TECHNICAL REPORT

ISO/TR 19601

First edition
2017-07

Nanotechnologies — Aerosol generation for air exposure studies of nano-objects and their aggregates and agglomerates (NOAA)

Nanotechnologies — Génération d'aérosols pour réaliser des études d'exposition à l'air des nano-objets et de leurs aggrégats et agglomérats (NOAA)

Reference number
ISO/TR 19601:2017(E)

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Foreword

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

Introduction

Inhalation is a primary route of exposure to aerosolized nano-objects and their aggregates and agglomerates (NOAA). The NOAAs include nano-objects with one, two or three external dimensions in the nanoscale from approximately 1 nm to 100 nm, which might be spheres, fibres, tubes and others as primary structures. NOAAs can consist of individual primary structures in the nanoscale and aggregated or agglomerated structures, including those sizes larger than 100 nm. To evaluate the inhalation toxicity of NOAA, it is important to consider certain parameters that make the toxicity testing relevant to human exposure. The three critical aspects to consider when designing and conducting nanomaterial inhalation toxicity study are

a) uniform and reproducible nano-object aerosol generation that is relevant to realistic exposures,

b) thorough characterization of nanomaterials throughout the duration of testing including starting and generated materials, and

c) use of occupational exposure limits (OEL) and reference concentrations (RfC) (as derived from existing studies and/or real-time exposure monitoring data) for dosimetry.

Therefore, to conduct *in vitro* and *in vivo* NOAA, it is important to choose an appropriate NOAA aerosol generator and use online and off-line techniques for nano-object characterization.

Aerosol generation techniques are well established and have been used in laboratory studies, inhalation therapy and industry for many years. A number of aerosol generation techniques are routinely used for other materials that can be adapted for nano-object inhalation toxicity studies. In principle, aerosol generation involves application of some form of energy to the material to reduce its size or to form small particles that are dispersed in a gas stream.

This document provides the status of nano-object aerosol generators. This document further discusses the advantages and limitations of the respective nano-object generators, which can aid in choosing the appropriate generator when conducting the nano-object inhalation toxicity study. No matter what generation system is used for toxicity study, the generated atmospheres should be thoroughly characterized in order to allow for comparison to occupational exposure atmospheres so that a valid risk assessment/occupational exposure limit (OEL) can be developed. Therefore, this document will also provide nano-object aerosol size information generated from respective generators along with the proper nano-object characterization methods. This document complements the work of the Organization for Economic Cooperation and Development (OECD) Working Party on Manufactured Nanomaterial (WPMN) and other related framework documents. Recommendations and guidelines to assist investigators in making appropriate choices of an aerosol generator for their target NOAAs to be tested are presented in this document.

Nanotechnologies — Aerosol generation for air exposure studies of nano-objects and their aggregates and agglomerates (NOAA)

1 Scope

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

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/TS 80004-1, *Nanotechnologies — Vocabulary — Part 1: Core terms*

ISO/TS 80004-2, *Nanotechnologies — Vocabulary — Part 2: Nano-objects*

ISO/TS 80004-4, *Nanotechnologies — Vocabulary — Part 4: Nanostructured materials*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 80004-1, ISO/TS 80004-2, and ISO/TS 80004-4 and the following 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
aerodynamic diameter
diameter of a spherical particle with a density of 1 000 kg/m^3 that has the same settling velocity as the *particle* (3.29) under consideration

Note 1 to entry: Aerodynamic diameter is related to the inertial properties of aerosol particles and is generally used to describe particles larger than approximately 100 nm.

[SOURCE: ISO/TR 27628:2007, 2.2]

3.2
aerosol
metastable suspension of solid or liquid *particles* (3.29) in a gas

[SOURCE: ISO/TR 27628:2007, 2.3]

3.3
agglomerate
collection of weakly bound *particles* (3.29) or *aggregates* (3.4) or *mixtures* (3.17) of the two where the resulting external surface area is similar to the sum of the *surface areas* (3.32) 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* (3.31) and the original source particles are termed *primary particles* (3.30).

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

3.4
aggregate
particle (3.29) comprising strongly bonded or fused particles where the resulting external surface area may be significantly smaller than the sum of calculated *surface areas* (3.32) 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* (3.31) and the original source particles are termed *primary particles* (3.30).

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

3.5
coagulation
formation of larger *particles* (3.29) through the collision and subsequent adhesion of smaller particles

[SOURCE: ISO/TR 27628:2007, 2.6]

3.6
differential electrical mobility classifier
DEMC
classifier that is able to select aerosol particles according to their electrical mobility and pass them to its exit

Note 1 to entry: A DEMC classifies aerosol particles by balancing the electrical force on each particle with its aerodynamic drag force in an electrical field. Classified particles are in a narrow range of electrical mobility determined by the operating conditions and physical dimensions of the DEMC, while they can have sizes due to difference in the number of charges that they have.

[SOURCE: ISO 15900:2009, 2.7]

3.7
differential mobility analysing system
DMAS
system to measure the size distribution of submicrometre aerosol particles consisting of a *DEMC* (3.6), flow meters, a particle detector, interconnecting plumbing, a computer and suitable software

[SOURCE: ISO 15900:2009, 2.8]

3.8
dustiness
propensity of a material to generate airborne dust during its handling

[SOURCE: EN 1540:2011]

3.9
engineered nanomaterial
nanomaterial (3.21) that is rationally designed manufactured

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

3.10
hazard category
division of criteria within each *hazard class* (3.11) as used in Globally Harmonized System of Classification and Labelling of Chemicals (GHS)

3.11
hazard class
nature of the physical, health or environmental hazard as used in Globally Harmonized System of Classification and Labelling of Chemicals (GHS)

[SOURCE: GHS, 2015]

3.12
geometric mean diameter
GMD
measure of central tendency of particle size distribution using the logarithm of particle diameters

Note 1 to entry: The GMD is normally computed from particle counts and when noted may be based on *surface area* (3.32) or particle volume with appropriate weighting, as:

$$\ln(\mathrm{GMD}) = \frac{\sum_{i=m}^{n} \Delta N_i \ln(d_i)}{N}$$

where

d_i is the midpoint diameter for the size channel, i;

N is the total concentration;

ΔN_i is the concentration within the size channel, i;

m is the first channel;

n is the last channel.

[SOURCE: ISO 10808:2010, 3.5, modified]

3.13
geometric standard deviation
GSD
measure of width or spread of particle sizes, computed for the *DMAS* (3.7) by

$$\ln(\mathrm{GSD}) = \sqrt{\frac{\sum_{i=m}^{n} N_i \left[\ln d_i - \ln(\mathrm{GMD})\right]^2}{N-1}}$$

[SOURCE: ISO 10808:2010, 3.6]

3.14
count median diameter
CMD
diameter equal to *GMD* (3.12) for particle counts assuming a logarithmic normal distribution

[SOURCE: ISO 10808:2010, 3.7, modified]

3.15
mass median aerodynamic diameter
MMAD
calculated *aerodynamic diameter* (3.1) which divides the *particles* (3.29) of an *aerosol* (3.2) in half based on mass of the particles

Note 1 to entry: 50 % of the particles by mass will be larger than the median diameter and 50 % of the particles will be smaller than the median.

[SOURCE: EPA IRIS Glossary]

3.16
manufactured nanomaterial
nanomaterial (3.21) intentionally produced to have specific properties or composition

[SOURCE: ISO/TS 80004-1:2015, 2.9, modified]

3.17
mixture
solution composed of two or more *substances* (3.33) in which they do not react

Note 1 to entry: A solution is also a mixture.

[SOURCE: GHS, 2015]

3.18
mobility
<aerosols> propensity for an aerosol particle to move in response to an external influence, such as an electrostatic field, thermal field or by diffusion

[SOURCE: ISO/TR 27628:2007, 2.9]

3.19
nano-aerosol
fluid nanodispersion with gaseous matrix and at least one or more liquid or solid nanophase (including *nano-objects* (3.22))

[SOURCE: ISO/TS 80004-4:2015, 3.5.4]

3.20
nanofibre
nano-object (3.22) with two similar external dimensions in the *nanoscale* (3.25) and the third dimension significantly larger

Note 1 to entry: The largest external dimension is not necessarily in the nanoscale.

Note 2 to entry: The terms nanofibril and nanofilament can also be used.

[SOURCE: ISO/TS 80004-2:2015, 4.5, modified]

3.21
nanomaterial
material with any external dimension in the *nanoscale* (3.25) or having internal or surface structure in the nanoscale

Note 1 to entry: Generic term covering both *nano-object* (3.22) and *nanostructured material* (3.27).

EXAMPLE Nanocrystalline materials, nanoparticle powder, materials with nanoscale precipitates, nanoscale films, nano-porous material, nanoscale emulsions and materials with nanoscale textures on the surface. End products containing nanomaterials (e.g. tires, electronic equipment, coated DVDs) are not themselves nanomaterials.

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

3.22
nano-object
discrete piece of material with one, two or three external dimensions in the *nanoscale* (3.25)

Note 1 to entry: The second and third external dimensions are orthogonal to the first dimension and to each other.

[SOURCE: ISO/TS 80004-1:2015, 2.5]

3.23
nanoparticle
nano-object (3.22) with all external dimensions in the *nanoscale* (3.25) 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* (3.20) or *nanoplate* (3.24) may be preferred to the term nanoparticle.

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

3.24
nanoplate
nano-object (3.22) with one external dimension in the *nanoscale* (3.25) and the other two external dimensions significantly larger

Note 1 to entry: The larger external dimensions are not necessarily in the nanoscale.

[SOURCE: ISO/TS 80004-2, 4.6, modified]

3.25
nanoscale
size range from approximately 1 nm to 100 nm

Note 1 to entry: Properties that are not extrapolations from a larger size will typically, but not exclusively, be exhibited in this size range. For such properties, the size limits are considered approximate.

Note 2 to entry: The lower limit in this definition (approximately 1 nm) is introduced to avoid single and small groups of atoms from being designated as *nano-objects* (3.22) or elements of *nanostructures* (3.26), which might be implied by the absence of a lower limit.

[SOURCE: ISO/TS 80004-1:2015, 2.1, modified]

3.26
nanostructure
interrelation of the constituent parts of a material in which one or more of those constituent parts belong to the *nanoscale* (3.25)

[SOURCE: ISO/TS 80004-1:2015, 2.6, modified]

3.27
nanostructured material
material having internal or surface structure in the *nanoscale* (3.25)

Note 1 to entry: If external dimension(s) are in the nanoscale, the term *nano-object* (3.22) is recommended.

[SOURCE: ISO/TS 80004-1:2015, 2.7, modified]

3.28
nanotube
hollow nanofibre

[SOURCE: ISO/TS 80004-2:2015, 4.8]

3.29
particle
minute piece of matter with defined physical boundaries

Note 1 to entry: A physical boundary can also be described as an interface.

Note 2 to entry: A particle can move as a unit.

Note 3 to entry: This general definition applies to particle *nano-objects* (3.22).

[SOURCE: ISO/TS 26824:2013, 1.1]

3.30
primary particle
original source particle of *agglomerates* (3.3) or *aggregates* (3.4) or *mixtures* (3.17) of the two

Note 1 to entry: Constituent particles of agglomerates or aggregates at a certain actual state may be primary particles, but often the constituents are aggregates.

Note 2 to entry: Agglomerates and aggregates are also termed *secondary particles* (3.31).

[SOURCE: ISO 26824:2013, 1.4]

3.31
secondary particle
particle (3.29) formed through chemical reactions in the gas phase (gas to particle conversion)

[SOURCE: ISO/TR 27628:2007, 2.17]

3.32
surface area
area of external surface plus the internal surface of its accessible macro- and mesopore

Note 1 to entry: Includes mass-specific surface area or volume-specific surface area.

