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Development of International Standard on Nano Aerosol Generation for Inhalation Toxicology Study

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Abstract

Development ISO TR 19601 : Aerosol generation for NOAA (nano-objects and their aggregates and agglomerates) air exposure studies is completed recently. The technical report (TR) reviews methods for generating aerosols of NOAA for *in vivo* and *in vitro* inhalation studies. The goals of this technical report is to aid in selecting appropriates NOAA aerosol generator to perform a planned toxicology design. The TR describes how to approach air exposure study design after considering workplace exposure scenario with providing a flow chart to select a proper NOAA generator for aimed study. The TR presents variety of NOAA generator currently used, and describes the principles of operation, advantage at limitation of the NOAA generators. This TR will assist investigators on NOAA inhalation toxicity testing how to design inhalation exposure study with selection of proper generators. This mini-review summarizes contents of the technical report and provides the current status of science in NOAA aerosol generation.

Keywords: Aerosol generation; Inhalation study; NOAA; ISO; Nanoparticle

Introduction

Inhalation is a main route of exposure to aerosolized nanomaterials. New term, nano-objects and their aggregates and agglomerates (NOAA), is frequently used instead of nanomaterials. The NOAA include nano-objects with one, two, are three dimensions in the nanoscale from approximately 1-100 nm which might be spheres, fibers, tubes and others as primary structures. NOAA consists of individual primary structures in the nanoscale and larger than 100 nm with aggregated or agglomerated structure [1]. The toxicity of nanomaterials has been frequently tested based on the OECD test guidelines (TG). Currently acute, subacute and subchronic inhalation test guidelines are under revision. OECD TG 403 [2], is an acute inhalation toxicity test guidelines to obtain LC50. OECD TG 436 [3], is a new acute toxic class method. OECD TG 412 [4], is a repeated dose inhalation toxicity TG for 14-28 day study. OECD TG 413 [5], is a subchronic inhalation toxicity TG. Recently these test guidelines are being revised to accommodate traditional chemical exposure as well as nanomaterial exposure, because existing inhalation TGs are not sufficient to satisfy nanomaterial inhalation toxicity testing. Along with OECD test guideline revision activities, ISO TC 229 (International Organization for standardization technical committee 229-Nanotechnologies) initiated a standard for aerosol generation for NOAA from 2014. This TR complements the activities of the Organization for Economic Cooperation and Development (OECD) working party on manufactured nanomaterials (WPMN) and relevant documents. This TR assists scientists to choose appropriate aerosol generator for their target NOAAs to be tested. This standard ISO TR (technical report) 19601 provides a status of science in producing aerosols of NOAA for inhalation study. Appropriate generation of NOAA aerosols determines a successful inhalation toxicity tests which cost a lot of resources and time.

The TR deals with three critical aspects to consider when designing and conducting nanomaterial inhalation toxicity study : 1) uniform and reproducible nano-objects generation that is relevant to realistic exposures 2) thorough characterization of nanomaterials throughout the duration of testing including starting and generated materials and 3) use of occupational exposure limits (OEL) and reference concentrations (RfC) for dosimetry.

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Scope of Standard

The technical report reviews methods for producing aerosols of NOAA for *in vivo* and *in vitro* air exposure studies. The purpose of the document is to aid in selecting an appropriate aerosol generator to fulfil a proposed toxicology study design. The document describes characteristics of aerosol generation methods, including their advantages and limitations. This TR does not provide guidance for aerosol generation of specific nano-objects.

