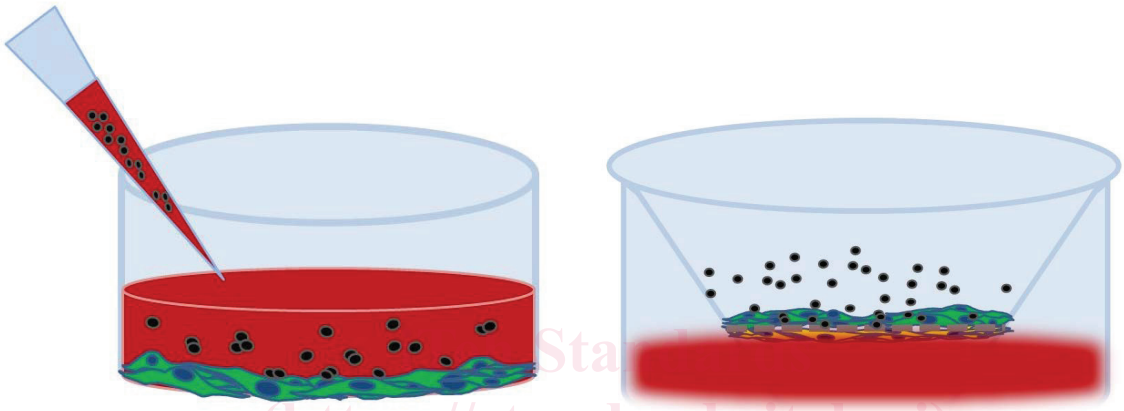
5.1 Background

Nano-objects can be incidental or are manufactured from a variety of materials (e.g. metals, polymers, metal oxides) and come in many different morphologies and combinations. Testing the toxicity of inhaled NOAA using in vitro systems involves considerations of several parameters, including the appropriate mode of exposure (see 5.2), characterization of test material (see 5.3) and choice of cell types (see 5.4).

5.2 Modes of exposure

5.2.1 General

Both direct and indirect methods have been used to assess the aerosolized nano-objects using in vitro systems. Direct methods involve exposing the test aerosol, generated using aerosol generators, to the cells directly under submerged, intermittently submerged (e.g. on a rocking platform) or air-liquid interface (ALI) conditions[3][4][5]. Figure 1 presents the diagrammatic representation of exposure to NOAA under submerged and ALI conditions. Indirect methods often involve collection of aerosols (e.g. road-side ambient particles or nano-objects) using special apparatus (e.g. wetted rotating vane impactors or liquid impingers) or on a filter-like substrate followed by suspension of the collected sample in a culture medium before exposing the cells[3][6][7][8][9].



a) Submerged culture b) Air-liquid interface culture

Figure 1 — Diagrammatic representation of submerged and air-liquid interface (ALI) cultures

The indirect method allows for the identification of the potential toxicity of air borne particles. However, the steps needed to prepare the suspensions of collected test material (when using indirect methods) and the interaction of the test material with the medium in submerged systems (when using direct methods) can cause changes to the properties of the test material leading to false assessments. This is especially applicable to nano-objects due to the fact that both their agglomeration state and movements are impacted by the use of a suspending medium, with their movement being controlled by Brownian diffusion or sedimentation based on their physico-chemical parameters such as size and density[10][11]. As a result, the probability of agglomerates settling down onto the cell culture is higher than for single particles. Additionally, dissolution kinetics of nano-objects under submerged conditions might differ considerably from what the particles undergo in in vivo conditions. Since the size, form, and solubility are the main parameters that determine nano-object toxicity, the toxicity outcomes observed in the submerged systems might not capture what happens in the lung after nano-object exposure. Another limitation of submerged systems is that the phenotype of the cells is usually different than that found under in vivo conditions as seen by the lack of formation of tight junctions and mucus in epithelial cells, an issue which is particularly important for inhalation models[12][13].

To overcome the limitations associated with the submerged systems, ALI systems are used and are preferred for inhalation toxicity testing because of their physiological relevance[14][15][16][18]. An ALI system constitutes cells cultured on a semi-permeable membrane where their basal surface is exposed to the culture medium and the apical surface is exposed to air. Such configuration is considered to be physiologically relevant as it has been shown to drive differentiation of the cells that mimics their in vivo phenotype[12][13][19]. In addition to having the cells in an “in vivo-like” configuration/phenotype, ALI systems allow a relatively direct deposition of NOAA on the cells that more closely mimics the particle deposition that results following inhalation, which might be used to derive a concentration-response relationship[20]. It should be noted that, similar to liquid conditions, the generation of an

aerosol can also have an effect on particle characteristics. Thus, during and after aerosol generation, proper characterization of the particles should be performed.

Figure 1 shows exposure of NOAA to cells under submerged conditions and at the ALI. Under submerged conditions, the NOAA have to span the depth of the medium to reach the cells. At ALI conditions, the NOAA settle directly on the cells due to minimal fluid layer over the cells. Several studies have compared submerged and ALI systems exposed to NOAA and have reported differences in cellular responses[15]. For example, a higher expression of interleukin 6 (IL-6), IL-8, and hemeoxygenase-1 was observed in cells exposed to NOAA at ALI as compared to submerged cultures[20][21][22][23]. A few studies have observed contradicting outcomes for the aforementioned biomarkers[24][25][26]. Despite the differences between the two modes of exposure, both the systems are still used to assess the toxicity of NOAA. Submerged cell cultures can be used for identifying the toxic potency of air borne (e.g. environmental or exhaust) particles. However, for evaluation of engineered and manufactured nano-objects, especially when considering occupational exposure, the ALI culture system more appropriately represents factors dealing with lung exposure.

5.2.2 Considerations for ALI exposure systems

5.2.2.1 General

Treating the cell systems at ALI requires exposure systems that can deliver the material to be tested as an aerosol to the cell system in a form that is relevant to human exposure scenarios. The exposure systems used to test NOAA could either be “closed-box” or “flow-through” type and typically involve two basic components: an aerosol generator that generates the test atmosphere and the exposure chamber that houses the cell system. Flow-through systems (as shown in Figure 2) also typically consist of connectors and peripherals that transport, dilute, characterize and condition the aerosol before delivering it to the cells inside the chamber, and an exhaust, which could be used for test atmosphere sampling.

Figure 2 depicts a basic diagrammatic configuration of a system including an aerosol generator and an exposure chamber for exposing cells to aerosolized (or nebulized) substances at the ALI. The materials are aerosolized (or nebulized) using an aerosol generator (or nebulizer), which is connected to the exposure chamber. Deposited concentration of NOAA can be determined by incorporating quartz crystal microbalance (QCM) and/or electron microscopy (EM) grids in the wells without cells (A). The cells are cultured on membrane inserts and exposed to dry aerosols or nebulized material at the ALI (B). Cells treated with air only can be used as a negative control (C). Sampling ports can be included at several check points to obtain aerosol or medium samples. The various components of the exposure system and their applicability to the assessment of NOAA are described in 5.2.2.2 to 5.2.2.3.