[SOURCE: ISO/TR 13014:2012, 2.28]

3.33
substance
chemical elements and their compounds in the natural state or obtained by any production process, including any additive necessary to preserve the stability of the product and any impurities deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition

[SOURCE: GHS, 2015]

3.34
ultrafine particle
particle (3.29) with a nominal diameter (such as geometric, aerodynamic, *mobility* (3.18), projected-area or otherwise) of 100 nm or less

Note 1 to entry: The term is often used in the context of particles produced as a by-product of a process (incidental particles), such as welding fume and combustion fume.

[SOURCE: ISO/TR 27628:2007, 2.21]

3.35
reference concentration
benchmark estimates of the quantitative dose–response assessment of chronic non-cancer toxicity for individual inhaled chemicals

[SOURCE: EPA, 1994]

4 Abbreviated terms

ADS	aerosol dilution system
ADAGE	acoustic dry aerosol generator elutriator
AERCON	aerosol control unit
ALI	air–liquid interface
APS	aerodynamic particle sizer
CMD	count median diameter
CNT	carbon nanotube
DEHS	diethylhexyl sebacate
DEMC	differential electrical mobility classifier
DI	deionized
DMAS	differential mobility analysing system
DOP	dioctyl phthalate
EDX	energy dispersive X-ray analyser
ELPI	electrical low pressure impactor
EM	electron microscopy
EPA	Environmental Protection Agency
EU	European Union
FBG	fluidized bed aerosol generator
GD	guidance document
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GLP	good laboratory practice
GMD	geometric mean diameter
GSD	geometric standard deviation
MAD	mutual acceptance of data
MFC	mass flow controller
MMAD	mass median aerodynamic diameter
MOUDI	micro-orifice uniform deposit impactor
MWCNT	multi-walled carbon nanotube
NOAA	nano-objects and their aggregates and agglomerates
NM	nanomaterial

OECD	Organization for Economic Cooperation and Development
OEL	occupational exposure limit
OPC	optical particle counter
OPPTS	office of pollution prevention and toxic substances
PSL	polystyrene latex
RfC	reference concentration
RPM	revolutions per minutes
SEM	scanning electron microscope
SNPS	scanning nanoparticle sizer
SPSF	standard project submission form
SSPD	small scale powder disperser
SWCNT	single-walled carbon nanotube
TEM	transmission electron microscope
TEOM	tapered element oscillating microbalance
TG	test guideline
TGA	thermogravimetric analysis
VAG	vilnius aerosol generator
WPMN	working party on manufactured nanomaterials

5 Study design considerations

5.1 General

Inhalation toxicity studies are important for evaluating the health risk of workers and the general population exposed to aerosolized NOAA. In designing an inhalation study for NOAA and selecting the proper generator, it is important to consider possible workplace exposure scenarios and existing inhalation toxicity testing guidelines.

5.2 Workplace exposure scenario

When designing an inhalation toxicity study for NOAA exposure, the actual workplace exposure scenario should be considered. Generation of NOAA aerosol should simulate actual workplace NOAA emissions and exposure in terms of concentration (mass or number based if known), shape, size and distribution size of NOAA, frequency of exposure, and handling and manufacturing conditions. For inhalation experiments, the starting NOAA could be in powder form, well or poorly suspended in liquid media or solid-state material as generated by condensation/evaporation or spark generation. Various methods of NOAA generation could be adopted to generate NOAA aerosols to simulate an actual exposure situation.

5.3 Existing inhalation toxicity testing guidelines

Generation of aerosols should be adapted to the needs described in existing inhalation testing guidelines, such as OECD Test Guideline (TG) 412, 413, 436 or Guidance Document (GD) 39[6][7][8][14][15] or equivalent or relevant national or international guidelines. The OECD is currently (2016) adapting the inhalation toxicity TGs to nanomaterials, adding investigations of effects in the lung after exposure to nanoparticles. The particle sizes described also for nanoparticles have an unchanged mass median aerodynamic diameter (MMAD) of up to 2 µm with a geometric standard deviation (GSD) up to 3.

5.4 Globally harmonized system of classification and labelling of chemicals (GHS)

The outcomes of the inhalation toxicity study may be used for hazard evaluation, classification and labelling according to GHS. In GHS, concentration ranges are used to describe the severity of toxicity, and this include ranges for inhalation toxicity, also valid for NOAA in a GHS context. The concentration units used are milligram per litre (mg/l) per time unit, which can be hour, 4 h or day, and the values triggering classification are from 0,05 to 5. See Table 1 to Table 3. For further information on GHS, consult the United Nations website at https://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev04/English/ST-SG-AC10-30-Rev4e.pdf.

Table 1 — Inhalation toxicity hazard category

Exposure route	Category 1	Category 2	Category 3	Category 4
Dust and mist (mg/l)	0,05	0,5	1,0	5

Table 2 — Guidance values for specific target organ toxicity (single exposure)

Route of exposure	Units	Category 1	Category 2
Inhalation (rat) dust/mist/fume	mg/l/4 h	$C \leq 1,0$	$5,0 \geq C \geq 1,0$

Table 3 — Guidance values for specific target organ toxicity (repeated exposure)

Route of exposure	Units	Category 1	Category 2
Inhalation (rat) dust/mist/fume	mg/l/6 h/day	$C \leq 0,02$	$0,02 < C \leq 0,2$

6 Considerations in selection of proper generators

6.1 Basic scheme

6.1.1 Flow chart

The basic scheme is described in Figure 1.

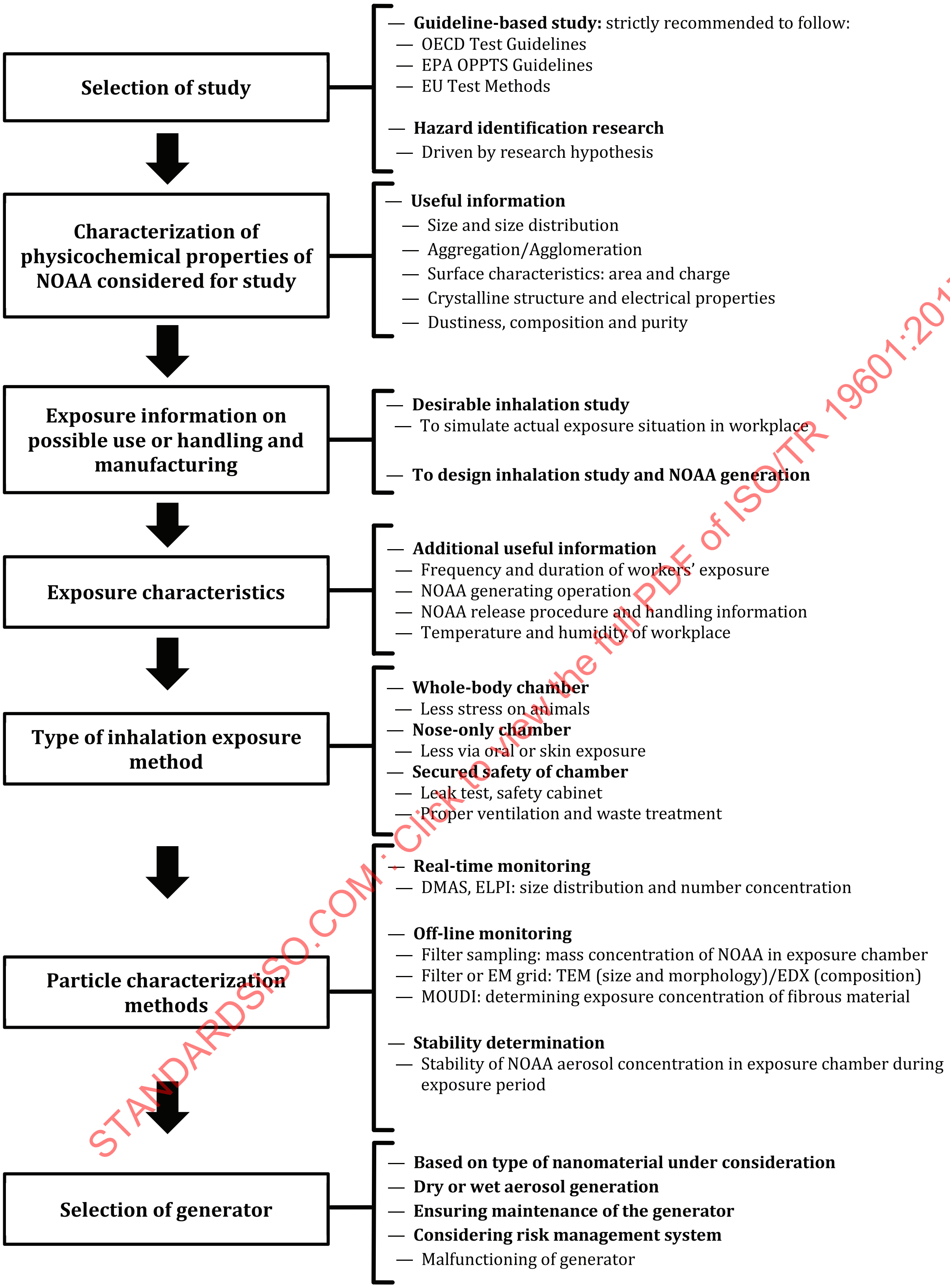


Figure 1 — Basic scheme in selection of proper generators

6.1.2 Selection of study

Studies should be conducted based on the standardized testing guidelines such as the ones put forth by OECD (TG 403, 412, 413 and 436)[6][7][8][14], EPA OPPTS (870.1300, 870.3465 and 870.4100[16][17][18]) or the EU. It is recommended to strictly follow relevant test guidelines to determine the number of animals in the study, duration of exposure and the observation period and test material characterization (including concentration and particle size measurement and frequency of measurements). A study can be conducted according to good laboratory practice (GLP), which is regulatory requirement for new studies in some regions, (preferred) or non-GLP.

Also, studies from scientific research sources can be used for hazard identification of NOAA and the study design is usually more flexible than a test guideline based study. Of note here is that such studies are usually performed to prove a research hypothesis and are not recognized by the OECD mutual acceptance of data (MAD) principle. The study results can be published in a scientific journal or retained for in-house use.

6.1.3 Characterization of physicochemical properties of nanomaterials

The physicochemical characterization of the nanomaterial (NM) as manufactured is important before generation of a NOAA aerosol or *in situ* generation. NMs are manufactured using diverse synthesis procedures that impart those unique properties designed for specific applications. As a result, the materials might have a complex structure, include impurities and have different surface properties (coatings or other modifications). Such physicochemical properties dictate the toxicological output of NMs and should therefore be thoroughly characterized. Useful information on physicochemical properties includes, but is not limited to, particle size, size distribution, shape, aggregation/agglomeration, surface characteristics such as area and charge, crystalline structure, dustiness, composition and purity. Trace impurities such as presence of endotoxin, metal catalyst residue or impurities from the raw materials might affect the toxicity outcome[12][19]to[25]. Therefore, characterization of NM is critical to ascertain that the NM properties match the claims made by the manufacturer before any study initiation. Several international standards are currently available to characterize NMs. For instance, for characterization of single-wall carbon nanotubes (SWCNT), there are standards for using transmission electron microscopy (TEM)[26], scanning electron microscopes with energy dispersive X-ray spectroscopy (SEM-EDX)[27], near infrared (NIR)[28], thermogravimetric analysis (TGA)[29], characterization of volatile components in SWCNT samples using evolved gas analysis/gas chromatograph-mass spectrometry[30] and characterization of multi-wall carbon nanotubes (MWCNT) — mesoscopic factors[31]. Some other methods, such as Raman spectroscopy, that are not internationally standardized can be found in ISO/TR 13014. However, experimentation with the test NOAA is required before any study initiation to ascertain that the particle size distribution is representative of the size distribution observed during typical handling and use. Agglomerates of primary particles are present in most workplace exposures.

6.1.4 Exposure information on possible use or handling and manufacturing

A desirable inhalation study should simulate an actual exposure situation. Exposure information on NOAA in terms of particle mass, concentration, number, size, dispersion (dispersed or aggregated/agglomerated) or shape is very useful in designing the inhalation study. Depending upon this information, particle shape and concentration similar to realistic exposures could be determined.

