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Nanotechnologies — Health and safety practices in occupational settings

Nanotechnologies — Pratiques de santé et de sécurité en milieux professionnels

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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

This second edition cancels and replaces the first edition (ISO/TR 12885:2008), which has been technically revised.

The main changes compared to the previous edition are as follows:

- widespread reference to 'nano-objects, and their aggregates and agglomerates greater than 100 nm' ('NOAAs'), in place of alternative terms;
- addition of annexes addressing:
    - primary chemical composition of nanomaterials;
    - nanomaterial-specific animal and cell culture toxicity studies;
    - characteristics of selected instruments and techniques for monitoring nano-aerosol exposure;
    - characteristics of biosafety cabinets;
    - advantages and disadvantages of different types of air-purifying particulate respirators;
- consolidation of bibliographical information.

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

Introduction

The field of nanotechnologies is advancing rapidly and is expected to impact virtually every facet of global industry and society. International standardization on nanotechnologies should contribute to realizing the potential of this technology through economic development, improving the quality of life, and for improving and protecting public health and the environment. One can expect many new manufactured nanomaterials coming to the market and workplace. The introduction of these new materials into the workplace raises questions concerning occupational safety and health. This document assembles useful knowledge on occupational safety and health practices in the context of nanotechnologies. Use of the information in this document could help companies, researchers, workers and other people to prevent potential adverse health and safety consequences during the production, handling, use and disposal of manufactured Nano-Objects, and their Aggregates and Agglomerates greater than 100 nm (NOAAs). This advice is broadly applicable across a range of NOAAs and applications.

This document is based on current information about nanotechnologies, including characterization, health effects, exposure assessments, and control practices. It is expected that this document will be revised and updated and new safety standards will be developed as our knowledge increases and experience is gained in the course of technological advance.

Nanotechnology involves materials at the nanoscale. ISO/TC 229 defines the "nanoscale" to mean size range from approximately 1 nm to 100 nm (ISO/TS 80004-1:2015)[1]. To give a sense of this scale, a human hair is of the order of 10 000 to 100 000 nm, a single red blood cell has a diameter of around 5 000 nm, viruses typically have a maximum dimension of 10 nm to 100 nm and a DNA molecule has a diameter of around 2 nm. The term "nanotechnology" can be misleading since it is not a single technology or scientific discipline. Rather it is a multidisciplinary grouping of physical, chemical, biological, engineering, and electronic processes, materials, applications and concepts in which the defining characteristic is one of size.

The distinctive and often unique properties which are observed with nanomaterials offer the promise of broad advances for a wide range of technologies in fields as diverse as computers, biomedicine, and energy. At this early stage the potential applications of nanomaterials seem to be limited only by the imagination. New companies, often spin outs from university research departments, are being formed and are finding no shortage of investors willing to back their ideas and products. New materials are being discovered or produced and for some, astonishing claims are being made concerning their properties, behaviours and applications.

While much of the current "hype" is highly speculative, there is no doubt that worldwide, governments and major industrial companies are committing significant resources for research into the development of nanometer scale processes, materials and products.

Ordinary materials such as carbon or silicon, when reduced to the nanoscale, often exhibit novel and unexpected characteristics such as extraordinary strength, chemical reactivity, electrical conductivity, or other characteristics that the same material does not possess at the micro or macro-scale. A huge range of nanomaterials have already been produced including nanotubes, nanowires, fullerene derivatives (buckyballs).

A few manufactured nanomaterials were developed already in the 19th and 20th centuries, at a time when the word "nanotechnology" was unknown. Among such nanomaterials are zeolites, catalyst supports such as $MgCl_2$, pigments and active fillers such as carbon black and synthetic amorphous silica. Market size of these commodity materials is well above the billion US dollars or million tons threshold.

Nanotechnologies are gaining in new commercial application. Nanomaterials are currently being used in electronic, magnetic and optoelectronic, biomedical, pharmaceutical, cosmetic, energy, catalytic and materials applications. Areas producing the greatest revenue for nanomaterials are chemical-mechanical polishing, magnetic recording tapes, sunscreens, automotive catalyst supports, electro-conductive coatings and optical fibres.

Among other factors, due to the great variability of physical and chemical properties of nanomaterials, our abilities to accurately predict the impact of some nanomaterials exposures on worker health are limited at this time. Similarly, there might be insufficient information about human exposures during work and our abilities to measure nanomaterials in the workplace (or more generally) are limited by current technologies. Overall, there is currently limited knowledge on chronic health effects of nanomaterials. In the case of some nanostructured materials, such as carbon black and synthetic amorphous silica, toxicological and epidemiological data are available.

A subset of nanomaterials, NOAAs are of particular concern in the workplace as they can be dispersed in the air and can represent health risks via inhalation exposures. NOAAs include structures 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 with sizes larger than 100 nm. An aggregate is comprised of strongly bonded or fused particles (structures). An agglomerate is a collection of weakly bound particles and/or aggregates.

There are many gaps in current science about identifying, characterizing, and evaluating potential occupational exposures in the nanotechnology context. These gaps in our knowledge are best addressed at a multidisciplinary level. Occupational health practitioners and scientists and practitioners in the toxicology field including medical scientists and environmental scientists have vital roles to play in safeguarding health in this fast-moving field. Collaborative studies — ideally with international coordination — are essential in order to provide the critical information required within a reasonable time frame.

Nanotechnologies — Health and safety practices in occupational settings

1 Scope

This document describes health and safety practices in occupational settings relevant to nanotechnologies. This document focuses on the occupational manufacture and use of manufactured nano-objects, and their aggregates and agglomerates greater than 100 nm (NOAAs). It does not address health and safety issues or practices associated with NOAAs generated by natural processes, hot processes and other standard operations which unintentionally generate NOAAs, or potential consumer exposures or uses, though some of the information in this document can be relevant to those areas.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the terms and definitions given in the ISO/TS 80004 series apply.

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

- ISO Online browsing platform: available at https://www.iso.org/obp/ui
- IEC Electropedia: available at http://www.electropedia.org/

4 Symbols and abbreviated terms

ACGIH	American Conference of Governmental Industrial Hygienists
AIDS	acquired immune deficiency syndrome
APF	assigned protection factor
APR	air-purifying respirator
BEI	biological exposure index
BET	Brunauer-Emmett-Teller
BMD	benchmark dose
BSC	biological safety cabinet
CNF	carbon nanofibre
CNT	carbon nanotube
COSHH	control of substances hazardous to health
CPC	condensation particle counter
DC	diffusion charger

DEMS	differential electrical mobility sizer
DMAS	differential mobility analysing system
DNA	DNA
DOE	U. S. Department of Energy
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EPA	U. S. Environmental Protection Agency
GI	gastro-intestinal
GSD	geometric standard deviation
HEI	Health Effects Institute
HEPA	high efficiency particulate air filter
HSE	U. K. Health and Safety Executive
HVAC	heating, ventilation and air conditioning
EHS	environment, health and safety
ELPI®	Electrical Low Pressure Impactor
ICON	International Council on Nanotechnology
ICP-MS	inductively coupled plasma mass spectrometry
ICRP	International Commission on Radiological Protection
ICSC	international chemical safety cards
IDLH	immediately dangerous to life or health
ILSI	International Life Sciences Institute
IRSST	Canadian Institut de recherche Robert-Sauvé en santé et en sécurité du travail
LEV	local exhaust ventilation
LPI	low pressure impactor
MCDA	multi-criteria decision analysis
MMAD	mass median aerodynamic diameter
MPPS	most penetrating particle size
MWCNT	multiwall carbon nanotube
NIOSH	U. S. National Institute for Occupational Safety and Health
NMAM	U. S. NIOSH manual of analytical methods
NOAA	nano-objects, and their aggregates and agglomerates greater than 100 nm
NOAEL	no-observed-adverse-effect Level

NRV	nano reference value
OSHA	U. S. Occupational Safety and Health Administration
PAPR	powered air-purifying respirator
PPE	personal protective equipment
PTFE	Polytetrafluoroethylene
RDECOM	research, development and engineering command
RPE	respiratory protection equipment
SAR	supplied-air respirator
SCBA	self-contained breathing apparatus
SCENIHR	E. C. Scientific Committee on Emerging and Newly Identified Health Risks
SDS	safety data sheet
SEM	scanning electron microscopy
SOP	standard operating procedures
SPE	skin protective equipment
SWCNT	single-wall carbon nanotube
TEM	transmission electron microscopy
TEOM	Tapered Element Oscillating Microbalance
USACHPPM	U. S. Army Center for Health Promotion and Preventive Medicine

5 Nanomaterials: Description and manufacturing

5.1 Manufactured nanomaterials

Manufactured nanomaterials are nanomaterials intentionally produced to have selected properties or composition[1]. Manufactured nanomaterials encompass nano-objects and nanostructured materials (see Figure 1). The former are defined as discrete piece of materials with one (nanoplate), two (nanofibre) or three external dimensions (nanoparticle) in the nanoscale (i.e. length range approximately from 1 nm and 100 nm)[1][2]. Examples of nanostructured materials are nanocomposites composed of nano-objects embedded in a solid matrix or nano-objects bonded together in simple random assemblies as in aggregates and agglomerates or ordered as in crystals of fullerenes or carbon nanotubes[3]. Discussion in this document focuses primarily on nano-objects and their simple assemblies.

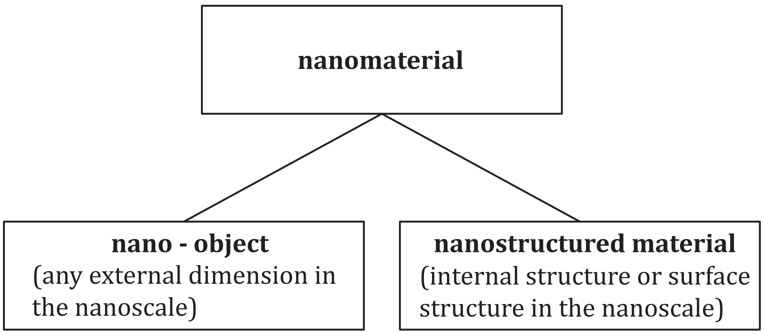


Figure 1 — Nanomaterials framework (Based on ISO/TS 80004-1:2015)[1]

Relatively simple nanomaterials presently in use or under active development can be classified in terms of dimensionality and the primary chemical composition. However, even simple nanomaterials are often coated and have complex chemical and physical structure. Any attempt to classify nanomaterials is highly artificial with many materials falling into several classification categories. Thus, the following description is for organizational purposes only.

Quantum dots and fullerenes are confined to the three-dimensional nanoscale domain. Nanotubes (i.e. hollow nanofibre), nanowires (i.e. electrically conducting or semi-conducting nanofibre), nanorods (i.e. solid nanofibre), other nanofibres and nanofibrils have at least two nanoscale dimensions, while nanoplates such as nanoscale surface coatings, thin films and layers have at least one nanoscale dimension (see Figures 2 and 3). In Annex A, nanomaterials are described according to the primary (or core) chemical composition of nano-objects: carbon nano-objects (e.g. fullerenes, carbon nanotubes); oxide nanomaterials (e.g. TiO_2 and ZnO); metal nanomaterials (e.g. Au); semiconductor nanomaterials (e.g. quantum dots); organic polymeric nanomaterials (e.g. dendrimers); and bio-inspired nanomaterials (e.g. capsid nanoparticles, the protein shell of a virus). Within these classes, different nanomaterials are listed in the order of decreasing necessary number of dimensions in nanoscale from 3D particles to fibres to layers.

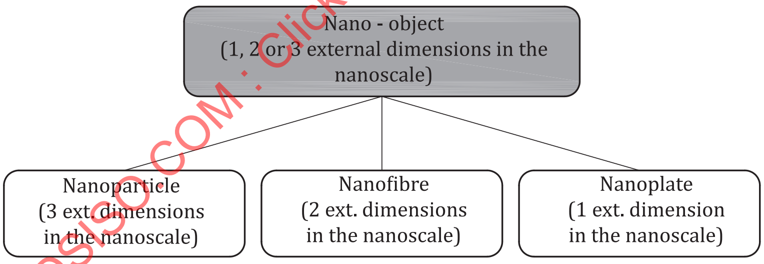
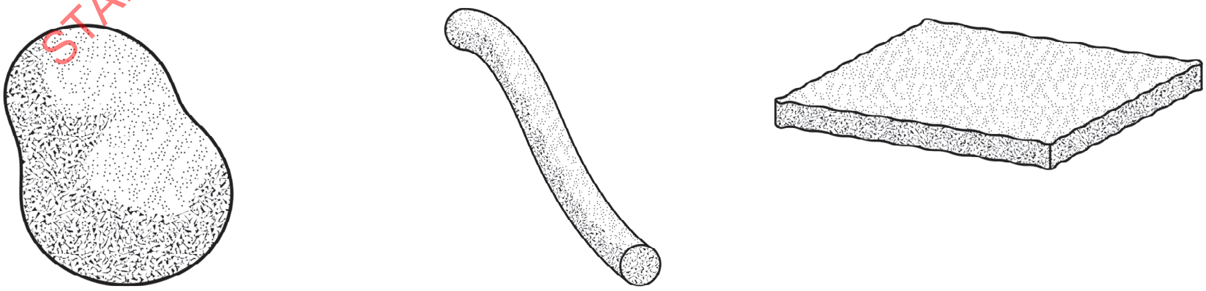


Figure 2 — Fragment of hierarchy of terms related to nano-objects (ISO/TS 80004-2:2015)[2]



a) Nanoparticle (3 external dimensions in the nanoscale)

b) Nanofibre (2 external dimensions in the nanoscale)

c) Nanoplate (1 external dimension in the nanoscale)

Figure 3 — Schematic diagrams showing some shapes for nano-objects (ISO/TS 80004-2:2015)[2]

5.2 Production processes

5.2.1 Typical production processes

Examples of methods typically used for the manufacturing of nanomaterials are:

- aerosol generation such as flame pyrolysis, high temperature evaporation and plasma synthesis;
- vapour deposition;
- liquid phase methods: colloidal, self-assembly, sol-gel;
- electropolymerization and electrodeposition;
- electro-spinning for polymer nanofibre synthesis;
- mechanical processes including grinding, milling and alloying.

5.2.2 Aerosol generation methods

The aerosol generation method is used to produce a wide range of nanomaterials. This method is based on homogeneous nucleation of a supersaturated vapour and subsequent particle growth by condensation, coagulation and capture[4]. The formation of vapour typically occurs within an aerosol reactor at elevated temperatures, where often a super saturate of a solid is cooled into a background of gas. The methods used to produce nanomaterials are usually categorized by the heating or evaporation process and include[5]:

- flame pyrolysis;
- furnace/hot wall reactors;
- laser induced pyrolysis.