Inhalation Study Considerations

In designing an inhalation study for NOAA, an actual workplace exposure scenario should be considered, because the health risk of workers is evaluated by inhalation toxicity study. Appropriate NOAA aerosol generation should reflect actual workplace NOAA exposure and emissions in terms of mass or number concentration, particle size, shape and size distribution, frequency and duration of exposure, and handling and manufacturing conditions. Various methods of NOAA from powder form and suspension in liquid media to solid state materials could be used to generate NOAA aerosols. The NOAA aerosol generation should be in line with existing inhalation testing guidelines such as OECD TG 403, 436, 412, 413 and guidance document (GD) 39 (OECD, 2009) or relevant national or international guidelines. Newly revised OECD TGs for nanomaterials describe that MMAD (mass median aerodynamic diameter) is up to 2 micrometer with a geometric standard deviation (GSD) up to 3. In addition, this TR recommends to consider GHS (Globally harmonized system of classification and labelling of chemicals) categorization when an inhalation study might be used for hazard evaluation, classification and labelling.

Considerations in Selection of Proper Generators

When conducting guideline based study, the standardized testing guidelines such as OECD, EPA OPPTS or EU. The study should be conducted following guidelines including number of animals, duration of exposure, observation period, and test material characterization. Studies driven by research hypothesis are more flexible than test guideline based studies. The basic scheme of study consideration is described in the Table 1. The physicochemical characterization of the pristine or manufactured nanomaterial is important before generation of a NOAA aerosol. Because nanomateial are manufactured by various synthetic procedures that impart those unique properties designed for specific applications, the nanomaterial could have a complex structure including impurities and different surface properties. The physicochemical properties nanomaterial influence toxicity of nanomaterials. Useful physicochemical properties nanomaterial include, but not limited to particle size, size distribution, shape, aggregation/agglomeration, surface characteristics, crystalline structure, dustiness, composition and purity. NOAA exposure information on use or handling and manufacturing in terms of particle mass, concentration, number, size, dispersion or shape is very important in designing the inhalation study. Particle shape and concentration similar to workplace exposure should be determined for NOAA inhalation study. Exposure characteristics including duration and frequency of exposure, worker activities, NOAA manufacturing handling and release or emission scenarios would be very useful in designing an inhalation study. Two types of inhalation exposure chambers, whole-body and nose-only are widely used. Nose-only exposure is a principle method of exposure recommended in the OECD TGs, reduces skin and oral exposure potential and consumes less quantity of test nanomaterials, while whole-body is more relevant to human exposure and causes less pain. NOAA particle should be characterized by real-time and off-line monitoring devices. Real-time monitoring of particle size and number including DMAS (differential mobility analyzing system) and ELPI (electrical low pressure impactor) will give particle size distribution and particle number concentration in real-time. Off-line filter sampling can be used to determine mass concentration of NOAA. In addition, off-line filter or EM grid sampling can be prepared for transmission electron microscope (TEM) observation for size and shape of NOAA and analyzed for composition by EDX (energy dispersive X-ray analyzer). The filter sampling can be further analyzed for chemical composition. The stability of NOAA aerosol concentration in inhalation chamber during exposure period should be monitored regularly according to test guidelines or test protocols. OECD TG recommends to concentration deviates within 20% during exposure period.

Step	**Considerations**
Selection of study	Guideline based study : strictly recommended to follow TG
	Hazard identification research
Characterization of physicochemical properties of NOAA considered for study	Size and size distribution
	Aggregation/agglomeration
	Surface characteristics: area and charge
	Crystalline structure
	Electrical properties
	Dustiness
	Composition and purity
Exposure information on possible use or handling and manufacturing	Simulating actual exposure situation in workplace
	Depending on particle shape and concentration

Exposure characteristics	Frequency and duration of workers' exposure
	NOAA generating operation
	NOAA release procedure and handling information
	Temperature and humidity of workplace
Type of inhalation exposure method	Whole-body chamber: less stress on animals
	Nose-only chamber: less via oral or skin exposure
	Secured safety of chamber
Particle characterization methods	Real-time monitoring
	Off-line monitoring
	Stability determination
Selection of generator	Based on type of nanomaterial under consideration
	Dry or wet aerosol generation
	Ensuring maintenance of the generator
	Considering risk management system: malfunctioning of generator, protection of dust explosion

Table 1: Basic scheme in selection of proper generators (summarized form of TR 19601).