6.1.5 Exposure characteristics

In addition to the exposure information described above, information on exposure characteristics such as frequency and duration of workers' exposure, worker activities, NOAA manufacturing operation, NOAA source release scenarios and handling information would be very useful in designing an inhalation study. Additional information on workplace temperature and humidity is also useful information.

6.1.6 Types of inhalation exposure methods

Two types of exposure chambers are currently widely used for *in vivo* inhalation studies: whole-body chamber and nose-only chamber. The preferred method is whole-body exposure as it is more relevant to human exposure and causes the least pain, suffering and distress to animals. Nose-only exposure reduces oral and skin exposure potential and requires lesser quantity of test materials as compared with whole-body exposure. The choice of the exposure chamber should be based on the study design. The chambers should be secured with safety measures, such as leak-proof construction and leak testing, use of a safety cabinet, proper ventilation with exhaust emissions control and waste treatment.

6.1.7 Particle characterization method

6.1.7.1 Real-time monitoring

Use of real-time particle size and number monitoring devices are desirable. Such real-time monitors, including differential mobility analysing system (DMAZ) and electrical low pressure impactor (ELPI) will give particle size distribution information, as well as particle number concentration in real-time. From these instruments, the concentration stability of test atmospheres can be monitored in real time.

6.1.7.2 Off-line monitoring

Off-line filter sampling can also be used to determine mass concentration of NOAA in the inhalation chamber. For instance, the micro-orifice uniform deposit impactor (MOUDI) could be used to determine time-resolved aerosol particle size exposure concentration by mass including fibrous materials. In addition, off-line filter or EM grid sampling can be processed for TEM observation for size and morphology of NOAA and analysed further for composition by energy dispersive X-ray analyser (EDX). Furthermore, filter samples can be analysed for chemical composition.

6.1.7.3 Stability determination

Nanomaterial concentration should be determined according to test guidelines or test protocols. The stability of NOAA aerosol concentration in the inhalation chamber during the exposure period should be monitored regularly, desirably in real-time. If the concentration deviates more than 20 % during exposure period, stability needs to be improved[8].

6.1.7.4 Selection of generator

The selection of an aerosol generator should consider the following 5.1 to 5.4, electrical charge neutralization and production of undesirable by-products during the generating process requiring dilution or removal. After selecting the generator for NOAA, the maintenance of the generator should be ensured. In prevention of situations of unacceptable risk, such as malfunctioning of the generator, dust explosions and fire, a risk management approach should be considered and risky situations avoided. The description of the types and characteristics of the generators in Clause 6 should be used to select an appropriate generator for the study. It is important to assess the generator's performance for the proposed study and NOAA to be generated with regard to time of generation required, ease of maintenance and refilling.

7 NOAA aerosol generators

7.1 General

Advantages and limitations of generation techniques are listed in Table 4.

Table 4 — Advantages and limitations of generation techniques

Mode of generation	Generation techniques	Advantage	Limitation
Dry dissemination	Wright dust feeder	— 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
	Brush type aerosol generator	— 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
	Small scale powder disperser	— 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 — 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	— 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 — fibrous test substances may exhibit breaking of individual fibres
	Acoustic dry aerosol generator elutriator	— generates a stable aerosol — suitable for less cohesive powder such as silica (SiO_2) — possible to use the test material itself	— affected by the ambient humidity
	Vilnius aerosol generator	— 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

Table 4 *(continued)*

Mode of generation	Generation techniques	Advantage	Limitation
	Rotating drum generator	— 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 — 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	— 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
	Electrostatic assist axial atomizer	— 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
Phase change	Evaporation/ condensation generator	— 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
	Spark generator	— can generate nanoparticle aerosols in the entire range (1 nm to 100 nm) — produced nanoparticles can be completely contamination free and composed of one or more materials depending on requirements and the system used	— few commercially available electrodes for aerosol generation — differences in the properties with the actual NOAA exposed to workers in workplace air

Table 4 *(continued)*

Mode of generation	Generation techniques	Advantage	Limitation
	Condensation nano-aerosols	— 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
Chemical reaction	Chemical reaction	— 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
Liquid phase filtration/dispersion	Critical point drying and direct injection	— highly dispersed particles without changing size and length distribution	— surface residue and modification needs to be considered

7.2 Dry dissemination

7.2.1 Wright dust feeder (see Figure 2)

7.2.1.1 Principle of operation

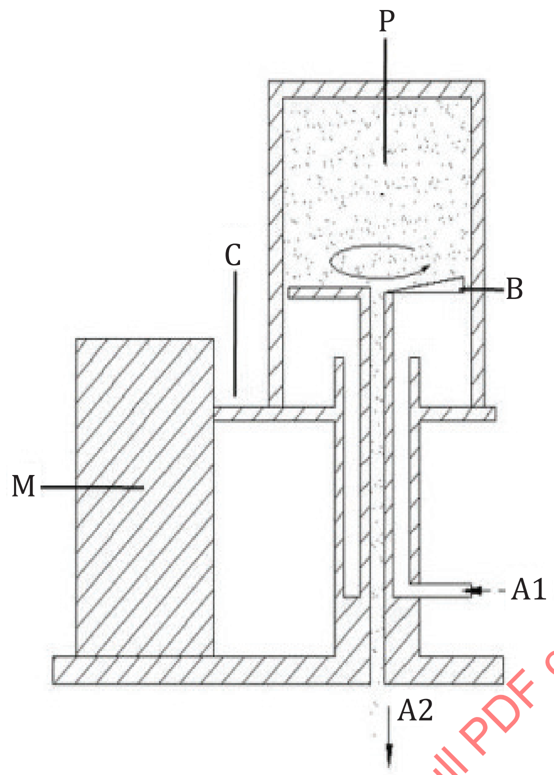
The dry powder is packed into the cylinder during preparation. During usage, the cylinder rotates while descending, which allows the compacted powder to be scraped by a knife and transported along the blade into a central tube. Compressed air introduced at A1 suspends the powder forming an aerosol. The aerosol exits at A2. The A2 is connected to the remaining system for introduction into the exposure area. The concentration of aerosol generated is determined by the rotational speed. For example, size distribution results; see Annex A[32][33].

7.2.1.2 Advantages

Advantages include suitability for small amounts of material required for generation. The structure and size of generator are small, simple and compact. Manufactured nanomaterials can be dispersed. The particle size distributions of CNTs measured by cascade impactor are MMAD 2,2 µm~2,9 µm and GSD 1,7~2,6 and for APS are MMAD 1,9 µm~2,0 µm and GSD 1,6~1,7[32].

7.2.1.3 Limitations

Limitations include unstable concentration. Uniformity of feed is dependent upon the way the dust is packed in the dust cup. The feeder cannot be used for all types of dust, especially soft materials with high cohesive forces (e.g. coal dust). The degree of agglomeration is subject to the concentration of water vapour in the air[34].



Key

A1 clean air
A2 aerosol
B rotating blade
C cylinder
M motor
P powder

Figure 2 — Schematic diagram of wright dust feeder

7.2.2 Brush type aerosol generator (see Figure 3)

7.2.2.1 Principle of operation

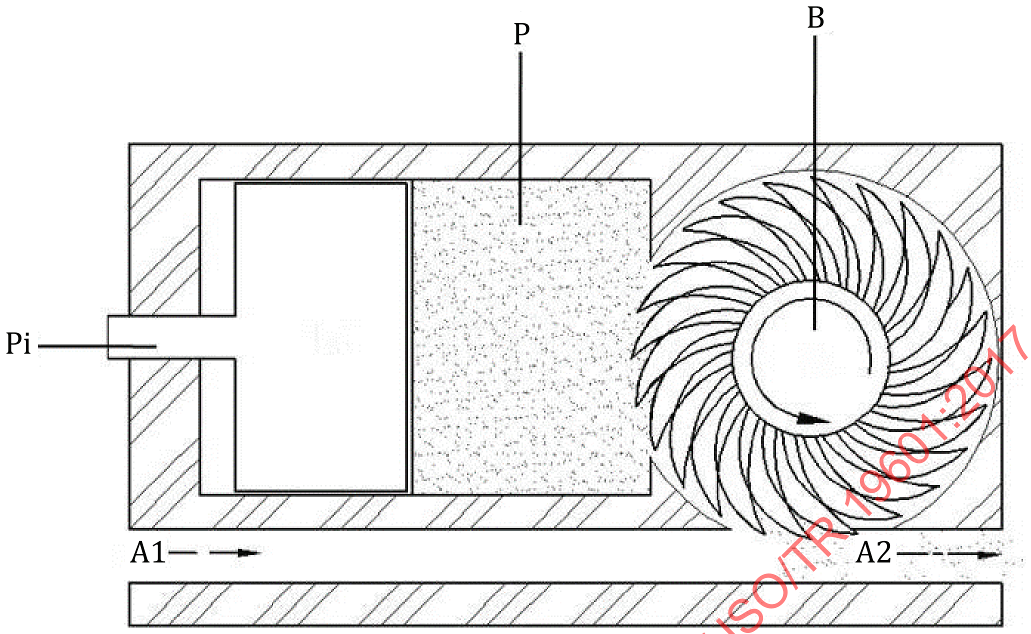
The kinetic energy from the metal bristles on a rotating circular wire brush dislodges and disperses the particles. Compressed air is used to suspend and transport the material. Adjustments of the amount of dust generated is made by altering the rotational speed of the brush per minute and powder supply rate[33][35][36].

7.2.2.2 Advantages

Advantages include simple structure, compact and small size. It is possible to use the test material as it is manufactured. Sufficient energy can be applied to disperse the particles. Less test material is required than nebulization and it is more appropriate for dry powders[37].

7.2.2.3 Limitations

Possible triboelectric charging may occur from friction while brushing off materials from a pellet. The charging may influence the generated particle size[37].



Key

A1 clean air

A2 aerosol

B brush

P powder

Pi piston

Figure 3 — Schematic diagram of brush type generator

7.2.3 Small scale powder disperser (SSPD; see Figure 4)

7.2.3.1 Principle of operation

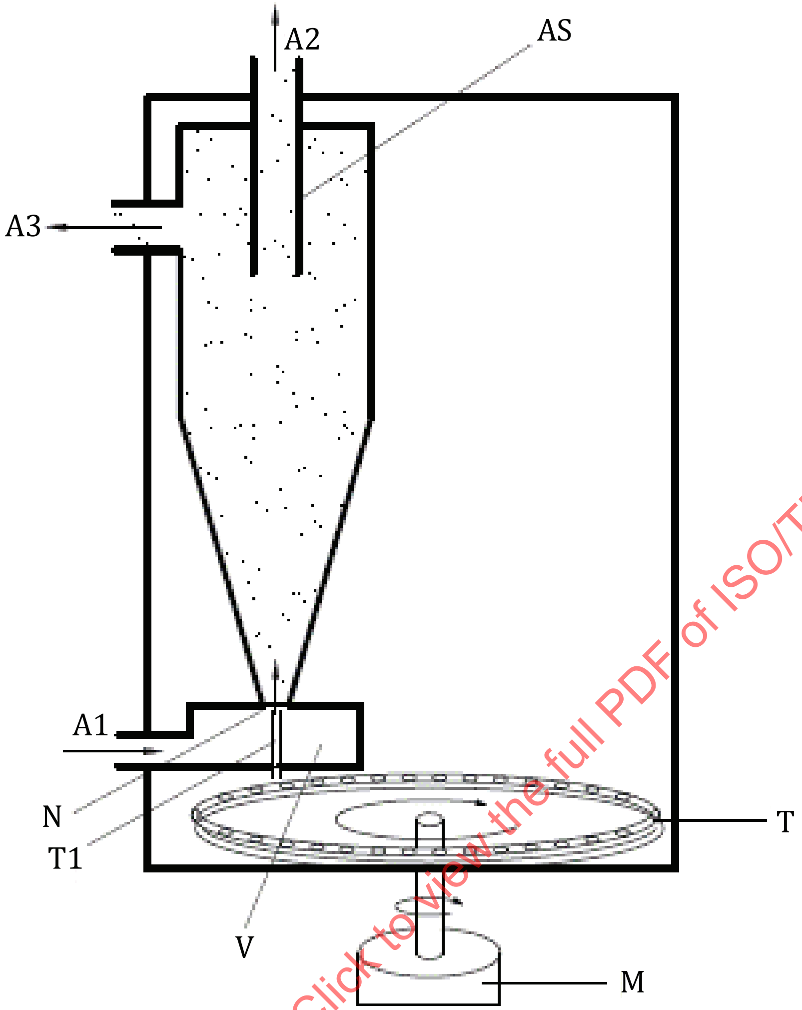
The generator consists of a gas ejector and a turntable that has a spiral groove filled with powder to provide a steady source of particles as the turntable rotates. Reduced pressure, generated by a source of compressed air in an ejector constructed from a tube located in a Venturi nozzle, vacuums the powder from the grove followed by mixing with air in the nozzle to form an aerosol. The output is controlled by rotational speed of the turntable and the pressure of the compressed air supply[39][40].