5.2.3 Vapor deposition methods

These methods are traditionally based on already well known and established methods for the manufacture of semiconductors. Here, vapour is formed in a reaction chamber by pyrolysis, reduction, oxidation and nitridation. The first step is the deposition of a few atoms. These first atoms form islands which spread and coalesce into a continuous film. Later, growth continues until thicker film develops[5][6]. Examples include[7]:

- physical vapour deposition (i.e. process of depositing a coating by vaporizing and subsequently condensing an element or compound, usually in a high vacuum);
- sputter deposition (i.e. physical vapour deposition technique employing energetic ions to transfer atoms from a target to a substrate);
- chemical vapour deposition (i.e. deposition of a solid material by chemical reaction of a gaseous precursor or mixture of precursors, commonly initiated by heat on a substrate).

These methods have been used to produce nanofilms including TiO_2, ZnO and SiC[5]. Vapour deposition processes mediated by a catalyst are used to produce carbon nanotubes commercially. Chemical vapour deposition emerged as an efficient graphene and synthetic diamond production method[8].

5.2.4 Colloidal/self-assembly methods

Self-assembly is defined as the autonomous action by which components organize themselves into patterns or structures[7]. The colloidal methods are also well established conventional wet chemistry precipitation processes in which solutions of different ions at required concentrations are mixed under controlled conditions of temperature and pressure which form insoluble precipitates[5].

Recently, a rapidly expanding sub-set of colloidal methods is the so-called sonochemistry method, which uses acoustic cavitation to control the process[9]. Cost-effective, eco-friendly, energy efficient, and nontoxic methods of producing nanomaterials using diverse biological entities have been receiving increasing attention in the last two decades in contrast to physical and chemical methods that use toxic solvents, generate unwanted by-products, and high energy consumption[10].

5.2.5 Electrodeposition

Electrodeposition is the deposition of material onto an electrode surface from ions in solution due to electrochemical reduction[7]. Polymer nanofibre and metal nanowire films as well as nanoparticle films can be fabricated on an substrate through a controlled electropolymerization (polymers) or electrodeposition (metals) process[11][12].

5.2.6 Electrospinning

Electrospinning is the use of electrical charge to induce drawing of very fine fibres from a liquid[7]. Electro-spinning method is a major method in the manufacture of polymer nanofibres.

5.2.7 Attrition methods

In attrition methods, size reduction is accomplished by grinding and milling and production of materials such as clay, coal, some metals and semiconductors have been made[13]. Production rates in the order of tons per hour can be obtained using these methods.

6 Hazard characterization

6.1 Health effects

6.1.1 General

The potential health risk of a substance is generally associated with the magnitude and duration of the exposure, the persistence of the material in the body, the inherent toxicity of the material, and the susceptibility or health status of the exposed person. Since hazard properties of some manufactured nanomaterials are not well characterized, there are uncertainties as to whether the unique properties of these nanomaterials also pose unique health risks. These uncertainties arise because of gaps in knowledge about the factors that are essential for evaluating health risks (e.g. routes of exposure, translocation of materials once they enter the body, and interaction of the materials with the body's biological systems). An important issue is whether the nanoscale version of a particular material poses risks that are significantly different in type or intensity than the micrometer-scale forms of the same material.

Results of existing studies in cell cultures (*in vitro*), animals (*in vivo*) or humans (epidemiological) on exposure and response to nanoscale or other respirable particles[14][15][16][17], as well as available toxicity information about a given material in microscopic form, provide a basis for preliminary estimates of the possible health effects from exposures to similar engineered materials on a nanoscale. However, it should be recognized that there are significant uncertainties and variables associated with predicting human health effects based on animal studies. Presently, *in vitro* cell culture methods are used mostly to delineate mechanisms of toxicity, and to screen and compare relative toxicities. There are only a limited number of *in vitro* assays validated for determination of safety of chemicals, which is, so far, mainly restricted to hazard identification (e.g. embryotoxicity, irritation testing).

NOTE Information on validated alternative methods for animal experiments can be found at for example: European Union Reference Laboratory for Alternative to Animal Testing (EURL-ECVAM)[18]; Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) of the National Toxicology Program, USA[19]; Japanese Center for the Validation of Alternative Methods (JACVAM)[20].

In general, these *in vitro* data cannot be extrapolated to humans without additional information (e.g. *in vivo* data). Initial experimental studies in animals have shown that the biological response (whether beneficial or detrimental) to certain incidental or manufactured NOAAs can be greater than that of the same mass of larger particles of similar chemical composition[21][22][23][24][25][26][27][28]. However, the dose expressed as mass might not be the best descriptor for the toxic effect observed. For lung inflammation induced by nano-TiO_2 and nanosilver the dose described as total surface area was a better dose descriptor than mass[29][30]. In addition to particle size, other particle characteristics might influence the biological response, including solubility, shape and aspect ratio, charge and surface chemistry (corona formation — proteins and lipids), catalytic properties, adsorbed pollutants (e.g. heavy metals or endotoxins), photoreactivity, as well as degree of agglomeration[29][31][32][33]. Often nano-object surfaces are intentionally modified with coatings or functionalized in order to prevent agglomeration of particles and to achieve desired properties, e.g. pharmacological activity. Such modifications, as well as the contamination of particle surfaces with impurities can lead to changes in biological responses. In addition, some nanomaterials are produced using rather toxic intermediates and/or solvents. So, when evaluating NOAA toxicity it should always be considered whether the solution used for NOAA dispersion and/or production residues present as contaminants might be responsible for the observed toxicity. In Annex B some further animal and cell culture studies upon NOAA are discussed in detail.

Recently OECD WPMN published the dossiers resulting from the nanomaterial safety testing sponsorship programme. In the dossiers, mainly animal testing results obtained by applying OECD test guidelines were collected and some *in vitro* genotoxicity studies were also included in the dossiers. Therefore, in Annex B, the results on the dossiers were summarized and readers of this TR can go into the dossier site[34] and look for detailed results.

Recent studies on histopathology show the occurrence of engineered and incidental nanoparticles inside pathological lesions in organs (such as liver, kidney, pancreas, bladder, brain affected by a few forms of cancer)[35][36] and their possible linkage to fetal malformations and miscarriages[37][38]. The authors suggest that these nanoparticles might have caused the harm.

6.1.2 Basic principles and uncertainties

The existing literature on particles and fibres provides a scientific basis from which to evaluate the potential hazards of manufactured NOAAs. While the properties of manufactured NOAAs can vary widely, the basic physicochemical and toxicokinetic principles learned from the existing studies are relevant to understanding the potential toxicity of NOAAs. For example, it is known from studies in humans that a greater proportion of inhaled nano-objects will deposit in the alveolar region of the respiratory tract (both at rest and with exercise) compared to larger particles[39][40]. In addition to the International Commission on Radiological Protection (ICRP) model[39], the Multiple Path Particle Dosimetry (MPPD) model can be used for modelling deposition in human and animal respiratory tract[41]. However, it has to be realized that nano-objects might agglomerate and that these agglomerates can deposit in other areas of the respiratory tract or possibly cannot be inhaled at all. Further, animal studies indicate that nano-objects after initial exposure can be translocated to other organs in the body, although it is not well known how this might be influenced by the chemical and physical properties of the nano-objects[42][43][44][45][46]. Additional uncertainties are introduced by the difficulties in predicting human health effects based on animal studies. There might also be the potential for greater dermal and gastro-intestinal uptake of NOAAs when compared to larger particles, although in general uptake from the GI-tract of nano-objects was found to be relatively low[47][48][49][50]. Evidence from nanotoxicological studies (*in vitro* and animal studies) suggests that exposure to some NOAAs might have the potential to cause cell, tissue, or systemic toxicity. Due to their small size, nano-objects have the potential to cross cell membranes and interact with subcellular structures, such as mitochondria and the nucleus (and some nano-objects have been shown to cause oxidative damage and impair some function of cells in culture)[51][52]. Animal studies have indicated that some NOAAs are more biologically active due to their greater surface area per mass compared with larger-sized particles of the same chemistry when dose response relationships are expressed as mass[21][22][23][24][25][26][27][28][29][30]. The greater surface area per mass of NOAAs compared to larger particles is a fundamental contributor to the greater chemical reactivity and utility of nano-objects for industrial, commercial, and medical applications, but it also raises concern about the potential for adverse health effects in workers exposed to NOAAs. NOAAs that are able to shed ions and/or slowly dissolve, e.g. Ag-NP, nano-ZnO, nano-CuO, can have toxicity mechanism similar to the ionic form of these materials.

6.1.3 Potential relevance of health effects information about incidental or naturally-occurring NOAAs

While there is limited information on the health effects of manufactured NOAAs, there is a larger body of research on the health effects of incidental NOAAs (e.g. diesel exhaust particulate[53][54] and welding fumes[55]). The biological mechanisms of particle-related lung responses (e.g. oxidative stress, inflammation, and production of cytokines, chemokines, and cell growth factors) appear to be a consistent lung response to incidental respirable, including nanoscale, particles[29][56][57]. However, contaminants present such as transition metals[58] might also contribute to the lung response[59]. Although the composition and thus the physicochemical characteristics of incidental and manufactured NOAAs can differ substantially, the toxicological and dosimetric principles derived from studies of incidental NOAAs might be relevant to assessing the health effects of manufactured NOAAs especially for respiratory exposure which is considered to pose the highest risk.

There is a very large body of research on the health effects of certain respirable fibres. For example, it is well established that particular forms of asbestos are causative factors in otherwise rare, occupationally-derived, malignant mesotheliomas[60] and other lung disorders (including pulmonary interstitial fibrosis, pleural plaques, calcification and thickening)[61]. The harmful effects of fibres are driven by three important factors: length, diameter and persistence[62][63][64][65]. It is difficult to draw conclusions regarding the health effects of manufactured nanoscale fibres based on asbestos studies, but they suggest that particle properties of size, shape and composition are important factors influencing the toxicity of NOAAs. For some CNT and structures of CNTs with characteristics similar to blue asbestos (long fibres, rigidity, persistence) granulomatous inflammation and mesothelioma induction was observed[66][67][68]. In contrast, for CNT lacking these characteristics such reactions were not reported[69]. The International Agency for Research on Cancer (IARC) has recently classified multiwall carbon nanotubes (MWCNTs) other than MCWCNT-7 and MCWCNT-7 as Group 3 and Group 2B respectively (for additional details see B.1.2.1). There have been shorter-term studies of the effects of single-wall carbon nanotubes (SWCNTs)[70][71] and multiwall carbon nanotubes[72] in the lungs of rats and mice (administered by intratracheal instillation or pharyngeal aspiration). These studies have shown unusual inflammatory and fibrogenic reactions in the lungs, including transient inflammation followed by early onset of fibrosis at mass doses lower than those causing fibrosis from quartz or carbon black[70][71].

6.1.4 Relationship between toxicity and surface area, surface chemistry, and particle number

To the extent that NOAAs might pose increased hazards, the most significant factors might relate to the greater number or surface area of NOAAs compared with that for the same mass concentration of larger particles. This hypothesis is based primarily on the pulmonary effects observed in studies of rodents exposed to various types of poorly soluble nanostructured materials in the form of agglomerated and aggregated nano-objects or larger respirable particles (e.g. titanium dioxide, carbon black, barium sulfate, diesel soot, coal fly ash, and toner). These studies found that for a given mass of particles, poorly soluble nanostructured materials in the form of agglomerated and aggregated nano-objects produced greater observable effect than larger particles of similar chemical composition and surface properties. Dose-response relationships obtained in animal studies for poorly-soluble and low toxicity particles appear consistent across particle sizes when dose is expressed as particle surface area[21][22][23][24][25][26][27][28][29][30][73][74][75][76][77]. The mechanisms by which these materials exhibit higher levels of toxicity at smaller particle sizes (on a mass basis) appear to involve pulmonary inflammation, oxidative stress, and tissue injury[29][56][57]. The biological activity of particles is affected by their number and their physical and chemical properties, including size, surface area, solubility, shape, crystal structure, charge, catalytic activity and chemistry[21][22][23][24][25][29][31][78][79][80][81]. It should be considered whether a dose expressed as mass, although convenient when using NOAAs in experiments, is the proper metric to express dose response relationships for NOAAs. For several toxicological end points other dose metrics e.g. volume, surface area and/or number was found to better express the dose response relationship[29][30][82].

Through engineering, the properties of nanomaterials can be modified. For example, recent *in vitro* studies have shown that the *in vitro* cytotoxicity of fullerenes (measured as cell death) and carbon nanotubes can be reduced by several orders of magnitude by modifying the surface chemistry of the fullerene molecules and carbon nanotubes (e.g. by hydroxylation)[83][84]. Cytotoxicity studies *in vitro* with quantum dots have shown that the type of surface coating can have a significant effect on cell motility and viability[85][86][87].

6.1.5 Inflammatory response to NOAAs

A variety of NOAAs ranging from carbon-based combustion products to transition metals, having entered tissues and cells, can elicit generalized inflammatory and acute phase responses which incorporate the release of signalling molecules such as chemokines, cytokines, C-reactive protein and fibrinogen (a coagulant)[88][89][90][91][92][93][94]. In addition, macrophage and neutrophil activation is also associated with the production of reactive oxygen species. Macrophages are the well-known surveillance cells in the tissues which react with particulate matters, including NOAAs to produce these biological responses. Exposure to different NOAAs has been found to modulate, in different ways, the defence/inflammatory capacities of macrophages[95]. In response to cytokines and chemokines, plasma proteins and neutrophils migrate from the blood to start inflammation. One of the roles of neutrophils in the inflammation is to destroy foreign bodies by proteolysis as well as reactive oxygen species. However, the excess or prolonged defence reactions against foreign bodies by these cells damage the tissues, too. When the process of macrophage ingestion and (attempted) proteolytic breakdown go awry or when nanoscale particles become internalized in non-immune-related cell types (e.g. parenchymal cells), intracellular molecular defenses are initiated which result in the new expression of protective genes. When these protective mechanisms (e.g. antioxidants) are depleted, tissue injury and disease might occur[57][96]. Several broad intracellular protective responses are known, from cell culture and animal studies, to be induced by the exposure to particulate/fibrous materials (carbon black, carbon nanotubes, ambient air particles, and nanostructured titania): the pro-inflammatory cytokines and relevant genes; the antioxidant-response element-inducible genes and proteins; and the stress (or "heat shock") response proteins[88][89][90][91][92][93][94]. Consequently, effects such as persistent inflammatory responses and gene inductions are likely to represent precursors of downstream pathological conditions. It is important to note that contaminants, such as metal catalysts or bacterial endotoxins (lipopolysaccharides), contributed to the induction of inflammatory responses observed in experimental toxicological studies of NOAAs. Specifically, the unpurified single-wall carbon nanotubes which contained more than 20 % by weight of iron induced stronger pulmonary inflammation than purified counterparts[58]; and the conventional formulations of gold NOAAs which contained significant amounts of endotoxins stimulated immune cells *in vitro*, and the improvement of formulation process, which diminished the endotoxin contamination, also effectively reduced the biological responses[97].