NOAA Aerosol Generators

NOAA aerosol generators have several modes of generation: dry dissemination, wet dissemination, phase change, chemical reaction and liquid phase filtration/dispersion. The generation techniques, principle of operation, advantage and disadvantages are summarized in the Table 2.

Mode of generation	Generation techniques	Principle of operation	Nanomaterials	Advantage	Limitation	References
Dry dissemination	Wright dust feeder	The dry powder is packed into the cylinder during preparation. The cylinder rotates while descending, which allows the compacted powder to be scraped by a knife and transported along the blade into a central tube. Compressed air introduced and suspends the powder forming an aerosol.	CNTs	- small amount of material required for generation - small, simple and compact structure - manufactured nanomaterials can be dispersed	- unstable concentration - feeder also cannot be used for every kind of dust	Ellinger-Ziegelbauer and Pauluhn [9] Shvedova et al. [10]
	Brush type aerosol generator	Using kinetic energy from the metal bristles on a rotating circular wire brush dislodges and disperses the powder	CNTs Graphene TiO_2 Al_2O_3 CuO ZnO	- small, simple and compact structure - possible to use the test material as it is manufactured - less test material is required	- possible triboelectric charging may occur from friction while brushing off materials from a pellet	Bermudez et al. [11] Ellinger-Ziegelbauer and Pauluhn [9] Ma-Hock et al. [12] Ma-Hock et al. [13] Ma-Hock et al. [14] Myojo et al. [15]
	Small scale powder disperser (SSPD)	Generator consists of a gas ejector and a turntable that has a spiral groove filled with powder to provide a steady source of particles to rotating turntable. Reduced pressure, generated by compressed air in an ejector, vacuums the powder from the grove	SiO_2	- possible to use the test material as it is manufactured - small and compact structure	- unstable concentration, which is affected by shape or cohesiveness of the particle when vacuuming the particle loaded groove	Baron et al. [16] Scabilloni et al. [17]

		followed by mixing air in the nozzle to form aerosol.			- intertwined and tangled carbon nanotubes may not be vacuumed evenly or particles may stick together - applies relatively weak forces for dispersing an agglomerate	
	Fluidized bed aerosol generator	Particles are dispersed by fluidizing small beads by using high-pressure air. The particles tested clings to the surface of beads. The motion of the beads assists in dispersing the powder. The impact between beads breaks up test powder agglomerates into fine particles.	CNTs	- small, simple and compact structure - possible to use the test material itself	- variable aerosol concentration and alteration of the test substance - ambient humidity, shape and/or cohesiveness of the particles may cause unstable concentration - relatively weak mechanism for dispersing an agglomerate - relatively weak mechanism for dispersing an agglomerate	Fujitani et al. [18]
	Acoustic dry aerosol generator elutriator (ADAGE)	Disperses the test particles by acoustic energy from aggregates/ agglomerates into deaggregated/ deagglomerated particles depending on the frequency and amplitude of energy applied.	CNTs SiO_2 TiO_2	- generates a stable aerosol - suitable for less cohesive powder such as silica ($SiO2$) - possible to use the test material itself	- affected by the ambient humidity	Baron et al. [16] Chen et al. [19] McKinney et al. [20] Porter et al. [21]
	Vilnius aerosol generator	The generator consists of a small compartment with free rotating vanes and a vibrating bottom disperses a powder and generates a dry powder aerosol. The disperser uses a combination of inlet air jets, a vibrating membrane and an air-driven stirring turbine to break up and aerosolize the powder	Aluminum, Nanopowder, Lunar dust	- possible to use the test material as it is manufactured - suitable to generate an aerosol for small volumes of powder - simple structure - possible to generate large amount (1 mg/m^3 to 2 500 mg/m^3) of test aerosol for a long time (0,5 h to 6 h)	- unstable concentration - weak mechanisms for dispersing an agglomerate - test particles may adhere to the vanes, which will hinder the aerosol generation process - unsuitable for generating aerosols from fibrous material	Lam et al. [22] Hussain et al. [23]
	Rotating drum generator	The test particles carried up the side of the drum then dropped, simulating the pouring of a powder aerosolize the particle by the falling motion of powder within a rotating drum	Bentonite, barium sulfate, talc,	- possible to use the test material itself - small, compact and easy to use	- concentration of the generated aerosol is unstable and affected by shape or cohesiveness of the particles	Breum [24] Schneider and Jensen [25]