7.2.3.2 Advantages

The advantages include that it is possible to use the test material as it is manufactured, the SSPD is compact and it is small in size. The SSPD is also suitable for generating aerosols from fibrous materials[38].

7.2.3.3 Limitations

Limitations include unstable concentration, which is affected by shape or cohesiveness of the particle when vacuuming the particle loaded groove. Particles such as intertwined and tangled CNTs may not be vacuumed evenly or particles may stick together. The SSPD applies relatively weak forces for dispersing an agglomerate. The test substance may adhere to the turntable by particle to surface adhesion or electrostatic forces. The differences in amount of aerosol generated may depend on the cohesiveness of various powders. The SSPD is also sensitive to back pressure limiting the use of cyclones or impactors downstream to reduce the large particle fraction.



Key

A1 clean air
A2 aerosol
A3 excess flow
AS aerosol splitter
M motor
N nozzle
T turntable
T1 tube
V Venturi throat

Figure 4 — Schematic diagram of small scale powder disperser

7.2.4 Fluidized bed aerosol generator (FBG; see Figure 5)

7.2.4.1 Principle of operation

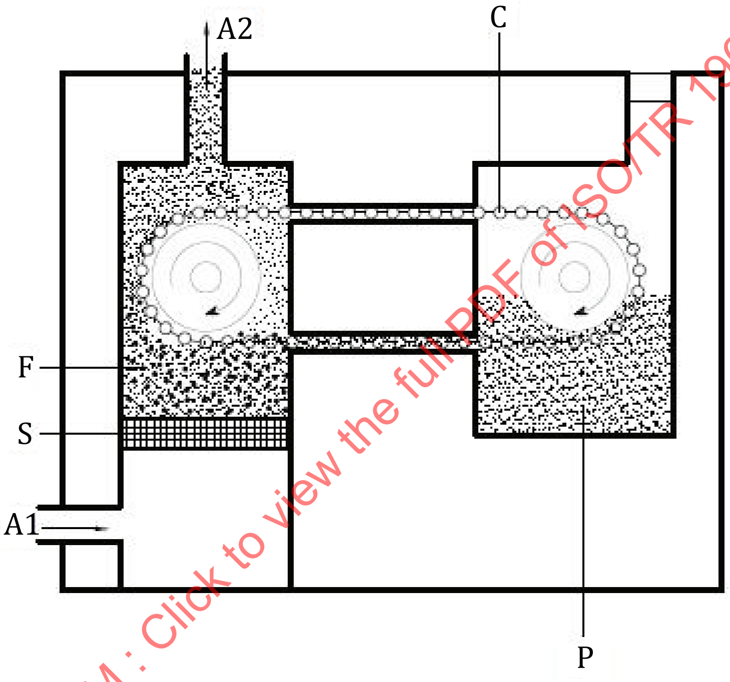
Particles are dispersed by fluidizing small beads (about 0,1 mm in diameter) by using high-pressure air. The substance tested clings to the surface of the bronze or silica beads (see Figure 5). The motion of the beads assists in dispersing the powder. The impact between beads breaks up test powder agglomerates into fine particles. The amount of aerosol generated can be adjusted by altering the revolutions per minutes (RPM) of the drive to the powder transport chain and supply flow rate[41].

7.2.4.2 Advantages

Advantages include simple structure, compactness and small size. It is possible to use the test material itself.

7.2.4.3 Limitations

Limitations include a variable aerosol concentration and alteration of the test substance. Ambient humidity, as well as the shape or cohesiveness of the particles may cause unstable concentration. For materials such as CNT, the change in concentration is largely dependent on time[41]. The relatively weak mechanism for dispersing an agglomerate is also an issue. Fibrous test substances may exhibit breaking of individual fibres.



Key

A1 clean air

A2 aerosol

C chain to transport powder

F fluidized beads

P powder

S porous plate

Figure 5 — Schematic diagram of fluidized bed aerosol generator

7.2.5 Acoustic dry aerosol generator elutriator (ADAGE; see Figure 6)

7.2.5.1 Principle of operation

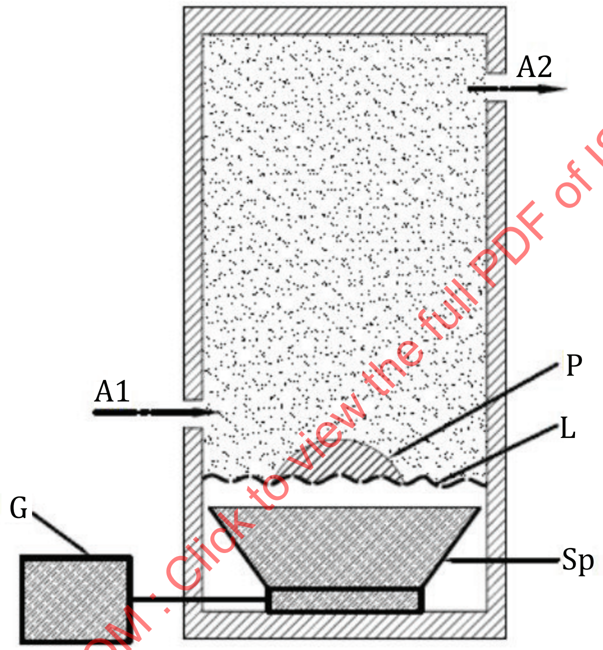
This generator disperses the test material by acoustic energy. Aggregates/agglomerates can be deaggregated/deagglomerated depending on the frequency and amplitude of energy applied. The amount of aerosol generated is adjusted by altering the frequency of the acoustic waves, quantity of test material and air supply flow rate[33][42][43][44].

7.2.5.2 Advantages

The ADAGE generates a rather stable aerosol and functions best as a dry aerosol generator. The ADAGE is, in particular, more suitable for less cohesive powder such as silica (SiO_2)[41]. It is also possible to use the test material itself.

7.2.5.3 Limitations

Limitations include that ADAGE is affected by the ambient humidity. The concentration of other materials may be time-dependent[41] compared with the generation stability of ADAGE and SSPD using TiO_2, SiO_2 and SWCNT. It was found that SiO_2 concentration generated by ADAGE used in the study fluctuated only about 4 % from the average concentration over a period of approximately 30 min. The generator is large and the structure is complex. The generation by ADAGE may be affected by the shape or cohesiveness of the particle.



Key

A1 clean air
A2 aerosol
Sp speaker
L latex sheet
G amplifier and frequency generator
P powder

Figure 6 — Schematic diagram of acoustic dry aerosol generator elutriator

7.2.6 Vilnius aerosol generator (VAG; see Figure 7)

7.2.6.1 Principle of operation

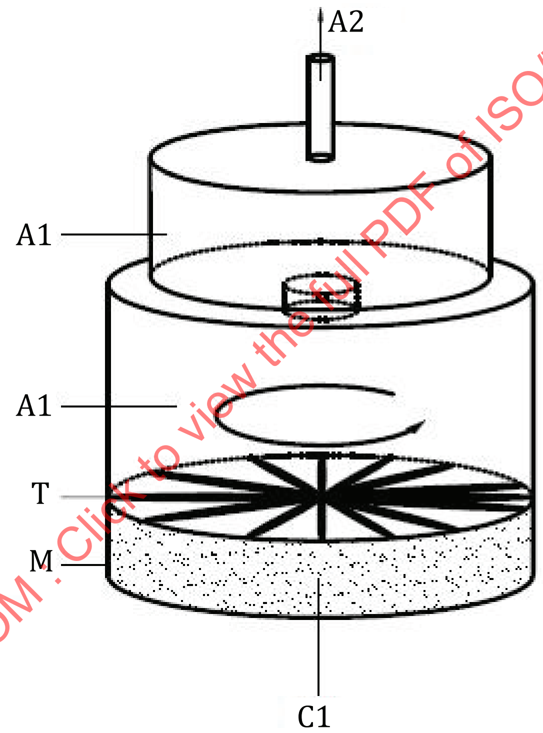
The generator consists of a small compartment with free rotating vanes and a vibrating bottom. The apparatus disperses a powder and generates a dry powder aerosol. The VAG consists of a controller and a disperser. The disperser uses a combination of inlet air jets, a vibrating membrane and an air-driven stirring turbine to break up and aerosolize the powder[45]. The aerosol is generated by four jets, which swirl and lift air-containing powder. Adjustments of the amount of aerosol are done through the flow rate of the jets.

7.2.6.2 Advantages

Advantages include that it is possible to use the test material as it is manufactured. Another advantage of the VAG is that it is suitable to generate an aerosol using small volumes of powder. The structure of VAG apparatus is simple. It is also possible to generate large amounts (1 mg/m^3 to 2 500 mg/m^3) of test aerosol for a long time (0,5 h to 6 h). It has been used for *in vitro* air exposure study[47] as well as a lunar dust inhalation exposure study[47].

7.2.6.3 Limitations

Limitations include unstable concentration that is affected by shape or cohesiveness of the particle. There are relatively weak mechanisms for dispersing an agglomerate. Test particles may adhere to the vanes, which will hinder the aerosol generation process. The VAG is unsuitable for generating aerosols from fibrous material. The differences in amount of aerosol generated also depends on the cohesiveness of the powder.



Key

A1 clean air

A2 aerosol

C1 dust chamber

T rotating vibrating turbine

M membrane

Figure 7 — Schematic diagram of vilnius aerosol generator

7.2.7 Rotating drum generator (see Figure 8)

7.2.7.1 Principle of operation

The generator aerosolizes the test material by the falling motion of powder within a rotating drum. The test material is carried up the side of the drum then dropped, simulating the pouring of a powder. The purpose is to generate dust representative with quantities and characteristics of industrial operations.