6.1.6 Observations from epidemiological studies involving fine and nanoscale particles

Initial epidemiological studies in workers exposed to aerosols, including incidental fine and nanoscale particles, have reported lung function decrements, adverse respiratory symptoms, chronic obstructive pulmonary disease, and fibrosis[14][15][16][17][55][98][99]. In addition, some studies have found elevated lung cancer among workers exposed to certain incidental nanoscale particles, e.g. diesel exhaust particulate[53][54] and welding fumes[55]. Human case studies have reported pulmonary oedema in workers exposed to polytetrafluoroethylene (PTFE) fume and an accidental death in a worker when an equipment malfunction caused overheating of the PTFE resin and release of the PTFE pyrolysis products in the workplace[100][101]. The implications of these studies for manufactured NOAAs, which might have different particle properties, are uncertain.

Epidemiological studies in the general population have shown associations between particulate air pollution and increased morbidity and mortality from respiratory and cardiovascular diseases[102][103][104][105]. Some epidemiological studies have shown adverse health effects associated with exposure to the incidental nanoscale particulate fraction of air pollution[106][107][108][109][110], although uncertainty exists about the role of incidental nanoscale particles relative to the other air pollutants in causing the observed adverse health effects. The associations in these studies have been based on measurements of the particle number or mass concentrations of particles within certain size fractions (e.g. PM2.5). In an experimental study of healthy and asthmatic subjects inhaling nanoscale carbon particles, changes were observed in the expression of adhesion molecules by blood leukocytes, which might relate to possible cardiovascular effects of incidental nanoscale particle exposure[111]. Controlled clinical studies in the laboratory have shown deposition of incidental nanoscale dusts throughout the pulmonary tree, accompanied by cardiovascular problems[29][112][113][114]. For diesel exhaust inhalation adverse vascular effects were attributed to the combustion derived nanoparticulates as demonstrated in a human volunteer study[115]. In addition, reducing personal exposure to air pollution using a highly efficient face mask appeared to reduce symptoms and improve a range of cardiovascular health measures in patients with coronary heart disease[116]. So, a link between inhalation exposure to incidental nanoscale particles and cardiovascular disease is likely.

Epidemiological studies aimed specifically at manufactured NOAAs have been performed only for TiO_2[28] and carbon black[15][16][17]. For both nanomaterials, epidemiologic studies found increase of lung cancer among workers, but did not find association with cumulative exposures to these nanomaterials. A recent 2016 meta-analysis did not find that carbon black exposure increases cardiac disease mortality[117]. The extrapolation of the findings associated with air pollution and particulates generally (which might include incidental nanoscale particles) to other manufactured NOAAs is uncertain. However, the potential risks from exposure to nanomaterials, including occupational exposure, should be considered.

6.2 Physical hazards

6.2.1 Fire (exothermic events)

Nanoscale combustible material might present a higher risk than coarser material of similar quantity due to exothermic events arising from catalytic reactions or due to a lowering of minimum ignition temperatures[118]. Decreasing the particle size of combustible materials can reduce minimum ignition energy and increase combustion rate, leading to the possibility of relatively inert materials becoming combustible. Dispersions of combustible nanomaterials in air might present a greater safety risk than dispersions of micro- and macro-materials with similar compositions. Dust explosiveness, minimum ignition energy and ignition temperature are typical means of characterizing safety relevant aspects of dusts[119].

Nano-objects and nanostructured porous materials have been used for many years as effective catalysts for increasing the rate of reactions or decreasing the necessary temperature for reactions to occur in liquids and gases. Depending on their composition and structure, some nanomaterials might initiate catalytic reactions and increase their fire and explosion potential that would not otherwise be anticipated from their chemical composition alone[120].

Additionally, some nanomaterials are designed to generate heat through the progression of reactions at the nanoscale. Such materials might present a fire hazard that is unique to manufactured NOAAs. The greater activity of NOAAs forms a basis for research into nanoenergetics. For instance, nanoscale Al/MoO_3 thermites ignite more than 300 times faster than corresponding micrometer-scale material[121].

6.2.2 Safety consideration in manufacturing NOAAs

The manufacture of NOAAs, as with any other novel materials, can be either on a small pilot scale, including research and development activities, or full scale. Presently, nanomaterial manufacturing can include several high-energy processes, such as flame pyrolysis, laser induced pyrolysis, laser vaporization, thermal plasma, microwave plasma, sputtering, and laser ablation, which present specific safety issues. Typical safety hazards in these processes include handling of high pressure cylinders, low pressure apparatus, inert and toxic gases, high temperature objects, operation of high electrical currents, electromagnetic radiation emitting devices, and high intensity light sources (including ultraviolet, infrared, and visible light) and lasers. Working under such hazardous conditions requires implementing appropriate work practices or following respective laboratory safety guidelines.

7 Exposure assessment to nanomaterials

7.1 General

This clause concerns the assessment of the exposure of workers as a result of the manufacture, handling and use of manufactured nano-objects and their agglomerates and aggregates (NOAAs). Occupational exposures to manufactured NOAAs can occur in a range of workplaces including construction, health care, energy, manufacturing and the chemical industry. New manufactured NOAAs are being developed and introduced into the workplace. One estimate has 400 000 workers worldwide employed in the field of nanotechnology[122]. The scientific framework for exposure assessment is discussed with a consideration of the routes of exposure and the most appropriate measurement metric to be used. This is followed by a summary of the instrumentation currently available and a discussion of possible sampling strategies.

Protecting workers' health involves minimizing occupational exposures to hazardous substances. At this time, there is insufficient information to determine specific occupational exposure limits (OELs) and/or reference values for many manufactured NOAAs[123][124][125]. However, first attempts have been made to propose occupational limit values which could be used in regulations. For example, provisional nano reference values (NRVs) have been developed in the Netherlands as pragmatic benchmark levels for different categories of nanomaterials[126][127][128][129]. Although NRVs are not health-based OELs, the Social and Economic Council of the Netherlands (SER) recommended their utilization as indicative guidance levels for exposure assessment until health-based OELs would become available. Some organizations have proposed OELs for selected manufactured NOAAs (see Clause 8 and Reference [130]). In addition, there are regulatory OELs for some traditional manufactured nanomaterials such as carbon black and synthetic amorphous silica.

Besides using these provisional OELs in assessing exposures, other approaches that are being used for exposure assessments include:

(1) Nanomaterial emission assessment approach: The nanomaterial emission assessment approach guides users in determining if there is a potential for exposure from such emissions. The nanomaterial emission assessment approach emphasizes identifying potential emission source(s) (i.e. area or workstation monitoring) and might not directly assess personal exposures. Upon determining the extent of emissions in the workplace, a control strategy (which might include the use of a control banding model) can be developed and implemented to minimize occupational exposures to manufactured NOAAs until there is sufficient scientific evidence to set an appropriate OEL on specific manufactured NOAAs in use. For example, OECD Safety of Manufactured Nanomaterials – No. 11 has suggested an (manufactured NOAAs) particle count level of 10 % above background levels[131].

(2) Tiered approach for exposure assessment: The tiered approach for exposure assessment is a formal methodology for conducting occupational exposure assessments and measurements of aerosols containing manufactured NOAA in work processes (see also 7.3.2). The tiered approach commonly contains three hierarchical tiers. Tier 1 focuses on data gathering prior to workplace assessment to identify possible sources of exposure. Exposure evaluation as part of the Control Banding approach (ISO/TS 12901-2:2014)[132] could be used in Tier 1. Tier 2 includes measurements and assessment of the workstation/breathing zone air and comparing it to the 'background'. Only if Tier 2 reveals a significantly increased particle number concentration, mass concentration or positive detection by electron microscopy or chemical analysis in the workplace, should Tier 3 comprehensive measurements be necessary (see, for example, Reference [133]).

Regardless of the approach used, measuring background levels is critical. One suggestion for the maximum background level to be used for monitoring purposes is three times the standard deviation of background particle number concentrations[124]. Further information on issues related to identifying and quantifying background can be found in review articles[124][134][135][136].

In one example, Genaidy et al.[137] conducted a detailed risk analysis of a CNF manufacturing process and suggested the following potential sources of workplace exposure to NOAAs:

- leakage and spillage from reactors and powder processing equipment;
- manually harvesting product from reactors;
- discharging product into containers;
- transporting containers of intermediate products to the next process;

- loading the powders into processing equipment;
- weighing out powder for shipment;
- packaging material for shipment;
- storing material between operations;
- cleaning equipment to remove debris stuck to side walls;
- changing filters on dust collection systems and vacuum cleaners;
- further processing of products containing NOAAs (e.g. cutting, grinding, drilling).

This detailed analysis identifies common processes that can lead to worker exposure to NOAAs.

7.2 Scientific framework for assessing exposure to nanomaterials

7.2.1 Routes of exposure

There are four main routes by which workers can be exposed to nanomaterials:

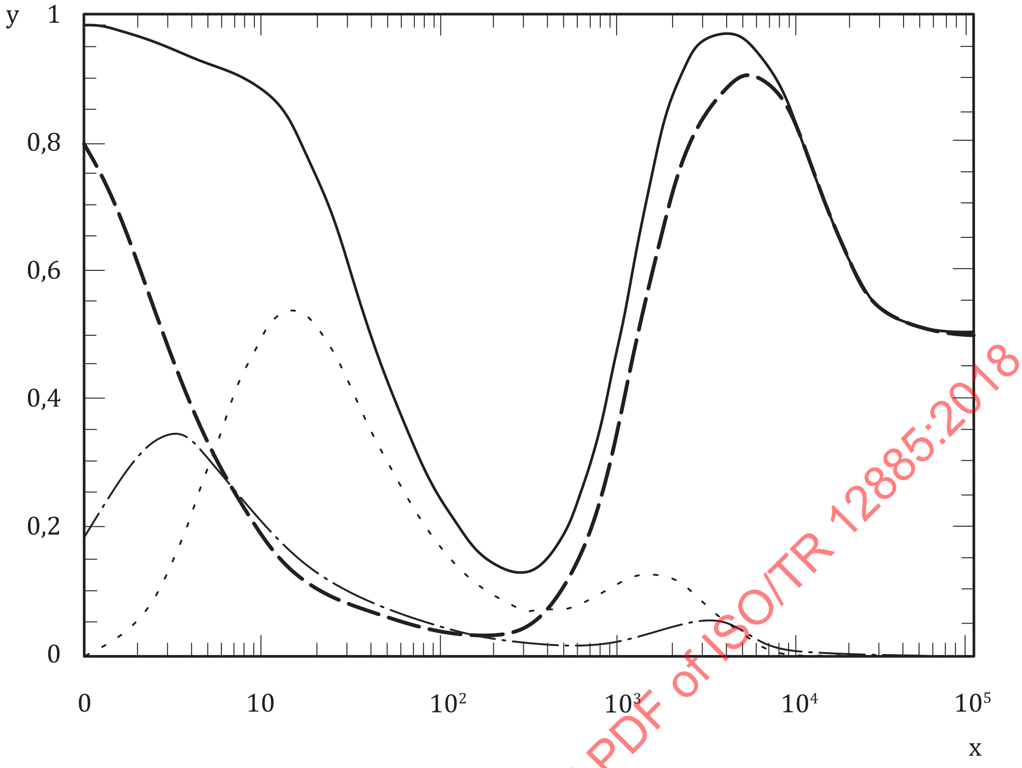
a) inhalation;

b) ingestion;

c) skin contact;

d) injection/parenteral route[29].

Inhalation. As with most particles in the workplace, inhalation is considered to be the primary route by which nanomaterials in the form of free, unbound, airborne particles (e.g. nano-objects and their agglomerates and aggregates) will enter the bodies of workers. Once inhaled, nanomaterials will deposit in the respiratory tract regions, depending upon their particle size. Specifically, nanoparticles will deposit in all regions of the respiratory tract. Figure 4 shows the fractional deposition of inhaled nanoparticles in the nasopharyngeal, tracheobronchial and alveolar regions of the human respiratory tract for nasal breathing, using the predictive mathematical model of the ICRP[138]. According to the ICRP model, 80 % of the 1 nm particles are deposited in the nasopharyngeal region, with 20 % in the tracheobronchial region and less than 1 % in the alveolar region. For 20 nm particles, however, 50 % deposit in the alveolar region and 25 % in the nasopharyngeal and tracheobronchial regions.



Key

x	particle diameter (nm)
y	deposition probability
————	total deposition
— — — —	head region
—·—·—	tracheobronchial
·········	alveolar

Note Deposition Fraction includes the probability of particles being inhaled (inhalability). The subject is considered to be a nose breather, performing standard work.

Figure 4 — Predicted total and regional deposition of particles in the human respiratory tract related to particle size using ICRP 66 model[138]

Depending on the physicochemical characteristics of the inhaled manufactured NOAAs, the respiratory system can be considered to be a pathway for inhaled substances to reach other organs that are distant from the lungs[139]. Nano-objects have been reported to translocate to different organs in the body after penetrating the cell epithelium and entering the blood or lymph systems (see 6.1.2 and Annex B). Even after inhalation a major part of the inhaled particles are taken up by alveolar and lung macrophages that are subsequently cleared by the mucociliary cascade and will end in the GI-tract.

Ingestion. In the workplace, NOAAs could be ingested by swallowing the mucous that traps and clears particles deposited in the airways; by ingestion of contaminated food or water; or by oral contact with contaminated surfaces or hand. Only a few studies have been carried out to investigate the uptake and deposition of NOAAs to the GI tract[29]. In general uptake from the GI-tract of objects was found to be relatively low[47][48][49][50]. It was found that

1) NOAAs can directly affect nutrients absorption through disruption of microvilli or alteration in expression of nutrient transporter genes;

2) food components directly might affect NOAAs absorption through physicochemical modification;

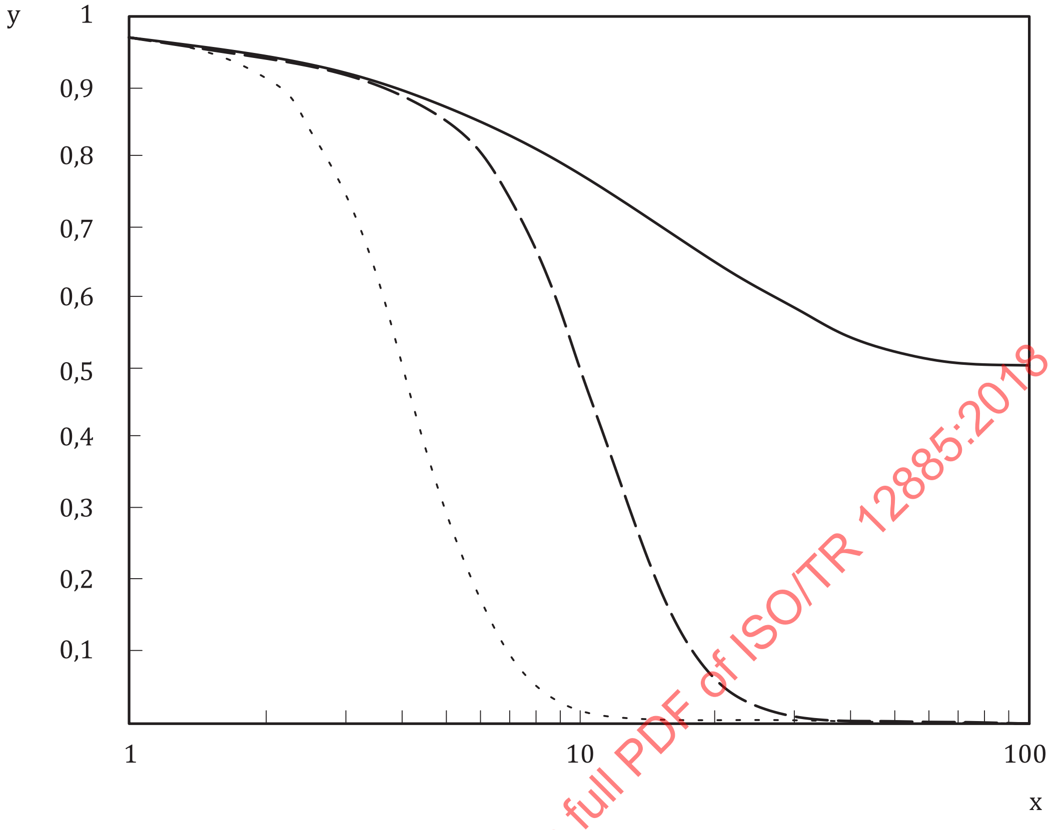
3) the presence of food components might affect oxidative stress induced by NOAAs[140].