					- relatively weak mechanisms for dispersing an agglomerate - unsuitable for generating aerosols from fibrous material - differences in concentration of aerosol generated over time	
Wet dissemination	Atomizer/nebulizer	Droplet generated by air pressure or ultrasonic vibration were dried after evaporation. The ultrasonic nebulizer having an electronic oscillator to generate a high frequency signal, that causes the mechanical ultrasonic vibration of a piezoelectric crystal and generates droplets.	CNTs, TiO_2, Nickel oxide, Ag, Au	- particles suspended or dispersed in liquid can be generated as aerosols - small, compact and easy to use	- particles may form from impurities in a solvent such as deionized (DI) water - possible to change the properties of nano-objects such as CNTs by contact with a liquid - concentration of aerosol can also increase over time as the liquid evaporates - difficult to generate particles when the particles are not well or uniformly dispersed	Morimoto et al. [26] Shvedova et al. [27] Pauluhn [28] Eydner et al. [29] Grassian et al. [30] Morimoto et al. [31] Herzog et al. [32] Kim et al. [33] Yu et al. [34] Han et al. [35]
	Electrostatical assist axial atomizer	The generator disperses the test particles by ultrasonic energy and applied electric fields. The concentration is adjusted by altering the solution concentration and flow rate	CNTs	- effective dispersing of CNT by using ultrasonic energy	- possibility of damage of the test substance by ultrasonic and introduction impurities such as biological agents from the DI water	Kim et al. [36]
Phase change	Evaporation/condensation generator	Contact-heater generates particles of pure material by thermal energy. The concentration is adjusted by altering the temperature of the heater and the flow rate	Ag, Au	- simple and stable method of generating metal nanoparticles - produced nanoparticles can be completely contamination free - can obtain high concentrated and non-aggregated nanoparticles	- difficult to generate materials with high melting temperature and low evaporation rate	Sung et al. [37] Jung et al. [38] Sung et al. [39] Ji et al. [40] Ji et al. [41]
	Spark generator	Creating sparks by supply a high voltage into an electrode bar, that is made of bulk material generates	Ag, Au, platinum, TiO_2, carbon black	- can generate nanoparticle aerosols in the entire range (1 nm to 100 nm)	- few commercially available electrodes for	Bitterle et al. [42] Takenaka et al. [43]

		nano-objects from the surface of the electrode bar (bulk material).		- produced nanoparticles can be completely contamination free and composed of one or more materials depending on requirements and the system used	aerosol generation - differences in the properties with the actual NOAA exposed to workers in workplace air	Kreyling et al. [44] Diabaté et al. [45] Takenaka et al. [46]
	Condensation nano-aerosols	Solid nanoparticles (called nuclei) are generated with a few nanometres diameters, then mixed with an atmosphere of vapour produced by heating a semi-volatile material.	Diethylhexyl sebacate, dioctyl phthalate	- might be the only way to make a controlled source of aerosol of appropriate particle sizes and concentration	- limited to materials with appropriate vapour pressure-temperature characteristics and stable under the applied temperatures - coagulation with resulting particle size growth with time may limit the ability to generate high concentrations	Chen et al. [47] TSI [48]
Chemical reaction	Chemical reaction	The particle are generated by chemical reactions and thermal energy in the furnace. It is capable of generating particles with controlled composition and physical properties using various precursors.	SiO_2, Ag	- simple to use, effective method for generating nanomaterials	- by-products are generated - use of inert gas may affect inhalation tests, dilution and other gas conditioning may be required	Sayes et al. [49] Demokritou et al. [50] Ostraat et al. [51]
Liquid phase filtration/ dispersion	Critical point drying and direct injection	Consists of two steps: liquid phase filtration/dispersion followed by critical point drying and direct injection of dispersed dry sample to inhalation chamber. MWCNTs in tertiary butyl alcohol suspension that was in liquid phase were filtered by fine mesh to remove aggregates/agglomerates from the sample. Subsequently, sublimation of MWCNTs in tertiary butyl alcohol suspension allows samples to dry and to be loaded into the cartridge without re-aggregation by surface tension during the drying process. The sample loaded in the cartridge was injected into the subchamber connected upstream of the main whole-body inhalation chamber	MWCNT	- highly dispersed particles without changing size and length distribution	- surface residue and modification needs to be considered	Taquahashi et al. [52]