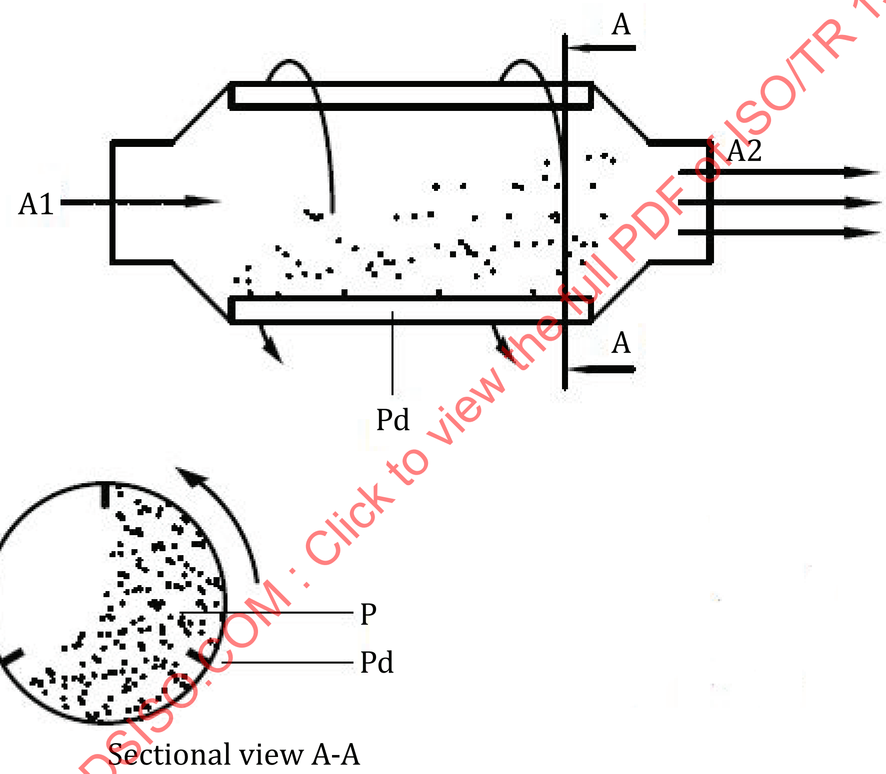
The flow of compressed air and RPM can be used to adjust the output[48][49][50]. The technique is used for standardized dustiness testing of powders[3].

7.2.7.2 Advantages

Advantages include that is it possible to use the test material itself and the rotating drum generator is small in size. It is also compact and simple to use. It might be desirable to relate an inhalation study to a standard dustiness test method.

7.2.7.3 Limitations

Limitations include that the concentration of the generated aerosol is unstable and affected by the shape or cohesiveness of the particles. The rotating drum has relatively weak mechanisms for dispersing an agglomerate. It is unsuitable for generating aerosols from fibrous material. There are also differences in concentration of aerosol generated over time.



Key

A1 clean air

A2 aerosol

P powder

Pd paddle

Figure 8 — Schematic diagram of rotating drum generator

7.3 Wet dissemination

7.3.1 Atomizer/nebulizer (see Figure 9 to Figure 11)

7.3.1.1 General

The atomizer/nebulizer is suitable for generating aerosols from soluble material in solution (e.g. NaCl and KCl) and non-soluble particle suspensions [e.g. polystyrene latex (PSL) and CNT]. Atomizers and nebulizers are classified according to the method by which they generate droplets depending on how the energy is delivered to the liquid, for example, air pressure or ultrasonic vibration. The atomizer/nebulizer can generate aerosols of different particle concentrations by altering the number of nozzles and the airflow rate. Conventional atomizers require a source of compressed air available from either in-house facilities or a portable compressor. The size of the resulting dry particles after evaporation depends on the concentration of suspended particles and dissolved solids and the size of the droplet generated by the nebulizer[51]to[56]. The size distribution of generated droplets is usually quite disperse with a geometric standard deviation of about 2[57]. If only dissolved solids are nebulized, dry residue particles after evaporation of the liquid will have a similar geometric standard deviation as the original droplets and sizes depending on the volume of the droplet lost by evaporation. The results for insoluble particles are more complex. The large droplet fraction of the droplet size distribution may result in aggregates formed from the original particles after drying because several particles may be contained in the original droplet. The small droplet fraction of the droplet size distribution may contain only dissolved solids and result in much smaller particles after drying. Usually, the concentration is adjusted for particle suspensions so that the majority of the dry residue particles contain only one of the original particles[38].

Another type of nebulizer is the ultrasonic nebulizer. The ultrasonic nebulizer has an electronic oscillator to generate a high frequency signal, which causes the mechanical ultrasonic vibration of a piezoelectric crystal. This vibrating element is in contact with a liquid reservoir and its high frequency vibration is sufficient to produce a mist[58].

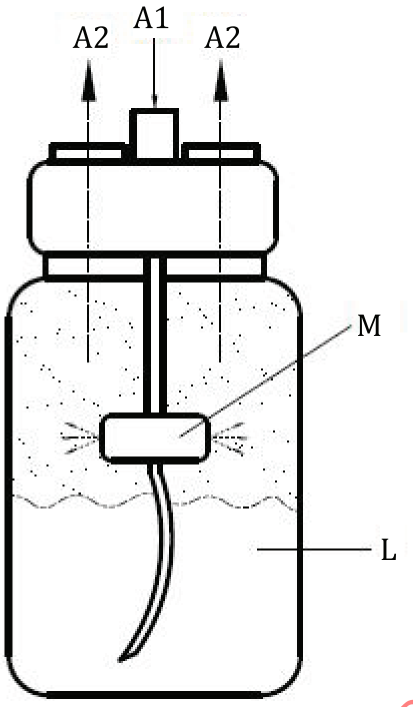
7.3.1.2 Advantages

Advantages of the atomizer/nebulizer include that particles suspended or dispersed in liquid can be generated as aerosols. The atomizer/nebulizer is also small in size, compact in shape and easy to use compared with dry disperser.

In comparison, ultrasonic nebulizers generate aerosols by ultrasonic vibration. They are lightweight generators. Another advantage is that the ultrasonic vibration is almost silent.

7.3.1.3 Limitations

The limitations include the possibility of particles formed from impurities in a solvent such as DI water (in this case, biological impurities). It might be possible to change the properties of nano-objects such as CNTs by contact with a liquid. The concentration of aerosol can also increase over time as the liquid evaporates. Atomizers/nebulizers have difficulties to generate particles when the particles are not well or uniformly dispersed.



Key

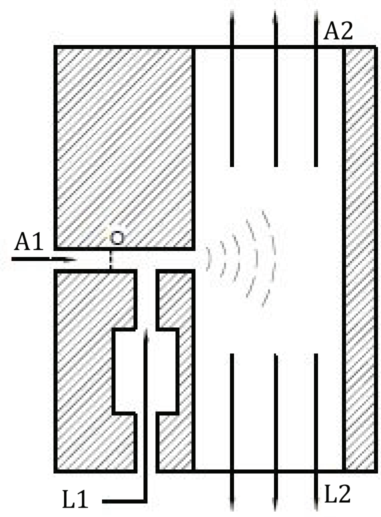
A1 clean air

A2 aerosol

L liquid suspension/solution

M multi-jet nozzles

Figure 9 — Schematic diagram of multi-jet nozzle atomizer



Key

A1 clean air

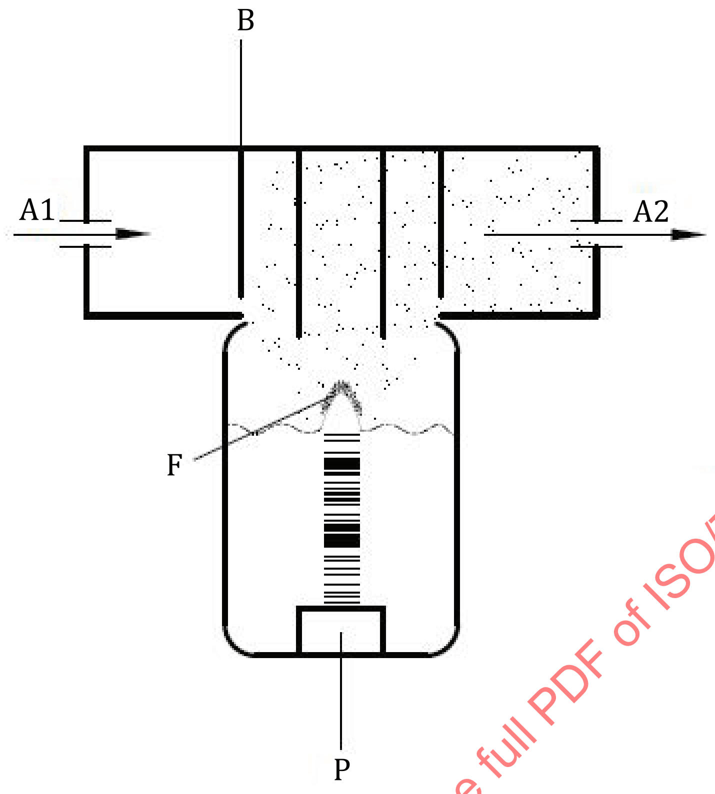
A2 aerosol

L1 liquid in

L2 excess liquid

O orifice

Figure 10 — Schematic diagram of atomizer nozzle cross-section



Key

A1 clean air

A2 aerosol

B baffle

F fountain, generated

P piezoelectric transducer

Figure 11 — Schematic diagram of ultrasonic nebulizer

7.3.2 Electro-static assist axial atomizer (see Figure 12)

7.3.2.1 Principle of operation

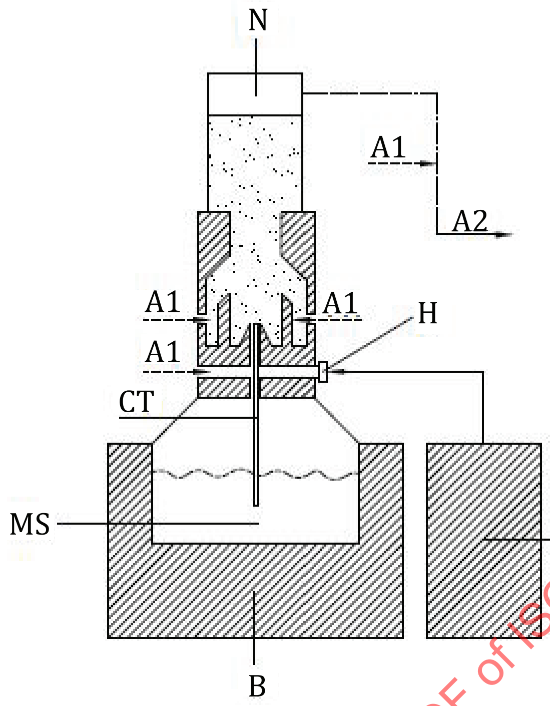
The generator disperses the test material by ultrasonic energy and applied electric fields. The concentration is adjusted by altering the solution concentration and flow rate[59].

7.3.2.2 Advantages

Advantages include effective dispersing of CNT by using ultrasonic energy. The clogging of capillary tubes is prevented by using a relatively large diameter tube.

7.3.2.3 Limitations

The limitations include the possibility of damage of the test substance by ultrasonic and the introduction of impurities such as biological agents from the DI water.



Key

A1 clean air
A2 aerosolized MWCNT
B ultrasonic and heating bath
CT capillary tube
H high voltage connector
MS MWCNT suspension
N neutralizer
P high voltage power supply

Figure 12 — Schematic diagram of CNT and MWCNT aerosol generator

7.4 Phase change

7.4.1 Evaporation/condensation generator (see Figure 13)

7.4.1.1 Principle of operation

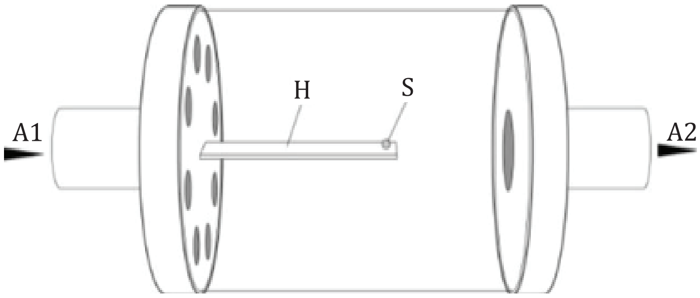
The generator uses a contact-heater. It generates particles of pure material by thermal energy (Ag, Au, etc.) The concentration is adjusted by altering the temperature of the heater and the flow rate[60][61][62][63].

7.4.1.2 Advantages

This is a simple and stable method of generating metal nanoparticles. The produced nanoparticles can be completely contamination free. With air as the carrier gas under atmospheric pressure, metal nanoparticles such as silver and gold can be easily generated. This simple method can obtain high concentrations and non-aggregated nanoparticles. The size distribution of nanoparticles can be precisely controlled by changing the surface temperature of the heater[65]. The geometric mean diameter and the total number of concentration of nanoparticles remaining can stay stable for about 24 h[60].