Skin contact. In the workplace, skin might be exposed to NOAAs during their manufacture or use or by contact with contaminated surfaces. It is still under discussion if and to what extent NOAAs in general are able to penetrate the intact skin, which, depending on the nanomaterial, might cause adverse effects[141][142]. Most of the reported work has been carried out with individual materials, such as TiO_2 and ZnO, on intact skin. A limited number of studies explored the effects of flexing the skin, skin damage and nanoparticle surface functionalities on skin penetration by nanoparticles[143][144][145][146]. The role of solvents and normal force applied to the skin in dermal uptake of NOAAs in the occupational setting is also yet to be fully explored. Ocular route might be another important route of exposure requiring additional studies.

Injection/parenteral route. Exposure through parenteral route can occur in the workplace primarily due to accidents such as needle-stick injuries. Workers who could be at risk from needle-stick injuries include, but are not limited to, those in workplaces in the health care sector, law enforcement and the construction industry. Manufactured NOAAs have potential applications as drug delivery systems and in medical diagnostic procedures involving injections[147]. Consequently, workers could be at risk of accidental injections of such manufactured NOAAs. Once the worker's skin is broken, punctured or scratched by needles or other sharp objects, manufactured NOAAs can enter the bloodstream and hence can translocate throughout the body.

7.2.2 Metric for assessing exposure to airborne nanomaterials

The traditional method of assessing worker exposure to airborne particles in workplaces involves the measurement of the mass concentration of health-related fractions of particles in the worker's breathing zone (ISO 7708:1995)[14] and their chemical composition. The health-related aerosol fractions relate to the probability of penetration of airborne particles to the various anatomical regions of the respiratory system and provide a specification for the performance of sampling instruments (see Figure 5). The inhalable convention is the fraction of total airborne particles that enters the nose or mouth during breathing, The thoracic convention is the fraction of inhaled particles that penetrate the respiratory tract beyond the larynx, with 50 % penetration at an aerodynamic equivalent diameter (AED) of 11,64 µm (equivalent to 10 µm when expressed as a fraction of total aerosol) and the respirable convention is the fraction of inhaled particles that penetrate to the alveolar region of the lung, with 50 % penetration at AED 4,25 µm (equivalent to 4 µm when expressed as a fraction of total aerosol).



Key

x particle diameter (nm)

y fraction of ambient particles in convention

——— inhalable

— — — — thoracic

. . . . respirable

Figure 5 — Health-related sampling conventions for workplace aerosols from ISO 7708[14]

The main exceptions to this methodology are particle-number-based metrics of exposure used:

a) for fibres such as asbestos (defined as of length > 5 μm, width < 3 μm and length: width aspect ratio ≥ 3:1, Note: the definition and sampling methodology of asbestos fibres can vary for different jurisdictions; this definition of fibre is based on World Health Organization definition[148]), for which the standard method is to collect them on a membrane filter sampler and to count them using optical or electron microscopy[149]; and

b) for microorganisms, for which the standard method is to collect them on a growth medium and to count the number of colony-forming units[150][151].

If not combined with other parameters, the mass dose metric (exposure concentration of air or lung dose) has been shown to be a poor predictor of toxicity for poorly soluble nano-aerosols/nano-objects (when compared with larger respirable particles), but it is a widely used metric. To be used in exposure assessment, this metric needs to be associated with other information like constituent/primary particles size, aggregation or agglomeration state, agglomerate density[152]. Particle surface area dose and particle volume have been reported to be better predictors of lung responses in rats or mice across a range of particle sizes[25][29][30]. Agglomeration can influence the deposited dose since airborne particle size determines the deposition efficiency in the respiratory tract[25]. The deposition probabilities are described in ISO 13138[153]. A number of studies have indicated that the toxicity of insoluble materials increases with decreasing particle size, on a mass for mass basis[29][154][155]. The mechanisms by which these materials exhibit higher levels of toxicity at smaller particle sizes have yet to be explained, although there are many hypotheses. A number of studies indicate that biological response depends on the surface-area of particles deposited in the lungs[25][26][29]. It has also been suggested that due to their small diameter, nano-objects are capable of penetrating epithelial cells, entering the bloodstream from the lungs[156], and even entering the brain via the olfactory nerves[40]. As particles in the nanometer size range have a high percentage of surface-atoms, and are known to show unique physicochemical properties, it would be expected that nano-objects would demonstrate biological behaviour closely associated with particle diameter, surface-area and surface activity.

It is apparent from the above discussion that measuring exposures to nano-aerosols in terms of mass concentration alone is not sufficient to assess potential health risk. In addition, there is strong evidence to suggest that occupational nano-aerosols should be monitored with respect to surface-area. However, in this context, aerosol surface-area is not well defined and it is dependent on the measurement method used. Geometric surface-area refers to the physical surface of an object, and is dependent on the length-scale used in the measurement. Measurement length-scale determines the upper size of features that are not detected by the measurement method. For example, methods utilizing molecular surface-adsorption have a length-scale that approximates to the diameter of the adsorbed molecules[157]. Similarly, biologically relevant surface-area will most likely be determined by the smallest biological molecule that interacts with particles within the body.

While a strong case might be made for using aerosol surface-area as an exposure metric, it is also necessary to consider characterizing exposures against aerosol mass and number concentration until further information is available. In the case of a nanomaterial with consistent composition, size and shape, one can measure the specific surface area and correlate it to mass concentration. However, the correlation coefficient will not be transferable to other nanomaterials with different distributions in composition, size and shape. For each of these exposure metrics, but particularly in the case of mass concentration, particle size selective inlets will need to be employed to ensure only particles within the relevant size range or in the particular respirable fraction, are sampled[158].

The actual particle cut-off size for assessing potential human health impact is still open to debate and depends upon particle behaviour and subsequent biological interactions. The currently proposed cut-off size for nanoparticles is 100 nm, although this is not derived from particle behaviour in the respiratory tract following deposition and it excludes larger particles of nanomaterials. However, at this scale it is thought that the properties of materials can be different from those at a larger scale. For instance, it could be possible to develop a health-related description of a nanoparticle based on the deposition probability in the lungs (see the curves in Figure 4). Particle deposition efficiency in the respiratory tract reaches a minimum at about 200 nm to 300 nm in diameter, and increases for particles of lesser diameter. In addition, as particles become smaller, surface curvature, the arrangement (and percentage) of atoms on the particle surface and size-dependent quantum effects play an increasingly significant role in determining physicochemical behaviour.

It is currently unclear whether the biological impact of discrete nanoparticles depositing within the respiratory system, is distinct from or similar to the impact of large agglomerates or aggregates of nanoparticles containing the same mass of material. Several factors can affect biological response. First, the location of deposition depends on aerodynamic particle size which will change with degree of agglomeration and aggregation. Next, if agglomerates or aggregates of nanoparticles either de-agglomerate or disaggregate completely following deposition, it is conceivable that the resulting biological impact will be similar to an equivalent exposure of discrete nanoparticles. In addition, if biological response is associated with the surface-area of the deposited aerosol, then for a given volume of material, the response to deposited agglomerates and aggregates with an open fractal-like structure will conceivably be similar to that from an equivalent dose of discrete particles. However if the nanostuctured particles do not de-agglomerate then it is likely that they will not translocate to other organs in the body as readily as the discrete nanoparticles and so the biological impacts will be different. So, knowledge of the ease with which the specific particles will de-agglomerate will be required before deciding at which particle size to exclude unwanted particles (bulk particles which are not nanostructured materials) and might vary with the particle properties.

7.3 Review of methods for characterizing exposure to manufactured NOAAs

7.3.1 General

The principal purpose for most particle sampling is for the protection of workers by controlling the emissions of hazardous substances to air. This usually forms part of a risk management strategy which is ideally based on: hazard identification, hazard assessment, exposure assessment and risk assessment (see 8.2). However, for NOAA the hazard and risk assessment information currently available is often very limited and this means that targeted exposure sampling can be carried out for various purposes:

1) assessment of personal exposure for compliance with regulations,
2) assessment of personal exposure for linking with potential adverse health effects in epidemiological studies,
3) identification of major emission sources for establishing targeted control plan,
4) assessment of efficiency of control systems deployed.

Each of these tasks requires specific and often different types of instrumentation. For example, for personal exposure measurements the best solution is to use small, battery-powered samplers, mounted with the inlets close to the worker's breathing zone, so that move with him/her during the working shift. For source identification, personal or hand-held portable monitors can be used, generally giving continuous measurements of concentration that can be correlated with details of the location, ventilation and the specific work processes being undertaken. In order to assess the nanoparticles present and/or the overall efficiency of control measures in the workplace, many different types of monitoring instruments can be used, including larger static, mains-driven research orientated instruments, depending upon the information required.

However, for assessing exposure to manufactured NOAAs a major confounding factor in most workplaces is that of incidental nanoscale objects derived from ambient aerosols that penetrate workplaces to differing degrees[159][160], and incidental nanomaterials generated within the workplace itself[161]. These confounding particles can directly impact measurements of particle count, but also rapidly coalesce with nano-objects, including the NOAAs of interest[162], requiring careful design of measurement strategy. Possible methods of discriminating between exposures to manufactured NOAAs and to ambient incidental nanoscale particles will be discussed later. While discrimination will facilitate analysis of exposures, effective control of both manufactured and incidental NOAAs is essential for effective occupational health and safety management.

Measurement techniques and strategies need to be optimally combined to enable sensitive yet cost-effective determination of airborne manufactured NOAAs in the workplace. Measurement strategies might consider using tiered approaches as this can facilitate cost effective screening at workplaces. In addition, sampling methods that have been developed and/or proposed are often conducted using multiple instruments designed to capture multiple metrics (such as mass concentration, surface area concentration and NOAAs count concentration).

One of the challenges in obtaining exposure assessments of manufactured NOAAs in the workplace is the lack of reliable, standardized reference materials for developing and validating potential exposure assessment tools (including standardized sampling and monitoring methods, calibration protocols and instrumentation). However, unimodal test aerosols of particles in the nanoscale and above can be produced: e.g. cubic particles (NaCl and CsCl), spherical particles (Ag and DEHS) and agglomerates (carbon, diesel soot and ZnO) can be generated. Bimodal test aerosols can also be generated for instruments inter-comparison, calibration and performance testing[163][164][165]. A calibration method for the absolute number concentration has been proposed by Koch et al.[166]. Reference materials are a means to provide assurances to the quality of measurements, and to improve or revise potential sampling and monitoring methods. For manufactured NOAAs, developing standardized reference materials can help assess in conducting exposure and risk assessments; this might involve sampling and measuring specific properties of the manufactured NOAAs of interest[167].

Ideally, according to Reference [168], the equipment for taking the occupational hygiene measurements should be:

- portable;
- capable of measuring multiple NOAA characteristics (particle count, mass, surface area, charge, size distribution, differentiate manufactured from background particles, temporal variation etc.);
- capable of obtaining breathing zone samples;
- capable of being used in industrial settings;
- battery-powered;
- real-time;
- relatively inexpensive.

At this time there is not a single instrument that meets all of these criteria. As well, many of the currently available hand-held, real-time aerosol instruments are more suited for measuring specific size-ranges of airborne spherical structures or NOAAs[169].

Particle number concentrations and particle number size distributions are some of the most commonly used metrics in many workplace exposure assessment studies. Advantages to this approach include the wide availability of measurement devices (e.g. CPC) and the instruments' high sensitivity to airborne nano-objects. Disadvantages include the inability to differentiate the background from the release of manufactured NOAAs from work-related practices. Challenges include the current lack of information of lower and upper detection limits (especially when compared with a measurement strategy being used). As well, uncertainty arises from the calibration of the devices which can differ by as much as 30 %[134].

A summary of currently available instrumentation is provided in Table 1, part of which is an updated version of that found in the ISO/TR 27628:2007 on Ultrafine, nanoparticle and nanostructured aerosols — Exposure characterization and assessment[170]. Some of the following subclauses contain information taken from ISO/TR 27628:2007, which will be updated periodically and might contain more up to date information than this document. Table 1 also integrates information from Reference [134] which contains a review of those instruments applied in occupational settings. Additionally a European Standard describes the different metrics in use and applicable instrumentation[171].

Table 1 — Selected instruments and techniques for monitoring nano-aerosol exposure

Metric	Devices/Techniques	Remarks
Mass directly	Size selective static sampler	The only devices offering a cut point around 100 nm are cascade impactors (Berner-type low pressure impactors, or Microorifice impactors). Allows gravimetric and chemical analysis of samples on stages below 100 nm.
	TEOM®<?> TEOM® is the registered trademark of a product supplied by Thermo Fisher Scientific Inc. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.	Sensitive real-time monitors such as the Tapered Element Oscillating Microbalance (TEOM) might be useable to measure nano-aerosol mass concentration online, with a suitable size selective inlet.
Mass by calculation	Electrical Low Pressure Impactor (ELPI®)<?> ELPI® is the registered trademark of a product supplied by Dekati Ltd. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.	Real-time size-selective (aerodynamic diameter) detection of active surface-area concentration giving aerosol size distribution. Mass concentration of aerosols can be calculated, only if particle charge and density are assumed or known. ELPIs can detect airborne particles between 6 nm – 10 µm. Size-selected samples might be further analysed off-line (as above).
	DMAS	Real-time size-selective (mobility diameter) detection of number concentration, giving aerosol size distribution. Mass concentration of aerosols can be calculated, only if particle shape and density are known or assumed.
Number directly	CPC	CPCs provide real-time number concentration measurements between their particle diameter detection limits. Without a nanoparticle pre-separator, they are not specific to the nanometre size range. Generally, different types of CPCs can detect airbonre particles between 5,5 nm – 9 µm. P-Trak has diffusion screen to limit top size to 1 µm. Particle number concentration.
	DMAS	Real-time size-selective (mobility diameter) detection of number concentration, giving number-based size distribution.
	Electron Microscopy	Off-line analysis of electron microscope samples can provide information on size-specific aerosol number concentration.
Number by calculation	Electrical Low Pressure Impactor (ELPI®)	Real-time size-selective (aerodynamic diameter) detection of active surface-area concentration, giving aerosol size distribution. Data might be interpreted in terms of number concentration. ELPIs can detect airbonre particles between 6 nm – 10 µm. Size-selected samples might be further analysed off-line.