Table 2: Principle of operation advantage and limitation of NOAA aerosol generators (modified from ISO 19601 Table 4).

Experimental Integration

The NOAA aerosol generator needs to be integrated exposure method with NOAA aerosol concentration, particle properties, electrostatic charge, flow rate, gas concentrations, temperature and relative humidity. The gas stream from the aerosol generator needs to be conditioned before and monitored before introduction to the exposure system. The objective of an NOAA air exposure study is to establish a quantitative relationship between toxicological result and

NOAA exposure in relation to nanomaterial characteristics, precise characterization of the NOAA is essential for an inhalation exposure study. Nanoparticle and nano-object composition number and mass concentrations, median and mean size and size distribution, surface area, electrical charge, surface properties, hygroscopicity and shape are important parameters for dosimetry [6].

Considerations for *In vivo* and *In vitro* Exposure Systems

During preparation of the NOAA aerosol generation system and exposure chamber, aerosol particle composition, size distribution, and purity should be measured. Stability of NOAA concentration in inhalation chamber should be ensured over exposure time period planned. Inhalation chamber and supporting equipment should be prepared in accordance with relevant test guidelines. NOAA aerosol can be deposited on chamber walls by Brownian diffusion and particle size can change due to aggregation/agglomeration. This deposition process depends on the particle size, electrostatic charge, particle number concentration and residence time. To reduce deposition losses, conductive tubing of the minimum length practical to use with the tubing diameter is selected to interface with instrumentation. All the measurement equipment should be calibrated. Recently *in vitro* air exposure study has been developed to reduce substantive time, cost, and animal numbers to substitute traditional *in vivo* study. To be predictive of human effects, *in vitro* air exposure study should include certain parameters in the assay design, 1) the choice of relevant cell types in a physiologically relevant configuration, 2) characterization of the test-material throughout the assay, including life cycle transformations, 3) the choice of realistic test-material concentration and form relevant to real exposures, 4) the use of context-specific dispersants and 5) the use of appropriate exposure route and duration. To compare and assess inhalation toxicity of NOAA, an ALI (air-liquid interface) cell exposure system (rather than submerged cell exposure systems) is preferred as it is more closely resembles *in vivo* conditions in the lungs and allows for physiologically relevant delivery of aerosolized nanoparticles to the cells [7].

Conclusions

Nanotechnology is developing rapidly and expected to affect every aspect of global industry and society. International standardization on nanotechnologies will contribute to improving quality of life, public health all environment, most of all, improving economic development. Currently, many new manufactured nanomaterials coming to market and workplace raise concerns on occupational safety and health. Inhalation is considered to be the primary route by NOAA entering the bodies of workers. Inhalation toxicity testing is a primary test in evaluating hazards of NOAA. To conduct appropriate inhalation toxicity testing, it is important to design or choose appropriate NOAA aerosol generator. This review presents NOAA aerosol generators described in the ISO TR 19601 [8]. The standard providing the status of NOAA aerosol generators, and further discuss the principles of generation the advantages and limitations of the respective NOAA generators.

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Declaration of Interest

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