7.4.1.3 Limitations

Limitations include difficulty in generating materials with a high melting temperature and low evaporation rate.



Key

A1 clean air

A2 aerosol

H heater

S metal pellet

Figure 13 — Schematic diagram of hot plate generator

7.4.2 Spark generator

7.4.2.1 Principle of operation (see Figure 14)

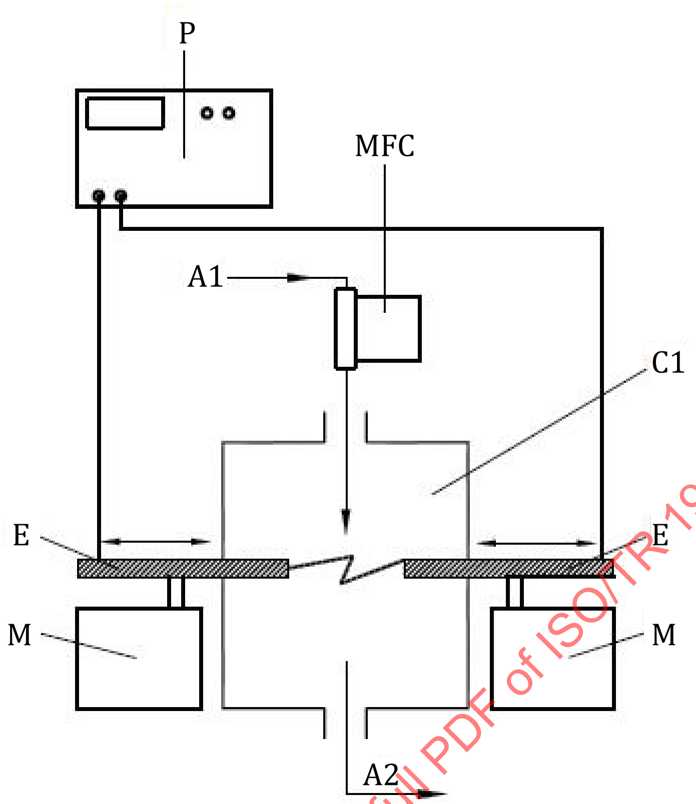
The generator creates sparks by supplying a high voltage into an electrode bar, which is made of bulk material (see Figure 14). Nano-objects are emitted from the surface of the electrode bar (bulk material) operated by stepping motor. The concentration is adjusted by altering the frequency of the spark and flow rate[65][66].

7.4.2.2 Advantages

The spark generator can generate nanoparticle aerosols in the entire range (1 nm to 100 nm). The produced nanoparticles can be completely contamination free and composed of one or more materials depending on the requirements and the system used[66].

7.4.2.3 Limitations

Limitations include few commercially available electrodes for aerosol generation and differences in the properties with the actual NOAA exposed to workers in workplace air.



Key

A1 carrier gas
A2 aerosol
C1 spark generator chamber
E electrode
M stepping motor
MFC mass flow controller
P power supply

Figure 14 — Schematic diagram of spark generator

7.4.3 Condensation nano-aerosols

7.4.3.1 Principle of operation

The other clauses of this document have covered NOAA formed from solid phase particles with low volatility. This subclause, included for completeness, covers nanoscale aerosols composed of semi-volatile particles. The principle of operation is a classic aerosol generation method described in Reference [67]. First, solid nanoparticles (called nuclei) are generated with diameters of a few nanometres. The nuclei are then mixed with an atmosphere of vapour produced by heating a semi-volatile material. Nearly monodisperse aerosol is formed by cooling the vapour and nuclei under controlled laminar flow conditions. By manipulating the generation conditions and materials, aerosols ranging from 30 nm to greater than 2 000 nm have been generated with a geometric deviation of 1,2 to 1,3 for concentrations as high as 10^{13} particles per m^3 [68]. A commercial version is available to generate aerosols with particle diameters greater than 100 nm using materials such diethylhexyl sebacate (DEHS), dioctyl phthalate (DOP), Emery 3004 or paraffin or carnauba waxes[69].

7.4.3.2 Advantages

The generation of condensation nano-aerosols may be required to achieve a specific study aim. The Sinclair LaMar technique might be the only way to make a controlled source of aerosol of appropriate particle sizes and concentration.

7.4.3.3 Limitations

Application of the method to materials not found in the related supporting literature might need a developmental effort. The technique is generally limited to materials with appropriate vapour pressure–temperature characteristics and with stability under the applied temperatures. Potentially, generating a novel material might require developing custom equipment. In health studies, consideration needs to be given to the material selected to generate the nuclei particles in addition to the condensed material. Since the aerosol generator involves thermal processes, the resulting aerosol needs to be cooled to temperatures suitable for toxicology studies. Coagulation with resulting particle size growth with time might limit the ability to generate high concentrations.

7.5 Chemical reaction

7.5.1 Combustion

7.5.1.1 Principle of operation

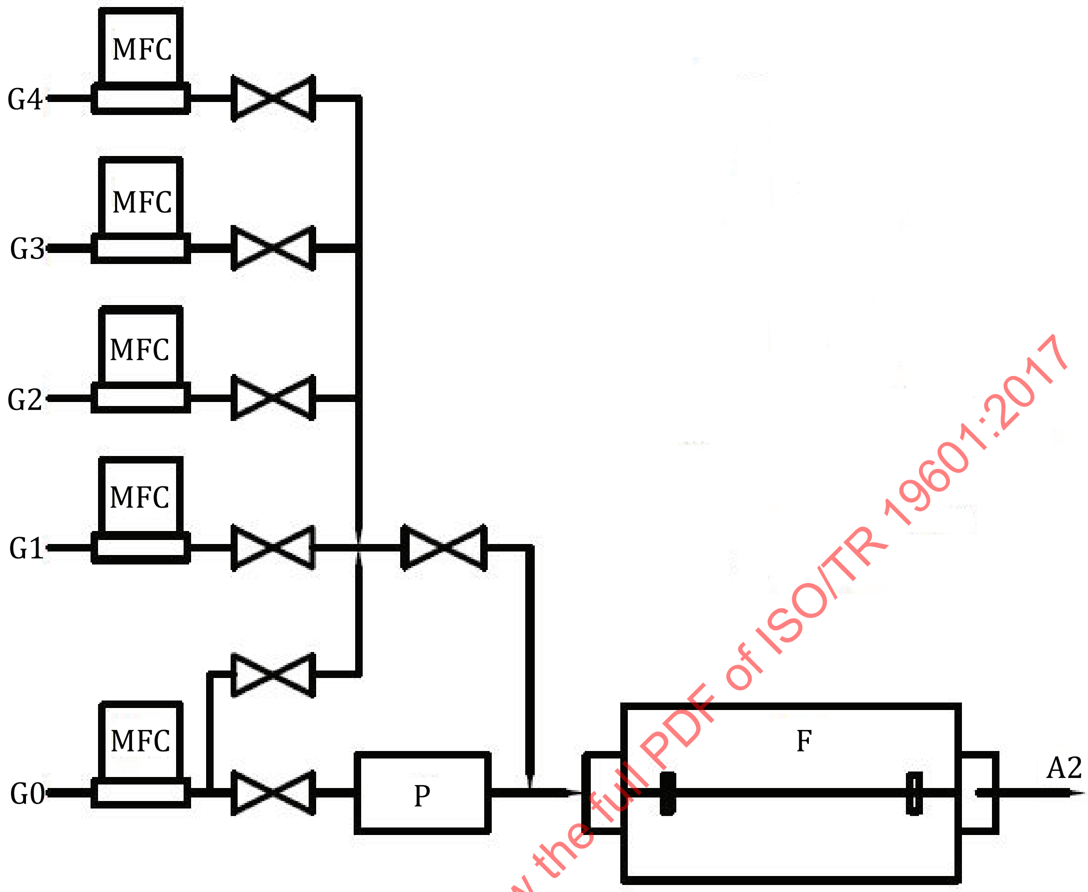
Figure 15 shows the schematic diagram of the furnace type generator. The particle generation uses chemical reactions and thermal energy in the furnace. Using various precursors, it is capable of generating particles with controlled composition and physical properties. Figure 16 shows the schematic diagram of particle generation in the flame type generator. The concentration adjustment is done by altering the temperature, flow rate and precursors[70][71][72][73].

7.5.1.2 Advantages

The advantages include its effectiveness in generating nanomaterial. It is also simple to use compared with other generation methods.

7.5.1.3 Limitations

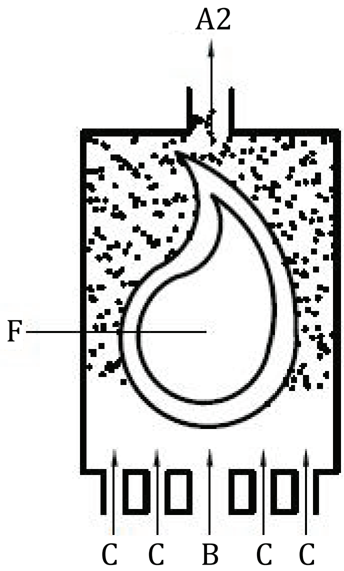
Limitations include differences in the properties of the laboratory-generated nanomaterial with the manufactured nanomaterial of interest. In the combustion process, by-products are generated due to reaction intermediates from precursors. The use of inert gas might affect the inhalation test. Dilution and other gas conditioning might be needed prior to exposure. Possibly, an approach might be to synthesize the nano-objects and capture on a filter and then use a dispersion technique for the toxicity study.



Key

A2	aerosol
F	furnace
G0	clean air
G1	reactive gas 1
G2	reactive gas 2
G3	reactive gas 3
G4	reactive gas 4
MFC	mass flow controller
P	precursor

Figure 15 — Schematic diagram of furnace generator



Key

A2 aerosol

B carrier gas + precursor

C working gas

F flame

Figure 16 — Schematic diagram of flame type generator

7.6 Liquid phase filtration/dispersion — Critical point drying (tertiary butyl alcohol sublimation) and direct injection system for whole-body inhalation studies

7.6.1 Principle of operation

This technique 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 (25 mm mesh for Mitsui MWNT-7) to remove aggregates/agglomerates from the sample and to generate highly dispersed particles without changing the size and length distribution (see Figure 17). 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 (see Figure 18). The sample loaded in the cartridge was injected into the subchamber connected upstream of the main whole-body inhalation chamber (see Figure 19 and Figure 20)[74].

7.6.2 Advantages

Using solvent recovery vacuum pump, the samples are easily prepared and are loaded into the direct injection cartridge, which can be used to introduce MWCNT aerosol into the inhalation exposure system. It is capable of generating target concentration of aerosol similar in size distribution to the primary particles in the bulk samples. Since the sample is in liquid phase until sublimation and confined to the vacuum chamber, it minimizes exposure to researchers. A minimum quantity of sample is required for the inhalation system tests because of insignificant losses during preparation.

7.6.3 Limitations

Limitations include possibility of adsorption of tertiary butyl alcohol to the dried sample. Depending on the purpose of the study, surface residue and surface modification needs to be considered. Heating during or after sublimation may be performed to minimize the residue[74]. Samples soluble to tertiary butyl alcohol will change particle size and shape during snap-freeze and sublimation process (in case of fullerene, very small nanofibre will form, depending on the concentration of the initial solution).