Table 1 *(continued)*

Metric	Devices/Techniques	Remarks
Surface-area directly	Diffusion Charger	Real-time measurement of aerosol active surface area. Active surface area does not scale directly with geometric surface area for particles larger than 100 nm. Note that not all commercially available diffusion chargers have a response that scales with particle active surface area for particles smaller than 100 nm. Diffusion chargers are only specific to nanoparticles if used with an appropriate inlet pre-separator.
	Electrical Low Pressure Impactor (ELPI®)	Real-time size-selective (aerodynamic diameter) detection of active surface-area concentration. Active surface area does not scale directly with geometric surface area for particles larger than 100 nm.
	Electron Microscopy	Off-line analysis of electron microscope samples can provide information on particle surface area with respect to size. TEM analysis provides direct information on the projected area of collected particles, which might be related to geometric area for some particle shapes.
Surface area by calculation	DMAS	Real-time size-selective (mobility diameter) detection of number concentration. Data might be interpreted in terms of aerosol surface area under certain circumstances. For instance, the mobility diameter of open agglomerates has been shown to correlate well with projected surface area[170].
	DMAS and ELPI® used in parallel	Differences in measured aerodynamic and mobility can be used to infer particle fractal dimension, which can be further used to estimate surface area.

Characteristics of the above mentioned instruments are given in Annex C.

7.3.2 Sampling strategy issues

Until it has been agreed which is(are) the most appropriate metric(s) for assessing exposure to manufactured NOAAs in relation to potential adverse effects, it has been recommended that a range of instrumentation be used to provide full characterization of the aerosols in workplaces where NOAAs are being produced, handled or used to make new materials[172]. This approach currently requires the use of relatively large and expensive "research" instruments, which is not conducive to the sampling procedures and strategies required to assess personal exposure for compliance with any exposure limit or for epidemiological purposes or for identifying major emission sources. A review on exposure related workplace measurements and laboratory studies is given in Kuhlbusch et al.[134].

However, new instruments are being continuously developed and there are small portable instruments for particle number concentrations, particle surface area concentrations and health-related surface area concentrations. While most of instruments are not yet truly personal, they are compact enough to be carried from location to location in the workplace and to be sited close to the worker at each location. Currently however, these instruments do not provide enough information for full characterization of the workplace, so static instruments such as the DMAS, ELPI® and devices for collecting particles for physical and chemical characterization should be included. Care should be taken in setting these static samplers as aerosol characteristics can change with distance from source, leading to spatial and temporal variation of nano-aerosol mass and number concentration. This is especially true for hot processes leading to particle nucleation from vapour that will often lead to variations in emission rate and concentration over time.

To improve the comparability of exposure data, the accepted practice of giving personal exposure as an eight-hour-shift value should also be observed in the case of nano-aerosols. In consequence, wherever possible exposure measurement results concerning shorter measurement intervals should be converted into shift data by time weighted recalculation. In all cases, where short-term exposure itself is the target of investigations, the time base of measurements needs to be documented. A time base of 15 min for short-term exposure measurements is recommended as it is generally used in occupational hygiene.

Selection of the most appropriate sampling location or locations is a key factor for a reliable interpretation of data in view of personal exposure. This requires analysis of tasks carried out by workers using or handling nanomaterials, identification of all the potential nano-aerosol-emitting sources in the workplace and an understanding of the ventilation system in the workplace to determine the potential for cross contamination. This could be a significant problem for NOAAs as they will remain airborne for considerable periods of time and be easily dispersed by the air currents in the workplace. For single sources, the relationship between aerosol emission and work activities should be clear, enabling the reliable assignment of exposure levels to be made.

However, unless the workplace is operating under clean room conditions or has high efficiency filters on the inlet air through well-defined inlets, outdoor sources of nano-aerosols (e.g. vehicle exhausts, other industrial activities, power stations, etc.) will penetrate indoors and result in overestimation of the levels of NOAAs emitted from the process under investigation. This will inevitably lead to an overestimation of the worker exposure to NOAAs derived from that process. One way to overcome this problem is to determine ambient or background particle counts prior to the commencement of manufacturing or processing of the NOAAs. However, it might not be possible to subtract the background particle counts from the exposure level counts since the background counts can fluctuate with time. Another method is to carry out simultaneous measurement of background concentrations using a duplicate set of monitoring equipment to monitor outside the workplace, and to subtract the outdoor levels from those measured inside the workplace. However this can be expensive and assumes that the ambient particles do not change during transport into the workplace[172].

An important component of assessing exposure in the workplace to manufactured NOAAs is the characterization of the background. Recent studies have been carried out whereby a suite of direct-reading instruments (e.g. CPC, SMPS, APS) were used to characterize the variability of the background nano-aerosol levels over time[134][136]. Where possible, measurement strategies should include the use of multiple direct-reading instruments to cover all the relevant measurands (e.g. particle size distribution, particle count, and particle surface areas).

Although relatively simple particle number and surface area measurements can be used for identifying the sources of leaks and major emission sources, at some point it becomes necessary to confirm that the measurements being made are related to the NOAA’s of concern. Sampling strategies such at NEAT 1.0[173] and NEAT 2.0[174] have made provision for the sampling and off-line analytical TEM analysis and chemical characterization of the particles collected. This information informs what percentage and in what size ranges the NOAA’s of concern contribute to the real-time measurement of particles. This ratio can then be used to calculate the surface area concentration of the manufactured NOAAs in the total surface area concentration values for all airborne NOAAs detected. The accuracy of this approach will obviously depend upon the manufactured NOAAs having at least one detectable element that is not present in outdoor aerosols. Application of this method to CNTs is described in C.6.

If there is no reliable characterization of the NOAA of interest, the real-time measurands have to be compared to determine whether a significant exposure or release might have occurred and the background is exceeded. Several decision rules can be used. Such decision rules, and even one combined decision rule, have been tested for a variety of exposure situations[175][176]. However, it was acknowledged that the sampling and analysis of additional aerosol samples to determine the occurrence of nano-objects will be a more reliable decision process.

- previously, many measurement strategies identify the need for:
  - tiered approach to facilitate exposure assessments;
  - tiered approach that is be easy to implement and cost effective;
  - approach based on established measurement methods;
  - approach capable of discriminating and quantifying manufactured NOAAs from background particles.

An example of an early tiered approach is described below and in Figure 6[124]:

- Tier 1 — Information Gathering. Tier 1 is focused on the gathering of information prior to a workplace exposure assessment. This is done to determine if additional assessment is needed in regard to potential occupational exposures to manufactured NOAAs.
- Tier 2 — Basic Assessment: If occupational exposure cannot be excluded following the Tier 1 analysis, a Tier 2 investigation will occur. Tier 2 involves detecting, monitoring and measuring possible release and detection of elevated particulate and NOAA concentrations by cost effective targeted monitoring. Assessment includes measuring nano-aerosol concentrations vs. background concentration levels to determine a realistic value for occupational exposures to manufactured NOAAs.
- Tier 3 — Comprehensive Assessment: Tier 3 becomes necessary if significant exposure can still not be excluded. In Tier 3, extended measurements are required for a more detailed assessment. This would include monitoring to identify the level of manufactured NOAAs, and would require the implementation of a control strategy to minimize occupational exposures.

This is just one example of several available tiered approaches to exposure assessment for nanomaterials. More recently, a “harmonized tiered approach” has been adopted by OECD[127]. Recommendations on how to perform an assessment of inhalation exposure to nano-objects and their agglomerates and aggregates (NOAA), including which measurement strategy to adopt, will be also provided in EN 17058[177].

Exposure data generated through the use of these approaches will be collected in suitable databases in order to reach a comprehensive description of exposure situations and use these data in control banding tools and for the derivation of future exposure limit values. Databases like NECID[178] or eNanoMapper are existing tools towards this aim. Calibration and the correct operation of the monitoring instruments is required to ensure data quality and enable such use of collected data (e.g. NanoGem[124] and Nanoindex[179]).

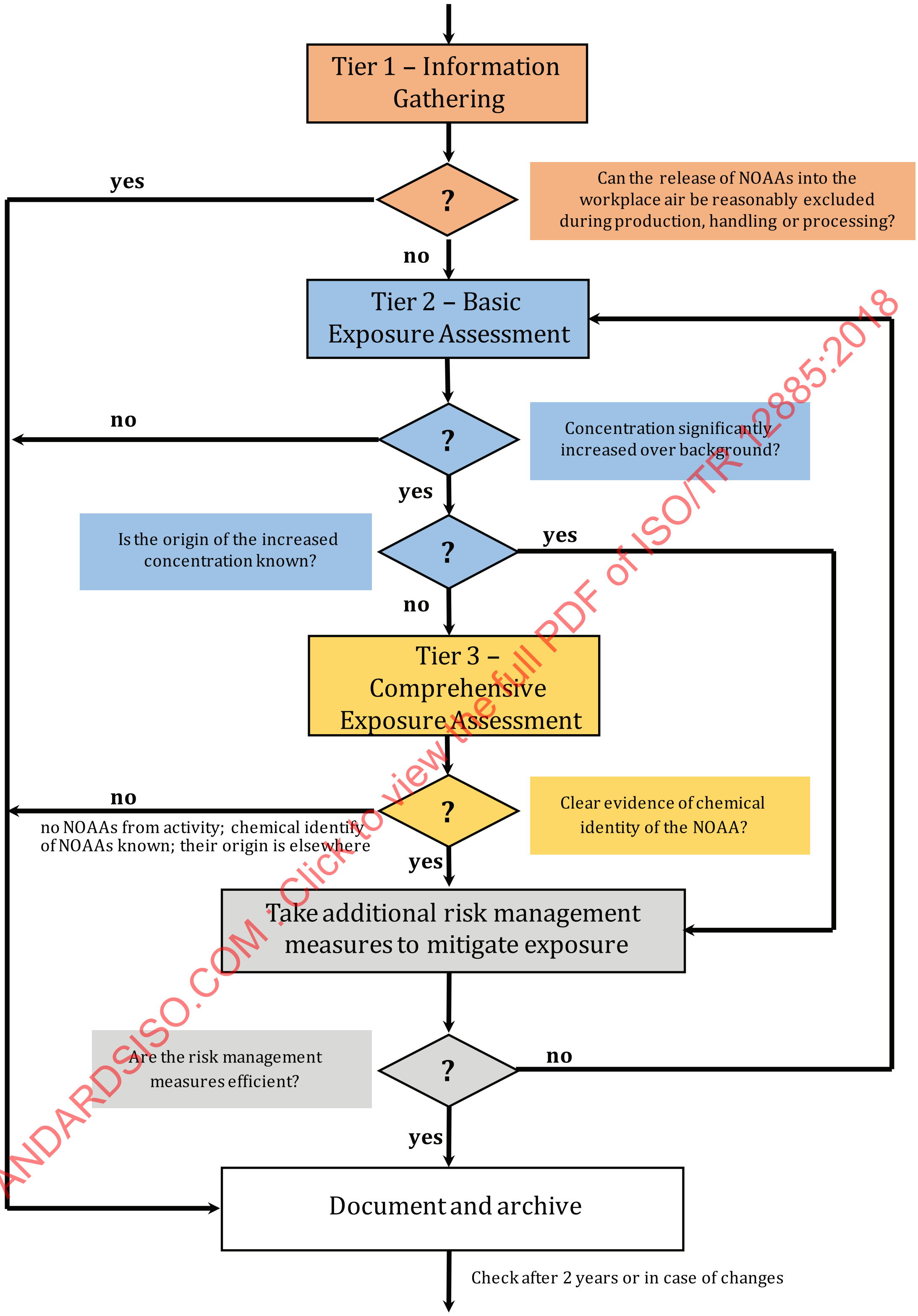


Figure 6 — Flow chart of a tiered approach (based on Figure 1 of Reference [124])

7.4 Dustiness assessment

7.4.1 General

The control of the emitted and released airborne nano-objects and particles during the handling and transportation of bulk materials is an important consideration for workers' exposure and the design and operation of many industrial or research processes. It is therefore important to obtain information about the propensity of bulk materials to release nano-objects and respirable particles and thus assist in assessing the risk for exposure to a hazardous material.

Dustiness data have been recommended for nanomaterials exposure assessment by the Organization for Economic Co-operation and Development[180] and are also already in use as an input parameter in some control banding tools for nanomaterials or to predict the likelihood of exposure by modelling. Finally, dustiness data can provide the manufacturers of nanomaterials with information that can help to improve their products (e.g. by selecting less dusty nanomaterials) or the users to improve their processes or their technical prevention approaches.

Dustiness depends on a number of factors including the physical state of the bulk material (e.g. powder, granules, pellets and moisture content), the physicochemical properties of the particles contained in the bulk material (e.g. size and shape, relevant density, type of coating, hydrophobicity and hydrophilicity properties, aggregation of particles), the environment (e.g. moisture, temperature), the condition of the bulk material, the type of aerosol generation (activation energy or energy input, time characteristics of the energy input) and the interaction between particles during agitation (e.g. friction shearing, van der Wall forces). Dustiness testing should simulate typical powder processing and handling. It should enable a comparison of the relative dust release potential of different bulk materials and data derived from dustiness testing could be used as input for qualitative or quantitative exposure assessment. Dustiness involves the application of a given type and amount of activation energy or energy input, to a stipulated amount of test material during a stipulated time, whereby particles are dispersed into the air and are described quantitatively.

7.4.2 Measurement methods

No single dustiness method is likely to represent and reproduce all the various types of processing and handling used in workplaces. Therefore, there are a number of methods for the design of dustiness devices and different values will be obtained by different test methods. However, the test and the variables including the sampling and measurement configuration need to be closely specified to ensure reproducibility. The results of pre-normative research[181] led to the development of four test methods: the rotating drum, the continuous drop, the small rotating drum and the vortex shaker.