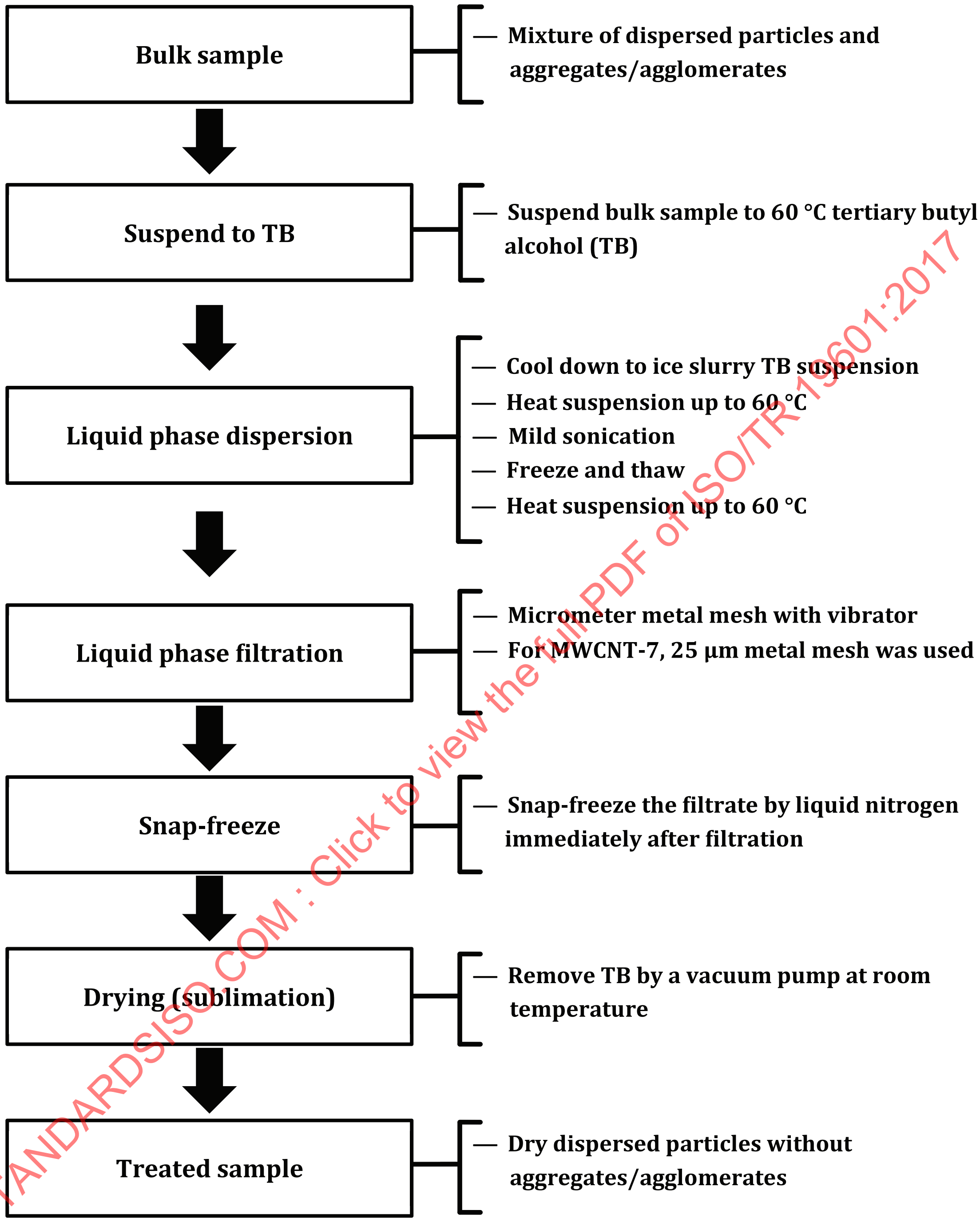
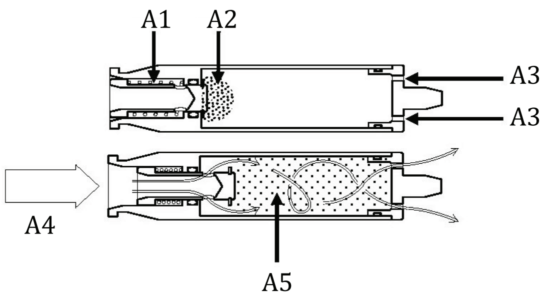
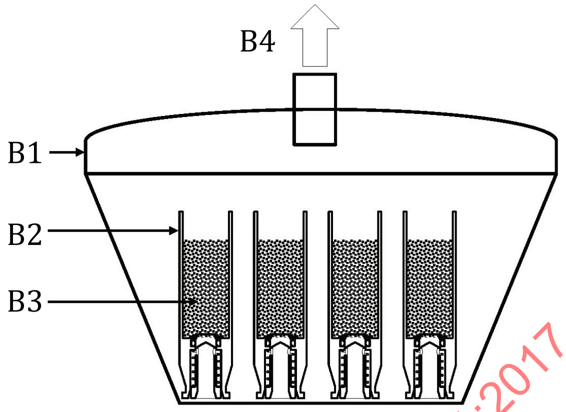


Figure 17 — Illustrated procedure of liquid phase filtering/dispersion — Critical point drying method (Taquann method)



a) Cartridge for direct injection



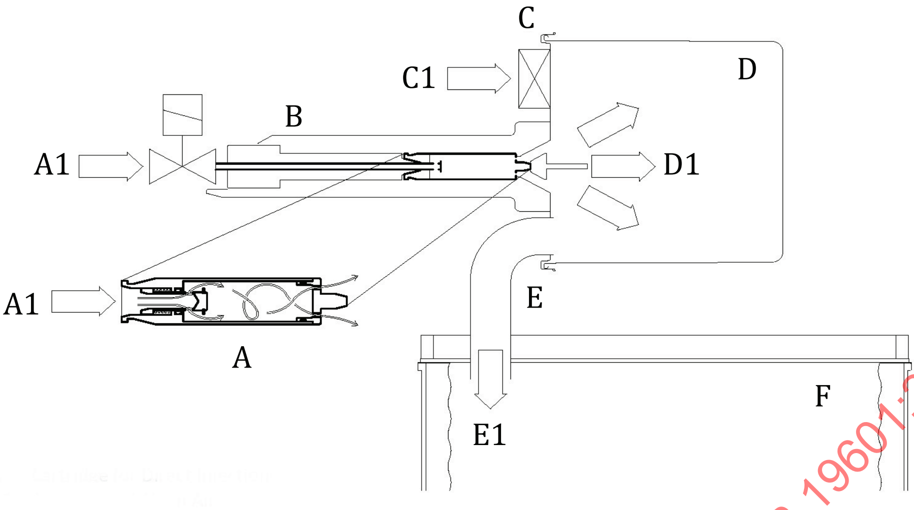
b) Sample loading to cartridges

Key

A1 slide valve
A2 dried dispersed samples
A3 outlets
A4 compressed clean air intake
A5 aerosol generation

B1 vacuum desiccator
B2 cartridge (without cap)
B3 frozen sample suspension of tertiary butyl alcohol
B4 connection to vacuum pump

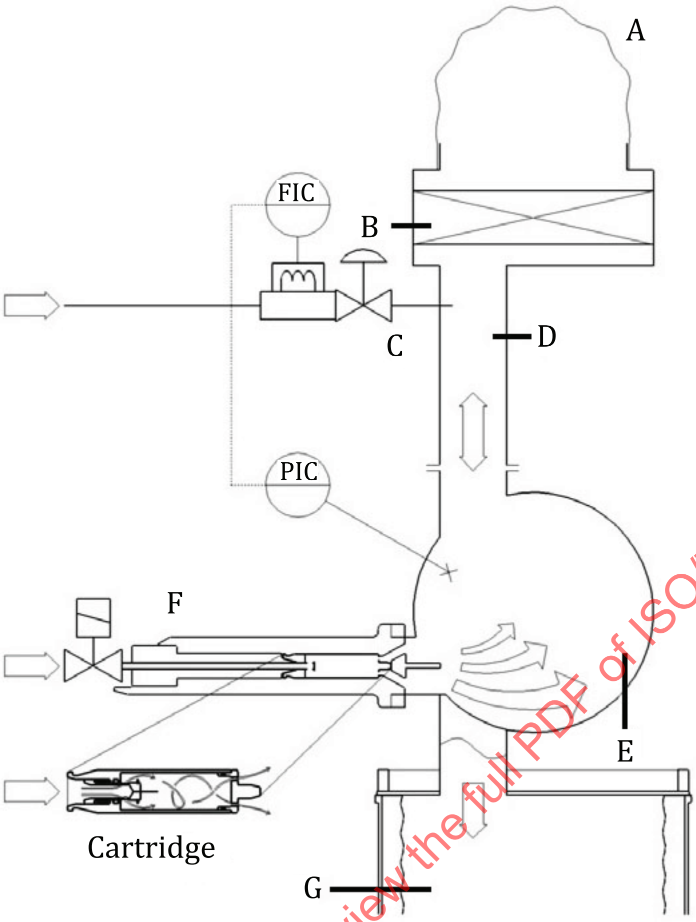
Figure 18 — Dispersion of filtrate (resuspended treated sample) containing measured amount of the sample to cartridges for applying to direct injection whole body inhalation system (Taquann direct injection system)



Key

A cartridge for direct injection
A1 compressed air for injection
B cartridge loader
C HEPA filter
C1 air of constant flow (room air)
D subchamber
D1 aerosol
E connecting pipe
E1 aerosol
F main chamber

Figure 19 — Schematic diagram of direct injection whole body inhalation system



Key

FIC	flow indicator/controller	C	main flow inlet shut off during injection
PIC	pressure indicator/controller	D	air pressure dumper duct
A+B+D	air pressure relief dumper	E	subchamber
A	flexible plastic film for insulation	F	cartridge loader/injector
B	ULPA filter unit	G	inhalation chamber (with animal cages) cartridge containing aliquot of dry dispersed sample

NOTE Advanced model shown as E and F.

Figure 20 — Cross-section diagram of the direct injection system

8 Experimental integration

8.1 General

Guidance in the previous clauses include the selection of a NOAA aerosol generator with respect to material characteristics and study design. However, the NOAA aerosol generator needs to be integrated into the rest of the experimental program. In particular, the atmosphere generated should be compatible with the exposure method, either *in vivo* or *in vitro*, with respect to NOAA aerosol concentration and particle properties, electrostatic charge, flow rate, gas concentrations, temperature and relative humidity. The gas stream from the aerosol generator may need to be conditioned before and monitored before introduction to the exposure system. An example dilution system described in Annex B might be useful. Consideration should be given to

— exposure characterization,

— particle properties, and

— exposure systems.

8.2 Exposure characterization

Precise characterization of the NOAA is essential for an air exposure study. The objective of an NOAA air exposure study is to establish a quantitative relationship between the observed toxicological outcome and the causative NM characteristics (e.g. dose and dose metrics used in terms of test substance and physical and chemical properties). Therefore, a precise characterization of the NOAA is essential for an inhalation exposure study.

8.3 Particle properties

NOAA should be thoroughly characterized to obtain information regarding their physicochemical properties such as composition, number and mass concentrations, median and mean size and size distribution, surface properties (e.g. area), electrical charge, surface properties, hygroscopicity and shape. The relevant chemical and physical properties of the nanoparticle should be determined to the extent possible; however, because these might not be known a priori as described in 6.1.2, as many parameters as practical should be determined. 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 might be important parameters for dosimetry. Some of the minimal characterization parameters items have been recommended in ISO/TR 13329 and in Reference [76]. (Essential parameters include composition, number and mass concentrations, shape, median and mean size and size distribution. Recommended parameters are surface area, electrical charge, surface properties and hygroscopicity.)

8.4 Considerations for *in vivo* exposure systems

8.4.1 During development of the NOAA generating system and prior to interfacing with the exposure chamber(s), measurements should be performed to verify aerosol particle composition, size distribution and purity and to establish stability over the exposure time period required.

During exposure tests, analysis should be conducted continuously and/or intermittently depending on the method of analysis to determine the consistency of particle size distribution without disrupting the inhalation exposure.

NOTE 1 Nanoparticle generating system for silver and other metals is described in ISO 10801.

NOTE 2 General guidance for aerosol generation in given in ISO/TS 12025.

8.4.2 Inhalation chambers and supporting equipment should be prepared in accordance with OECD GD 39, OECD TG 403, OECD TG 412 and OECD TG 413 or other recommended guidelines[6][7][8][16].