Conventional dustiness methods for micrometre size particles estimate the airborne dust generated in terms of dustiness mass fraction (e.g. respirable, thoracic, inhable) expressed in mg/kg. The current European standard EN 15051-1-3[182][183][184] for conventional dustiness provides two methods: the rotating drum method and the continuous drop method. Although these methods are accepted standards for micrometer size particles, the biological behaviour of nano-objects because of their small size and surface area has raised the question whether the dustiness can be adequately characterized by their mass fraction only. Therefore, particle number concentration and size distribution are other important measurands for measuring and characterizing the dustiness of bulk material containing nano-objects. ISO/TS 12025[185] provides general methodology for the quantification of nano-object release from powders as a result of treatment, ranging from handling to high energy dispersion, by measuring aerosols liberated after a defined aerosolization procedure. Further work to establish test methods that measure the dustiness of bulk materials containing nano-objects in terms of health-related index mass fraction, index number and emission rate, is taking place[186][187][188][189]. In addition, the new standards will also establish test methods that characterize the aerosol from its particle size distribution and the morphology and chemical composition of its particles.

7.5 Dermal exposure assessment

7.5.1 Sampling

7.5.1.1 General

Sampling of manufactured NOAAs deposited on skin in the workplace can be accomplished by adapting well established sampling methods developed for chemicals[190][191][192].

The direct assessment of dermal exposure to manufactured NOAAs can be accomplished by measuring the amount of the manufactured NOAAs in contact with the skin over a period of time. The methods developed for such purposes entail either the removal of accumulated contaminants from the skin[193] or interception of the material as contact occurs. The removal methods include uncertainties in the removal efficiency and require that the duration of contact be evaluated through independent means. Uncertainty is introduced by the interception methods through the use of materials that usually do not mimic the adherence characteristics of the skin accurately. These methods are summarized in the following subclauses.

7.5.1.2 Removal procedures

Rinse method. Various solvents (for example, solutions of surface active compounds) can be used to rinse the exposed skin and remove accumulated NOAAs. These solutions can then be analysed for the presence of NOAAs, followed by chemical, particle size and shape analyses.

Wipe method. Solvent impregnated materials can be used to wipe the skin and remove residues. The wipe material is then analysed for the NOAAs of concern. For example, metal concentrations might be measured using ICP-MS; other analytical methods would likely be required for most nanomaterials.

Tape stripping method. Adhesive tape can be applied to the skin for purposes of removing contaminant NOAAs both from the surface of the skin and from within the skin. ICP-MS or other analytical techniques can be used to estimate the amount of residue including NOAAs removed with the tape.

In all these methods, skin should be cleaned before potential NOAA exposure might occur. This confirms NOAA exposure if NOAA are identified after removal procedures.

7.5.1.3 Interception procedures

Patch method. Patches made from various materials such as cotton or polyester gauze, alpha-cellulose paper, polyurethane foam, or polypropylene film can be placed on the body to collect NOAAs as contact occurs. The method requires some fairly extensive assumptions, and in the occupational setting, it has been proven to be useful for screening purposes but is limited as a quantitative method. It is generally believed that amounts of contaminant recovered by either removal or interception methods do not accurately correspond to amounts of contaminant deposited on the skin. Collection efficiency is considerably lower via removal[194] than interception methods[195]. Results of these methods are more a reflection of skin loading than actual exposure; thus, measurement results at best provide indices of relative exposure.

Glove method. Absorbent gloves can be used to collect NOAAs contacting the hands[195][196][197].

Whole body dosimetry. This method involves the use of clothing covering the whole body (usually cotton, long underwear tops and bottoms and socks) to trap NOAAs. A problem with this method is the difficulty in extracting NOAAs from such a large collector. An advantage of this method over the patch method is that it is less likely to miss areas where exposure might occur. Another approach that can be used to estimate exposure is by using patches that are placed on multiple anatomical regions of the body[198].

7.5.1.4 Other procedures

Fluorescent tracers. This procedure involves modifying the NOAAs of concern with a nontoxic fluorescent tracer and then using video imaging to identify and quantify the points where the NOAAs contact the skin[199].

Contaminated surfaces. Contaminated surfaces, such as tools and equipment, represent another category of sampling. According to Fenske[200], surface sampling can be considered a first approximation of personal dermal exposure. This observation supports the value of controlling the migration of NOAAs in the workplace.

7.5.2 Sample characterization

Electron microscopy can be used to characterize size distribution, number concentration and shape of NOAAs collected on samplers. In wipe methods, use of mixed-cellulose ester filters as wipes could facilitate such analysis.

Light scattering, laser diffraction, size exclusion chromatography, acoustic techniques and field flow fractionation could be used to characterize size distribution and number concentration[201], while spectroscopic techniques can be useful in obtaining information about chemical composition and structure of NOAAs. These techniques can work with rinse sampling methods.

7.6 Dose (internal exposure) assessment

Internal exposure is more directly linked to adverse health effects. However, dose assessment involves analysis of tissues, body fluids, and exhaled air. In occupational settings, less invasive methods such as collection of hair, urine and exhaled air are used most commonly.

Dose can be determined by measuring amount of manufactured NOAAs of interest and/or their metabolites. The term “biomarker” is often used to describe a range of biological effects resulting from interactions between human biological systems and a toxicant. Biomarkers can provide direct evidence for the exposure to a particular toxicant if there is a unique correlation between a particular biomarker and a toxicant. One of the advantages of measuring biomarkers of exposure is that it provides information about combined exposure through multiple routes, including non-occupational. Measurements of biomarkers of exposure are used for screening and monitoring of workers.

Biomarkers of exposure to manufactured NOAAs are being developed and is complicated by great variety of chemical and physical properties resulting in a wide range of biological responses[202][203][204].

7.7 Discussion

As with all new and emerging technologies, the development of harmonized and validated measurement techniques for assessing and controlling exposure to manufactured NOAAs in the workplace will always be working from a position of insufficient knowledge until the suitability of current controls are assessed, the emission rates of manufactured NOAAs from those processes are determined and the exposures of the workforce to those NOAAs are characterized. Together with information on the toxicity of the NOAAs to human health, these parameters form the basis of the risk assessment process (see Clause 8. Risk Assessment of Nanomaterials) that informs legislation on its production, sale and use, allows the setting of appropriate occupational exposure limits and leads to guidance on the choice of suitable control procedures.

The area is moving fast and instrument manufacturers are currently developing new devices that they hope will become the mainstay of future manufactured NOAAs exposure assessments. Besides recently-introduced health-related and small portable diffusion charger surface area monitors, there are a number of developments in the pipeline, including: personal CPCs; small, portable instruments that provide particle number size distributions (similar to the information provided by the DMAS) and small, portable NOAA mass monitors. In addition, there are many other long-term developments including a possible portable device that should be able to discriminate between manufactured and incidental nanomaterials. So, assuming that international agreement can be obtained about which metric or metrics is the most appropriate to use as the basis of exposure assessment for inhalation of airborne nanomaterials, then the future looks promising that a wider range of personal monitors with improved functionality will be available. The choice of sampler or monitor depends upon the role for which it is to be used and a device for exposure assessment might be different from that used to determine sources and to assess the efficiency of control systems.

7.8 Summary

A possible approach to conducting exposure assessments and sampling for manufactured NOAAs in the workplace is to follow one of the recently proposed methodologies. Effective methodologies include the “nanomaterial emission assessment approach” and the “harmonized tiered approach”. These two methods build on an initial assessment with possible subsequent sampling and monitoring. The initial (Tier 1) assessment begins with an observational walk through survey of the workplace to gain a better understanding of each work process and to determine potential emission sources of manufactured NOAAs. This can be supplemented by cost-effective targeted measurements with personal or hand-held monitoring instruments to assess or to help determine whether significant airborne releases of manufactured NOAAs occur (e.g. comparing particle concentrations at the emission source to background particle concentrations or taking samples for electron microscopy particle characterization). For more complex situations a wider range of monitoring instruments and measurands (Tier 3) which requires the use of larger “research” instruments will provide more detailed measurements and insights into the nature of workplace exposure to NOAAs. Such approaches can provide a means for determining whether existing measures are adequate for controlling manufactured NOAAs emissions or if additional controls are needed. Finally, experience with the existing measurement strategies is under way and should be used to refine the currently used strategies.

See the Bibliography for further guidance for health and safety professional, specifically for industrial or occupational hygienists on exposure assessment strategies.

8 Risk assessment in occupational settings

8.1 Introduction and scope

The present clause describes the current state of the art of risk assessment for production and processing of nanomaterials. Thus, only occupational settings, e.g. production plants, pilot plants or laboratories, as well as, the professional use and manipulation (e.g. cutting, abrasion) of NOAA-containing products (e.g. coatings, paints), but not consumer product safety or environmental safety, are considered.

A sub-class of nanomaterials, free or unbound NOAAs, is of particular concern from an occupational safety and health perspective. Nanostructured materials, nanolayers or solids containing embedded NOAAs, e.g. polymer composites, coatings or finishings can also result in exposures. For example, in field studies it was shown that destructive processing (even using wet-saw) of polymer composites containing NOAAs generates substantial aerosol release, which includes some aggregated nanofibre material, but there was no evidence of release of unagglomerated nano-objects[205][206]. Such aerosols can contain both incidental and manufactured NOAAs. Therefore, it is important to properly characterize generated aerosols.

While physical hazards posed by the specific process, e.g. high temperatures, high voltage, etc. can be present in occupational settings dealing with NOAAs, this clause focuses more on toxicological hazards and less on fire and explosion hazards. Generally, acute effects should be avoided using typical principles and minimum requirements of occupational health and safety[207][208] provided that acute toxicity information is available for handled NOAAs. However, health risks due to chronic low level of exposures are more difficult to evaluate and therefore it can be more challenging to establish appropriate exposure mitigation programs[172].

Risk assessment is typically conducted by safety experts working in close contact with decision makers establishing risk management requirements. Risk assessment analysis requires detailed information both on products and processes. For example, European legislation requires risk assessments specific to individual substances and individual occupational settings[208][209]. Likewise, presently in many countries risk assessment for NOAAs is conducted according to existing regulations for individual materials and settings.

8.2 Risk assessment for NOAAs

8.2.1 General

Risk assessment is an analysis of the potential adverse health effects (current or future) caused by a hazardous agent in the absence of any additional actions to control or mitigate exposure to that agent. Risk assessment in occupational settings includes several elements: hazard identification, hazard assessment, exposure assessment, and risk characterization[210][211][212]. The goal of risk assessment is to evaluate whether existing risk in a specific workplace environment is above organization-specific acceptable level of risk and, therefore, to provide information to decision makers about the need to further strengthen risk management approaches.

Risk assessment can be quantitative or qualitative. However, components of the risk assessment process remain the same and include:

- Hazard Identification-identifies those hazards that make a significant contribution to risk.
- Exposure-Response Assessment-identifies the potential adverse health effects associated with the hazards of concern identified at the workplace.
- Exposure Assessment-evaluates the pathways by which individuals could be exposed to hazards present in a workplace.
- Risk Characterization-incorporates information from the three previous clauses to evaluate the potential risk to exposed individuals at the workplace.

Risk assessment in a specific workplace commonly starts with collection of information on hazard assessment. The process then continues in a logical process whereby hazard and exposure are assessed. Thus the step of risk characterization is the synthesis of hazard and exposure. The existing structure of risk assessment framework is flexible enough to be adapted to NOAAs.

8.2.2 Quantitative and qualitative risk assessment

Quantitative risk assessment. Quantitative risk assessment relies on the availability of quantitative exposure data expressed as the probability of exposure or exposure levels and quantitative exposure limits. Exposure limits are developed using dose-response relationships and signify exposure levels at which risk of adverse health effects or risk of an event leading to adverse health effects is below an acceptable level. Examples of exposure limits are Occupational Exposure Limits (OELs) for specific substances, Minimum Explosible Concentration for explosive dust clouds, and limits of power density for electro-magnetic fields. Another integral component of quantitative risk assessment is measuring and/or estimating actual exposures or probabilities of exposure in the workplace. In addition, good quality hazard (toxicity) data are necessary for a quantitative risk assessment.

In the absence of regulatory binding OELs, a good starting point for the exposure assessment might be the use of provisional NRVs or other proposed indicative OELs. Current challenges in establishing and implementing specific OELs for manufactured NOAAs include[123][125]:

- a lack of adequate *in vitro* and *in vivo* toxicity data, as well as epidemiology studies for many manufactured NOAAs (as such data are traditionally used to develop OELs).
- a lack of harmonized, validated and standardized methods for measuring airborne concentrations of manufactured NOAAs in the workplace. This is also confounded by uncertainties about the most relevant exposure metrics to use for exposure assessments and for number and surface area metrics how to distinguish manufactured NOAAs of interest vs. other airborne particles and contaminants in the workplace.

- the availability of portable and personal, simple-to-use, real-time instruments to measure airborne manufactured NOAAs have been limited.

Qualitative risk assessment. In the absence of data to utilize traditional quantitative risk assessment methods to evaluate risk, missing information can be obtained using various combinations of expert judgments and extrapolations from existing data for similar materials. In such qualitative risk assessment methods, safety professionals can be required to use their expert opinions in evaluating site-specific risk and in recommending implementation of exposure mitigation options. For example, in “Approaches to Safe Nanotechnology”[172] U.S. NIOSH recommends that “the decision to use respiratory protection should be based on professional judgment that takes into account toxicity information, exposure measurement data, and the frequency and likelihood of the worker's exposure.”.

More formalized techniques such as “expert elicitation” utilizing a systematic process of formalizing and quantifying experts' judgments about uncertain quantities[213][214] can be used in grouping nanomaterials according to their hazard properties and exposure potential. Such hazard groupings could facilitate the development of techniques utilizing banding to assess risks and recommend appropriate risk management techniques, such as Control Banding[215]. One example of such a risk assessment scheme groups nanomaterials according to their biopersistence, chemical activity and toxicity[216] (ISO/TS 12901-2:2014)[132]. Expert opinions can be also systematically utilized within decision analytical frameworks for nanomaterial risk assessment and management, such as Multi-criteria Decision Analysis (MCDA)[217].

Both quantitative and qualitative risk assessment models require regular re-assessment of available hazard and exposure information.

8.2.3 Hazard identification

Hazard identification calls for identifying and monitoring hazards that make a significant contribution to risk. In this case the focus is on developing a list of pertinent toxic hazards (chemicals or NOAAs) and physical hazards (strong electro-magnetic fields, high intensity light sources, high level of noise, flammable and explosive materials, high pressures and vacuum, etc.). Regardless of engineering controls, low potential of exposures, or low hazard, this step is to identify all hazards that are relevant to potential occupational exposures.

For the identification of the hazard, information can be obtained from typical sources such as speciality literature, Safety Data Sheets (SDS) and International Chemical Safety Cards (ICSC), vendor information beyond that supplied in the SDS/ICSC, government and trade association publications and proprietary information or test data. However, there is often a lack of nano-specific data or exposure limits. Thus, the information from these sources might not adequately characterize the hazard of specific NOAAs. When this information is not available from third parties, testing to generate data can be conducted.

8.2.4 Exposure-response assessment

Toxicological hazards. In occupational settings, protection from toxic effects is achieved by reducing exposures to the toxic substance below established “safe” levels resulting in an acceptable level of risk. Toxicological effects can be broadly characterized as threshold and non-threshold. For the former, it is possible to identify an exposure below which no adverse health effects are observed and for the latter, any exposure results in a non-zero probability of adverse health effect occurrence. For threshold toxicological effects, quantitative determination of “safe” levels includes the following steps:

1) determination of a No-Observed-Adverse-Effect Level (NOAEL) or a “Benchmark Dose” (BMD) using animal or human exposure-response data;

2) extrapolation of animal levels to human levels (recognizing the significant uncertainties introduced by such extrapolations): models translating environmental exposures to dose, such as a human lung dosimetry model, are used to calculate working lifetime exposure concentration;

3) derivation of occupational exposure limits upon consideration of technical feasibility, variability and uncertainties of models and approximations used and acceptable level of risk.

Toxicological properties of nanomaterials might arise from the intrinsic chemical composition of a material, such as those that would originate from bulk material. Apart from that, the scientific community is considering whether there is additional toxicity for NOAAs due to the particulate nature and due to unique properties associated with the nanoscale[218]. Toxicological studies are also conducted on novel NOAAs such as carbon nanotubes, which do not have bulk analogues.

The definition, design and standardization of adequate toxicological test protocols for NOAAs are in various stages of development including actual published standards. It is advised that the guidance document, ISO/TR 13014:2012[219], be consulted prior to undertaking any toxicological study. Available international accepted test guidelines (e.g. OECD) should be checked first concerning applicability to NOAAs and if necessary improvement and adaptation of these guidelines should be performed. Apart from the obvious toxicological issues, other major challenges being faced during experimental testing would be the representative formation of nano-object dispersions as well as their explicit characterization. At relatively high number concentration of particulate matter either in gas or liquid phase, nano-objects tend to form larger agglomerates very rapidly due to Brownian motion and relatively strong attractive interactions between nano-objects. For example, this can occur during unloading of nano-objects from a production line and packaging and in such circumstances exposure to agglomerates rather than individual nano-objects are likely.

Multiple toxicological findings have been reported and the number of studies being conducted is steadily increasing. The early toxicity studies were sometimes conducted with test materials which were not well characterized, mostly due to the technological limitations. In addition, scientists have proposed occupational exposure limits for certain NOAAs such as ultrafine titanium dioxide, carbon nanotubes and carbon nanofibres[130][220][221], while for other NOAAs a threshold could be derived using ISO/TR 18637:2016[222].

Hazards based on toxicological properties of nanomaterials are currently evolving as the volume of toxicological studies increases. However it is currently considered that:

— the toxicological properties of NOAAs cannot always be predicted from the known toxicity of the substance in bulk form alone; and

— for some nanomaterials, mass is not an appropriate metric for characterizing exposure and surface area (e.g. some poorly soluble low toxicity NOAAs), and particle number (e.g. fibrous NOAAs) have been proposed as better alternatives.

It should be noted that generally mass is used in the various studies as a convenient dose metric. However, one should also know the surface area and number concentration per mass for the NOAA in order to be able to convert the doses to these metrics for possible dose response relationship and clarification of toxic mechanisms.

Occupational exposure limits based on mass concentration, which are applied for bulk materials, might not be sufficiently adequate for nanomaterials of the same chemical composition[223][31]. For certain nanomaterials, occupational exposure limits based on other metrics, such as surface area or particle number, might be more suitable. However, until methods and exposure limits based on these metrics are sufficiently developed and accessible, the current, proposed mass-based limits, where they exist, must serve as guidance to assess exposure risk.

Physical hazards. Nanomaterial-specific fire and explosion hazards have been described[224][225]. Fire and explosion hazards posed by NOAAs could be more pronounced than those for larger particles or bulk materials, particularly if the larger particle congeners are combustible dusts. Therefore, additional tests are necessary to evaluate flammability, explosivity and reactivity of nanomaterials. Test protocols for these hazards are in place for bulk materials in dry powder form and are applicable to nanomaterials as well. These protocols include measurement of burning rate, self-ignition temperature and the characterization of the explosive properties such as minimum ignition energy, and explosion severity test (K_{st} and P_{max}). Flammability of nanomaterials can be also evaluated using tests developed for chemicals, for example, using ASTM E-918-83 Standard Practice for Determining Limits of Flammability of Chemicals at Elevated Temperature and Pressure[226]. The explosive properties are analysed through utilizing the results of the Fallhammer test (mechanical sensitivity, shock) and Koenen test (thermal sensitivity)[227]. Therefore, once the physical hazard data are available, risk assessment for fire and explosion hazards could be conducted using existing techniques.

Hazard assessment. Where there is a lack of applicable hazard data for nanomaterials, hazard grouping of nanomaterials based on expert opinions could be implemented. For example, in the ISO Control Banding approach for nanomaterials (ISO/TS 12901-2:2014)[132] five hazard groups are identified.

Given the great variety of possible nanomaterials and high costs of experiments assessing hazardous properties, computational models (such as Quantitative Structure Activity Relationship like models) predicting hazard properties of novel nanomaterials are expected to play an increasingly important role in risk assessment.

Further information about hazard characterization can be found in Clause 6 and Annex B.

8.2.5 Exposure assessment

Emission scenarios. The next step includes characterization of the quantity of hazard material or physical agent. The quantity of material handled or physical agent present in the workplace is an important factor affecting exposure potential. Naturally, workplace sites where only small amounts of material are being handled or processed would be expected to have lower exposure potential compared to sites handling larger amounts. Such an analysis of work places facilitates assessment of exposure potential among workers' populations with distinct work duties related to or within exposure-relevant distance to the work process of interest, thus leading to identification of populations of concern.

Emission identification also includes a survey of individual workplaces, worker procedures, manufacturing processes and the safety measures in place, including use of engineering controls and personal protective equipment. These will help in the description of the exposure and identification of potential for exposure among workers' populations with distinct work duties related to or within exposure-relevant distance to the work process of interest. If such a preliminary survey indicates potential for exposure, further collection and analysis of data to assess workplace exposure can be warranted. As the discussion about relevant metrics is ongoing, multiple tools to characterize exposure to nano-objects could be employed[172]. Such tools include traditional techniques to characterize mass concentration and airborne particle number concentration. This information might be supplemented by particle size distribution, surface area, chemical characterization data and/or electron microscopy (for further details please see Clause 7 and Annex C).

Exposure routes. Exposure to NOAAs can occur as a result of direct contact. In the case of nano-objects, the discussion of potential exposure scenarios should consider the liberation potential of the nano-objects. Liberation potential can be defined as the ability of individual nano-objects to be available for direct contact with human skin or other organs such as lungs. The following factors are to be considered for the release of airborne NOAAs: physicochemical properties and process characteristics. Physico-chemical properties of NOAAs might include: size, surface coating, charge, dustiness, etc. In considering process characteristics, the conditions of the material should be observed — that is, whether the material is contained in a liquid or solid matrix. Mechanical processing such as stirring, drilling, sawing, milling, grating and cutting can result in release of nano-objects and particles of nanostructured materials. Other processes which can result in exposures include spraying liquid formulations containing NOAAs and high-energy treatment of nanomaterials or nano-enabled materials, such as laser drilling or plasma welding resulting in vaporization of treated materials. Additionally, engineering processes, which range from closed contained systems to open air handling, can have a wide range of potential for exposure. Risk of exposure can be also affected by other factors such as incorrect use of and malfunctioning equipment — both manufacturing and exposure mitigating equipment (engineering controls and personal protective equipment); inadequate workplace practices; poor personal hygiene and unsafe individual worker's behaviour.

Realistic exposure scenarios need to be identified for exposure assessment. Inhalation and dermal exposure are typically the most common routes of exposure in the workplace. Oral exposure at the workplace is considered less likely (although ingestion is a component of inhalation exposure through mucocilliary clearance and swallowing of inhaled particles). Ingestion might also occur from unintentional hand to mouth transfer after dermal exposure[207][208]. Parenteral exposure could occur accidentally (e.g. needle stick). Some studies have reported that NOAAs do not penetrate intact porcine skin[228][229], while other studies have shown that nanoscale or microscale particles can penetrate the stratum corneum (particularly with mechanical skin flexing) and reach the dermal and epidermal layers of porcine or human skin[143][230]. Another potentially important, but underinvestigated route is ocular.

Inhalation exposure can be characterized by applying appropriate analytical methods, such as particle counters and sizers or related methods plus chemical analysis and electron microscopy, while dermal exposures can be characterized using handwipes sampling followed by chemical analysis and electron microscopy. These methods should be applied with appropriate validation and calibration, adequate consideration of ambient background levels, variations of analytical results due to humidity, and effects of particle aggregation and agglomeration on particle concentration. Particle size, number and distribution are likely to be important factors. These data might need to be supplemented by surface area or chemical characterization data. (For additional information see also Clause 7 and Annex C).

In the absence of real time exposure data, more qualitative techniques can be used to characterize exposure potential. For example, in the ISO Control Banding approach (ISO/TS 12901-2:2014)[132], exposure potential is characterized using four exposure bands. Exposure bands are selected based on the type of workplace process, the physical form of NOAAs (dry powder, liquid dispersion, solid composite) and the amount used in a particular occupational setting.

8.2.6 Risk characterization

Risk characterization includes review and integration of the hazard identification, exposure-response assessment, and exposure assessment steps. Quantitative risk estimates are evaluated for statistical and biological uncertainty. Risk characterization also provides site-specific evaluation of hazard and exposure, whether risks at a specific workplace exceed acceptable levels, and whether there are sensitive populations. Risk management measures might be recommended to reduce risks below acceptable levels. Typical measures might include elimination and substitution of hazardous NOAAs (though the opportunities to do so might be limited by the unique properties of the NOAAs) as well as technical measures, e.g. modifications to production processes and/or implementation of engineering controls, organizational measures, e.g. safety procedures, personal protective equipment and individual worker instruction (for further details see Clause 9).

8.3 Conclusions

Evaluating the health risk of nanomaterials in the occupational setting involves quantitative and/or qualitative risk assessment methods. In initial assessment (data gathering), only a qualitative risk assessment utilizing expert judgements and control banding tools is required. Quantitative risk assessment[205] can be feasible, when exposure and dose-response data are available (e.g. in a toxicological or epidemiological study). For the occupational exposure itself, in the absence of regulatory binding OELs, an acceptable starting point for the risk assessment might be the use of provisional NRVs or other proposed indicative OELs. Presently, quantitative health hazard and exposure data are not available for most nanomaterials. Therefore, health risk evaluation for the workplace currently relies to a great degree on professional judgments for hazard identification, potential exposures and the application of appropriate safety measures.

9 Risk mitigation approaches

9.1 Introduction

This clause examines current knowledge on control practices for mitigating or preventing exposure to NOAA in the workplace. It does not aim to address health and safety issues or practices associated with nanomaterials generated by natural processes, produced incidentally (e.g. during welding) or associated with potential consumer exposures or uses, though the information might be relevant to those areas as well. The clause covers control of both health hazards and safety (physicochemical) hazards, and specific examples of controls used in companies and research laboratories will be presented.

Information in this clause has been drawn from best practice documents developed by organizations around the world[231] to [237][172]. Information on current practices is derived primarily from a report by the University of California, Santa Barbara for the International Council on Nanotechnology (ICON) entitled "A Review of Current Practices in the Nanotechnology Industry — Phase two report: Survey of current practices in the nanotechnology workplace"[238]. The report presents the findings of an international voluntary survey of current environment, health, safety (EHS) and product stewardship practices in the global nanotechnology industry.

The control of emissions containing NOAAs in occupational settings is not a new subject. Controls are well established for preventing and controlling exposure to, for example, welding fumes and diesel emissions (which contain incidental NOAAs). What is new and unique is the need to control exposure to manufactured NOAAs in an increasing number of workplaces. Using existing knowledge for the control of fine and ultrafine particles (including incidental NOAAs) as a starting point, informed guidance is summarized for the control of manufactured NOAAs.

While it is expected that the controls described in this clause should be effective (to some extent at least — some will be very effective) in preventing exposure to manufactured NOAAs in specified situations, to date there is only limited evidence regarding the effectiveness of the control methods. However, based on existing knowledge and information, advice is provided on the likely effectiveness of different control strategies in preventing exposure[239].

Use of the information on control methodologies can help companies, researchers and other people prevent adverse health and safety consequences during the production, handling, use and disposal of manufactured NOAAs. This information covers a range of NOAAs and applications.

9.2 Implication of risk assessment in regard to control methodologies

9.2.1 Background

In considering the appropriate control strategies for NOAAs in the workplace, it is necessary to consider first the levels of risk associated with the workplace activities. Risk assessment was examined in Clause 8. Ideally, the control strategy should align with the known risk — as demonstrated in, for example, the *Control Banding* approach. (See also ISO/TS 12901-1 and ISO/TS 12901-2 dealing with occupational risk management of manufactured NOAAs [132][240])

NOTE Control banding (ISO/TS 12901-2:2014)[132] is a process in which a single control technology (such as general ventilation or containment) is applied to one range or band of exposures to a chemical (such as 1–10 mg/m^3) that falls within a given hazard group (such as practically non-hazardous and serious hazard). Five main control bands have been developed for exposure to NOAAs by inhalation:

- Band 1: Natural or mechanical general ventilation.
- Band 2: Local ventilation: extractor hood, slot hood, arm hood, table hood, etc.
- Band 3: Enclosed ventilation: ventilated booth, fume hood, closed reactor with regular opening.
- Band 4: Full containment: glove box/bags, continuously closed systems.
- Band 5: Full containment and seek expert advice.

There might potentially be health risks arising from both the unique and common chemical properties of manufactured NOAAs associated with occupational exposure to these materials. There are also potential safety risks of fire or explosion[241] during the manufacture, handling, storage and use of manufactured NOAAs. However at present, there are uncertainties in the extent of health and safety risks involved in working with most NOAAs, and thus knowledge-based control strategies are possible for only a few NOAAs such as CNTs, CNFs and TiO_2. While there are only a few specific workplace exposure standards set for airborne concentrations of free unbound NOAAs, exposure standards have been set for substances in a larger particle form, some of which have the same or similar chemical composition to manufactured NOAAs currently being manufactured or used.

9.2.2 Strategies for control

If we apply the precautionary principle to NOAAs, it follows that the lack of scientific certainty about potential health and safety risks associated with manufactured NOAAs should not prevent the utilization of cost-effective preventive measures to reduce potential risks.

There are two main forms of the Precautionary Principle: the strict form (based on the premise — first do no harm) requires inaction when action might pose a risk; whereas the active form calls for doing more rather than less — applying appropriate effort to mitigate risk by choosing less risky alternatives when they are available and taking responsibility for potential risks. The active form incorporates the following six components[242]:

1) taking precautionary action before scientific certainty of cause and effect;
2) setting goals;
3) seeking out and evaluating alternatives;
4) shifting burdens of proof and financial responsibility to the proponents;
5) accepting a duty to monitor, understand, investigate, inform, and act;
6) developing more democratic and thorough decision-making criteria and methods.