8.4.3 Inhalation chambers and supporting equipment should be prepared for nanoparticle exposure studies

NOTE 1 Aerosolized nanoparticles 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. See standard texts on aerosol science[79][80][81].

NOTE 2 Charge neutralization of the NOAA might be required depending on the purpose of the study.

If charge distribution is considered a characterization requirement, this should be specified and measured in the study.

NOTE 3 To reduce deposition losses, conductive tubing of the minimum length practical to use with the tubing diameter is selected to interface with instrumentation.

NOTE 4 In cases where water is introduced by the generation method, a diffusion dryer might be needed. In some cases, a bubbler might be needed to increase humidity.

Manifolds should be characterized to ensure compliance with OECD GD 39, OECD TG 403, OECD TG 412 and OECD TG 413 for determining any sampling bias.

NOTE 5 Sampling manifold consists of tubing, solenoid valves and/or other elements required for routing samples from each chamber to online monitoring equipment.

8.4.4 The DMAS should be routinely calibrated and the results documented. The initial calibration may be performed in the factory.

9 Considerations in use of nano-aerosol generator for *in vitro* study

As discussed in the 2007 US National Research Council report titled "Toxicity Testing in the 21st Century: A Vision and a Strategy" and due to the substantive time, cost and animal numbers required to conduct traditional *in vivo* toxicity tests, there is a need for further development and use of human cell-based methods[82][83]. For *in vitro* assays to be predictive of human effects, they should include certain parameters into the assay design including a) the choice of relevant cell types in a physiologically relevant configuration, b) characterization of the test-material throughout the assay, including life cycle transformations, c) the choice of realistic test-material concentration and form relevant to real exposures, d) the use of context-specific dispersants and e) the use of appropriate exposure route and duration. Specifically to assess inhalation toxicity of NOAA, an air-liquid interface (ALI) cell exposure system (rather than submerged cell exposure systems) is preferred as it more closely resembles *in vivo* conditions in the lungs and allows for physiologically relevant delivery of aerosolized nanoparticles to the cells[84]. For such a model to accurately reflect human-relevant *in vivo* exposure, it is critical to choose appropriate techniques for generating, exposing and studying aerosolized NOAA in cell cultures at the ALI.

Some of the aerosol generators described in this document have been used for generating NOAA for *in vitro* studies, where the aerosolized NOAA is administered to the cells, housed in an exposure chamber, at ALI[85][86][87][88][89]. A number of aerosol exposure systems [e.g. Cultex, Vitrocell, nano-aerosol chamber *in vitro* toxicity (NACIVT), etc.] are available that allow assessment of inhalation toxicity of NOAA and differ from each other primarily in the way the nanomaterials are deposited onto the cells (gravitational versus electrostatic)[86][87][89][90]. These *in vitro* inhalation exposure systems include engineering controls such as controlled temperature, humidity and gas exchange to provide a suitable environment for long-term culturing of cells. These exposure systems, in conjunction with the aerosol generators, enable the characterization of NOAA throughout the assay duration in terms of NOAA delivery, uptake and impacts on the lung cells during NOAA exposure.

Annex A
(informative)

NOAA generators, their particle size distribution and measurement methods

This annex contains information assembled from previously reported literature sources. Important details related to starting material characteristics, instrumentation parameters and material processing are included in those literature sources but are omitted from the table for brevity. These details are critical to understand in relation to the tabulated particle size distributions as the particle size distributions can be highly dependent upon initial material characteristics and processing conditions.

Subject nanomaterials	Type	Generators	Particle size distribution	Measuring method	References
Carbon nanotubes	Dry dissemination	Acoustic dry aerosol generator elutriator	~4 µm	Cascade impactor, DMAS, APS	[40]
Carbon nanotubes	Dry dissemination	Acoustic dry aerosol generator elutriator	Cascade impactor MMAD: 1,5 µm GSD: 1,65~1,70 µm Electrical low pressure impactor CMD: 0,45 µm GSD: 2,09 DMAS CMD: 0,36 µm GSD: 1,7	Cascade impactor, filter sampling, electrical low pressure impactor, DMAS	[43]
Carbon nanotubes	Dry dissemination	Bin feeder	MMAD: 1,9~3,3 µm GSD: 2,0~3,1	Cyclone, cascade impactor, filter sampling	[91]
Carbon nanotubes	Dry dissemination	Wright dust feeder	Cascade impactor MMAD: 2,2~2,9 µm GSD: 1,7~2,6 APS MMAD: 1,9~2,0 µm GSD: 1,6~1,7	Filter sampling, cascade impactor, APS	[32]
Carbon nanotubes	Wet dissemination	CNT generator	N/A	Filter sampling	[59]

Subject nanomaterials	Type	Generators	Particle size distribution	Measuring method	References
Carbon nanotubes	Dry dissemination	Brush type generator	Cascade impactor MMAD: 0,5~1,3 µm GSD: 3,1~5,4 DMAS CMD: 58~64 nm GSD: 1,7~1,8 OPC CMD: 0,6 µm	Cascade impactor, DMAS, OPC, filter sampling	[35]
Carbon nanotubes, carbon black, graphene	Dry dissemination	Brush type generator	Cascade impactor MMAD: 1,1~2,0 µm GSD: 3,0~3,7 DMAS CMD: 96~145 nm OPC CMD: 494~533 nm	Cascade impactor, DMAS, OPC	[93]
Carbon nanotubes	Dry dissemination	Acoustic dry aerosol generator elutriator	MMAD: 1,5 µm GSD: 1,7	Cascade impactor	[92]
MWCNT	Dry dissemination	Rotating brush generator	Count mean length 4 µm to 6 µm	SEM	[94]
Carbon nanotubes	Wet dissemination	Atomizer/ nebulizer (dispersion agent)	Geometric mean length: 0,7 µm (SD 1,7) Geometric mean width: 0,2 µm (SD 1,7)	TEM image analysis	[54]
Carbon nanotubes	Wet dissemination	Atomizer/ nebulizer (in ethanol), wright dust feeder	Cascade impactor MMAD: 2,7~3,4 µm GSD: 1,98~2,14 APS CMD: 1,7~2,2 µm GSD: 1,67~1,76	Cascade impactor, APS, DMAS	[95]
Carbon nanotubes	Wet dissemination	Atomizer/ nebulizer	25 nm~2 840 nm CMD: 209 nm GSD: 1,98	TEM	[52]
Carbon nanotubes	Dry dissemination	Wright dust feeder	Mass mode aerodynamic diameter: 4,2 µm	Cascade impactor	[33]
SWCNT	Phase change	Electrospray	35 nm, 80 nm	TEM	[96]
MWCNT	Phase change	Electrospray	20 nm to 500 nm in diameter	SMPS	[97]
MWCNT	Dry dissemination	Fluidized bed with cyclone	300 nm modal diameter 1 µm to 2 µm modal diameter	SMPS LPI	[98]

Subject nanomaterials	Type	Generators	Particle size distribution	Measuring method	References
Carbon black	Phase change	Spark generator	CMD: 90 nm~105 nm GSD: 1,53~1,55	DMAS	[99]
Carbon fibres	Dry dissemination	Small scale powder disperser	CMD: 3,66 µm (GSD: 1,11) CML: 14,83 µm (GSD: 4,00)	Image analysis	[100]
Titanium dioxide	Dry dissemination	Brush type generator	MMAD: 1,29 µm~1,44 µm GSD: 2,60~3,65	OPC, cascade impactor	[36]
Titanium dioxide	Wet dissemination	Atomizer/ nebulizer	MMAD: 0,7 µm~1,1 µm	Zeta potential meter	[56]
Titanium dioxide	Wet dissemination	Atomizer/ nebulizer	CMD: 128 nm GSD: 1,7	DMAS	[101]
Titanium dioxide	Dry dissemination	Brush type generator	Cascade impactor MMAD: 0,7 µm ~1,1 µm GSD: 2,3~3,4 DMAS CMD: 0,20 µm~0,25 µm OPC CMD: 0,45 µm	Cascade impactor, DMAS, OPC, filter sampling	[92]
Titanium dioxide, nickel oxide	Wet dissemination	Atomizer/ nebulizer	CMD: 139 nm (NiO) CMD: 51 nm (TiO_2)	DMAS	[102]
Titanium dioxide	Dry dissemination	Small scale powder disperser	Cascade impactor MMAD: 0,29 µm~0,44 µm	Cascade impactor, BET	[103]
Titanium dioxide	Phase change	Spark generator	CMD: 20 nm~25 nm GSD: 1,6	DMAS	[65]
Titanium dioxide, Y-zirconia, talc, etc.	Dry dissemination	Rotating drum generator		APS, DMAS	[49]
Silicon dioxide	Chemical reaction	Furnace generator	CMD: 37 nm~83 nm	DMAS	[71]
Silicon dioxide	Dry dissemination	Small scale powder disperser	~3 µm	Cascade impactor, OPC	[39]
Silver nanoparticle	Wet dissemination	Atomizer/ nebulizer	CMD: 33 nm	OPC	[89]
Silver nanoparticle	Phase change	Hot plate generator	CMD: 16 nm GSD: 1,4	DMAS	[104]
Silver nanoparticle	Chemical reaction	Furnace generator	CMD: 6 nm GSD: 2,0	TEM image analysis	[70]
Silver nanoparticle	Phase change	Hot plate generator	CMD: 18 nm GSD: 1,45	DMAS	[105]

Subject nanomaterials	Type	Generators	Particle size distribution	Measuring method	References
Silver nanoparticle	Phase change	Spark generator	CMD: 17 nm GSD: 1,38	DMAS	[106]
Platinum nanoparticle	Phase change	Spark generator	CMD: 25 nm GSD: 1,5	DMAS	[107]
Copper nanoparticle	Wet dissemination	Atomizer/ nebulizer	CMD: 60 nm GSD: 1,8	DMAS	[108]
Gold nanoparticle	Phase change	Hot plate generator	DMAS GMD: 5 nm TEM image analysis GMD: 2,47 nm GSD: 1,42	DMAS, TEM image analysis	[62]
Gold nanoparticle	Wet dissemination	Atomizer/ nebulizer	CMD: 76 nm ~ 79 nm	DMAS, electrical low pressure impactor	[109]
Gold nanoparticle	Phase change	Spark generator	CMD: 5 nm ~ 8 nm	DMAS	[64]
Gold nanoparticle	Wet dissemination	Atomizer/ nebulizer	TEM image analysis CMD: 12,8 nm GSD: 1,14	TEM image analysis	[110]
Lunar dust	Dry dissemination	Vilnius aerosol generator	Cascade impactor MMAD: 2,0 µm ~2,4 µm GSD: <1,6 APS MMAD: 2,4 µm~2,8 µm GSD: < 1,3	Cascade impactor, APS	[111]
Bentonite, barium sulfate, talc, etc.	Dry dissemination	Rotating drum generator		TEOM	[48]
Carbon nanotubes, titanium dioxide	Liquid phase filtration/ dispersion–critical point drying (tertiary butyl alcohol sublimation) and direct injection system for whole-body inhalation studies	Direct injection of dispersed sample by compressed air	Carbon nanotube (Mitsui MWNT-7) Arithmetic mean length 7,3 µm (SD 4,9, Max. 33,0 µm) Titanium oxide MMAD:0,7 µm ~0,9 µm GSD: 3,3~3,5	Filter sampling SEM image analysis Cascade impactor	[74]
Al_2O_3	Dry dissemination	Brush dust feeder	MMAD (GSD) 1,2 (2,6) OPC 0,5; SMPS 0,2	MMAD, cascade impactor	[37]
Aluminium nanopowder	Dry dissemination	Vilnius aerosol generator	MMAD 155 GSD 4,8		[46]
CuO	Dry dissemination	Brush dust feeder	MMAD (GSD) 0,52 (3,0) OPC 0,42; SMPS 0,15	MMAD, cascade impactor	[37]