NOTE Principle 15 of the United Nations Rio Declaration on Environment and Development[243] states that: "In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.".

The uncertainties about health and safety risks in the absence of NOAA-specific workplace exposure standards do suggest a precautionary approach is required to control risks for the manufacture, use, storage and handling of NOAAs. In relation to free nanoparticles and nanotubes, in 2004 the UK Royal Society and Royal Academy of Engineering recommended that factories and research laboratories treat manufactured nanoparticles and nanotubes without hazard data as if they were hazardous[5].

NOTE Control of Substances Hazardous to Health (COSHH) defines hazard "in relation to a substance, means the intrinsic property of that substance which has the potential to cause harm to the health of a person, and "hazardous" shall be construed accordingly."[244].

Similarly, ISO/TS 12901-2 defaults NOAAs, for which no hazard data are available and no bulk or analogous substance with assigned hazard band exists, to the most hazardous band E[132]. It has been suggested that strict prevention measures should be taken to limit airborne NOAA release into the occupational environment and the environment outside the business[234], and it is considered important to employ a broadly based risk management program to reduce workplace exposures and eliminate workers' exposure wherever possible[232]. Application of the precautionary principle does not imply that organizations should not use NOAAs until health and safety hazards are fully understood. As indicated above, precautionary actions should be determined according to the assessment of possible consequences of the NOAAs used, including the consideration of available hazard information and the concomitant uncertainties[245][246]. These precautionary actions should follow the established hierarchy of controls for protecting workers: eliminate the usage of NOAAs, redesign the process to reduce exposure to NOAA, and use of appropriate personal protection[247].

Choices for the control strategies should be informed by the available understanding of NOAAs and relevant information on similar larger particles. Where NOAA-specific information is known, the control approach can be varied according to the properties of the NOAAs involved. Appropriate work practices should be tailored to the processes and job tasks during which exposure might occur. The choice of strategy should also be informed by methods chosen for the control of incidental NOAAs.

In regard to NOAA-specific information, Clause 5 and Annex A described how NOAAs are not a single group of objects but a multiplicity of shapes, sizes and composition. Given the variation in properties (e.g. general chemistry, shape/size, surface area and surface reactivity), it is not surprising that some nano-objects have greater health and safety hazards associated than others (see Clause 6).

With an increasing understanding of health and safety risks, for some NOAAs it is becoming possible to modify prevention measures. For example, more health hazard information would allow to band risk (and hence controls) effectively based on differentiable health hazards in the control banding approach (ISO/TS 12901-2:2014)[132] — which might in some cases allow relaxation of strict prevention measures. However, as NOAA quantities manufactured, used, stored and handled increase, the health risks involved can be expected to increase in some situations. Measuring and evaluating the effectiveness of prevention approaches is an essential control strategy. A better understanding of the health risk is needed to install proper prevention measures (e.g. protection from inhalation by masks or containment with respect to induction of lung inflammation).

9.3 Examination of control methodologies

9.3.1 Exposure prevention

Exposure prevention measures and appropriate work practices are essential in occupational health and safety, and the production and use of NOAAs might involve various kinds of risks. Consequently, management, researchers and other employees might give priority to preparing an exposure prevention program designed specifically for the company or research facility[234]. Establishing and implementing an exposure prevention program should be an effective step in protecting the health and safety of employees and other people in the workplace.

A number of organizations have developed specific programs for handling NOAAs[238]. In developing the exposure prevention program, the company director should (among other things) clearly identify the responsibilities of various individuals in the company and ensure that senior management, research teams and laboratories are involved. A risk assessment approach is needed, with consideration of the specific NOAA, its form, toxicity and potential hazards. The approach should be used for each specific task undertaken. A number of organizations working with NOAAs reported[238] that their exposure prevention program depended on:

- the material form (powder, in suspension/solution or embedded in a matrix).
- specific known hazards (such as flammability, toxicity, carcinogenicity or high reactivity).
- elements of an exposure prevention (risk management) program might include, for example: Monitoring and recording the performance and effectiveness of control measures.
- monitoring workplace exposures to NOAAs.
- developing the criteria and procedures for installing engineering controls (e.g. equipment enclosure or local exhaust ventilation) at process locations where exposure might occur.
- providing effective training and instruction to the workforce on hazards, operating procedures, equipment manual, procedures for handling NOAAs, and effective protective measures. The ICON review reported that guideline documents are used by some organizations[238].
- obtaining safety data sheets from the producers or suppliers of the NOAAs used. The safety data sheet should provide indications about health hazards posed by the products, and the protective measures for the workplace.

  NOTE Examination of current safety data sheets for manufactured NOAAs has shown that information might be incomplete, and using solely the safety data sheet information can result in the implementation of incomplete protection measures[235].
- developing procedures describing the types of personal protective equipment that should be used (e.g. clothing and respirators), and when it should be used.
- developing procedures to include the frequency of changing or washing personal protective equipment (e.g. gloves and coveralls).
- maintenance of respirator including storage and keeping records where appropriate.

- developing procedures for cleaning and decontamination of equipment and enclosures etc.
- seeking expert advice, e.g. from occupational hygienists, to help guarantee a safe working environment.
- undertaking research projects focused on nanotechnology-based health and safety issues (the research might be undertaken in collaboration with government agencies).
- benchmarking and sharing practice knowhow with other organizations working with NOAAs.

The guide for preventing exposure to lead[248] is an example of a guide that sets forth the principle points for consideration in an exposure prevention program designed specifically for controlling exposure to hazardous dusts.

9.3.2 Control strategies

In general, the main approaches to risk control of hazardous materials in the workplace is focused on prevention of exposure by elimination of the hazard, substitution of the hazard, engineering control techniques, administrative control systems and use of personal protective equipment. These complementary approaches should be considered starting with the design stage of an industrial process[249]. The preferred order of options (starting with most preferred) is: elimination > substitution > engineering techniques > administrative means > personal protective equipment. In practice, an appropriate combination of these strategies will provide the best approach for a workplace to control exposures.

In general the highest risk for NOAA is considered to be respiratory exposure in view of the well-known lung toxicity of particulates. Control practices for the reduction of inhalable and respirable dust in the workplace are well-known and well established[172]. The efficiency of these methods for NOAAs has so far been only partially evaluated, but these measures seem useful as a starting point for the development of preventive measures[235][239]. Some adjustments might be needed to prevent potential exposure to NOAAs. Generally, organizations working with NOAAs are using conventional chemical safety methods, with some taking measures beyond those of conventional chemical hygiene[238]. Control measures are also based on the toxicity and physicochemical properties of other materials handled in the laboratory, e.g. in the ICON review, most respondents indicated their choice of gloves was based on which solvents were being used[238].

9.3.3 Reducing risk through effective design

Effective process design can make a very major contribution to preventing workplace exposures[249]. After installation it can be difficult to make modifications to correct deficiencies in original design.

In the design phase, special attention should be paid to positioning of plant, installations, processes, equipment activities and workstations. The designer can make a major contribution by:

- recognizing the exposure factors specific to the processes and production modes, and then designing to eliminate or reduce as much as possible these exposure factors;
- designing and recommending control measures.

The design stage include activities such as producing the building plans, and planning the procurement, production, packaging, warehousing, shipping and other systems. In addition to taking into account the regulatory requirements and production imperatives, layouts should be designed to eliminate situations involving risks from the process and for the workers. Careful design can help to prevent the generation of dusts and aerosols.

Regarding the design of engineering control systems, different processes will produce a range of particle sizes, which might include not only nano-objects, but also particles of their agglomerates and aggregates larger than the nanoscale size range in some cases. The velocities (face, capture, and transport) necessarily will depend upon the nature of the process and the size range of the particles produced[250][251]. For this reason, the engineering control systems intended to prevent or limit the emission or accumulation of airborne NOAAs in the work environment, such as process enclosure and ventilation, should be designed according to the gaseous and particulate properties of NOAAs.

A fundamental principle of good design is to avoid explosive situations. Dusts having explosive potential are to be avoided. In the worst case scenario, equipment might need to be blast-proofed[234].

Also, where appropriate, building design can incorporate physical separation of workplaces handling hazardous materials.

9.3.4 Substitution of raw materials, products, processes and equipment

Substitution is generally a very effective way to reduce risks to health and safety in the workplace. While the unique chemical and physical characteristics of individual NOAAs are likely to limit possibilities for straightforward substitution of one nanomaterial for another, as it is this uniqueness which will likely determine their application and commercial usefulness, there might be substitution opportunities in processes involving nanomaterials, e.g.:

- replacing more toxic raw materials with less toxic raw materials (e.g. reducing the presence of toxic impurities).
- consider whether NOAAs are necessary to the application or product.
- replacing more toxic products with products that are less toxic.
- changing the physical form of the product or material — use dispersions, pastes, granules or composites instead of dry powders or aerosols.
- process changes are very effective risk control methods, for example, changing from dry processes to wet processes, and the use of water can reduce dust emissions at some dry material drop-off or transfer points[249].
- substitution of equipment which involves the use or production of smaller quantities of toxic materials, or fewer toxic materials.
- substitution of equipment to avoid emissions, or to reduce and better control emissions.
- particle modification, e.g. the coating of quantum dots. In one study, the lack of observable genotoxicity of quantum dots was attributed to a silica coating, which successfully prevented the interaction of Cd, Se, Zn and S with proteins and DNA in the nucleus[252]. A further potential approach which has been examined is the possibility of modifying CdSe metalloid core structures to increase the thermodynamic stability and hence reduce the potential for breakdown into Cd and Se components[253].

9.3.5 Engineering control techniques

9.3.5.1 Background

For workplaces generally, the choice of engineering control technique(s) should include consideration of the level of risk involved. For example, in the UK's COSHH Essentials[244], the approach recommended for control of airborne contaminants is:

- Highest risk — seek specialist advice;
- High risk — use process containment;

  NOTE In this document, process containment is considered as an engineering technique.
- Less risk — use local engineering control [e.g. local exhaust ventilation (LEV)];
- Lowest risk — use general ventilation.

Currently for NOAAs there is limited understanding of the level of risk, and it is suggested that in situations where there is risk uncertainty, a precautionary approach should be utilized.

Engineering control systems can be used effectively to control powders and gases, and are in common use in the chemical, pharmaceutical and other industries[254]. Many of the processes used in pharmaceutical production are similar to those used in the nanomaterial industries discussed in Clause 5 and include blending, mixing, and handling of hazardous compounds in liquid and powder form[239]. The general control concepts required for working with hazardous materials include specification of general ventilation, LEV, maintenance, cleaning and disposal, personal protective equipment (PPE), industrial hygiene monitoring, and medical surveillance[255]. Particular work practices, such as using High Efficiency Particulate Air (HEPA) filtered vacuums instead of dry sweeping, are required. In addition, routine industrial hygiene and medical monitoring ensure that work practices and engineering controls are effective.

Source containment is considered the highest level in the containment hierarchy and is used by the pharmaceutical industry[247]. This category contains many options including elimination, substitution, product modifications, process modifications, and equipment modifications. These steps could include reworking the process to reduce the number of times material is transferred or keeping the product in solution to minimize aerosolization potential. The next level of control for capturing process emissions is the use of engineering controls such as glove boxes, downflow booths, and local exhaust ventilation. Table 2 shows a generic process list (column one) along with applicable engineering controls (column two) and references. The engineering control column provides a framework for identifying exposure controls for particular processes. The third column shows the industry in which these control approaches have been tested. References are listed in the fourth column for studies that apply to each of these processes and controls. A review of exposure assessment studies in NOAA production and downstream user facilities along with recommendations for specific engineering control approaches to minimize exposures to NOAAs can be found elsewhere[239].

Table 2 — Engineering controls and associated tasks for various industries[239]

Process/task	Engineering control	Industry	Reference
Reactor fugitive emissions	Enclosure	Nanotechnology	Tsai et al. 2009[256] Lee et al. 2011[257]
Product harvesting	Glovebox	Nanotechnology	Yeganeh et al. 2008[258]
Reactor cleaning	Spot LEV system/fume extractor	Nanotechnology	Methner 2008[259]
Small-scale weighing	Chemical fume hood	Nanotechnology	Tsai et al. 2009[260] Ahn et al. 2008[261] Tsai et al. 2010[262]
	Biological safety cabinet	Nanotechnology and laboratory	Cena and Peters 2011[263] Macher and First 1984[264]
	Glovebox isolator	Pharmaceutical	Walker 2002[265] Hirst et al. 2002[266]
	Nano fume hood	Pharmaceutical	
	Air curtain isolation hood	Nanotechnology/research	Tsai et al. 2010[262]
Product discharge/bag filling	Discharge/collar hood	Silica and pharmaceutical	ACGIH 2013[250] HSE 2003[267] Hirst et al. 2002[266]
	Continuous liner	Pharmaceutical	Hirst et al. 2002[266]
	Inflatable seal	Pharmaceutical	Hirst et al. 2002[266]

Table 2 *(continued)*

Process/task	Engineering control	Industry	Reference
Bag/container emptying	Bag dump station	Silica	HSE 2003[268] Heitbrink and McKinnery 1986[269] Cecala et al. 1988[270]
Large-scale weighing/ handling	Ventilated booth	Pharmaceutical	Hirst et al. 2002[266] Floura and Kremer 2008[271] HSE 2003[272]
Nanocomposite machining	High velocity-low volume	Woodworking	
	Wet suppression	Nanotechnology	Bello et al. 2009[273]
Air filter change-out	Bag in-bag out	Pharmaceutical	

9.3.5.2 Ventilation and general considerations

It is important to confirm that the LEV system is operating as designed by regularly measuring exhaust airflows[239]. A standard measurement — hood static pressure — provides important information on the hood performance, because any change in airflow results in a change in hood static pressure. For hoods designed to prevent exposures to hazardous airborne contaminants, the ACGIH Industrial Ventilation: A Manual of Recommended Practice for Operation and Maintenance recommends the installation of a fixed hood static pressure gauge[274].

In addition to routinely monitoring the hood static pressure, additional system checks should be completed periodically to ensure adequate system performance, including smoke tube testing, hood slot/face velocity measurements, and duct velocity measurements using an anemometer. A dry ice test is another method of evaluation designed to qualitatively determine the containment performance of fume hoods. These system evaluation tasks should become part of a routine preventative maintenance schedule to check system performance.

It is important to note that the collection and release of air contaminants might be regulated; companies should contact agencies responsible for local air pollution control to ensure compliance with emissions requirements when implementing new or revised engineering controls.

To reduce the risk of exposure to NOAAs, a few standard precautions should be followed in areas where exposures might occur:

— isolate rooms where NOAAs are handled from the rest of the plant with walls, doors, or other barriers.

— maintain production areas where NOAAs are being produced or handled under negative air pressure relative to the rest of the plant.

— when possible, place hoods away from doors, windows, air supply registers, and aisles to reduce the impact of cross drafts.

— provide supply air to production rooms to replace most of the exhausted air.

— direct exhaust air discharge stacks away from air intakes, doors, and windows. Consider environmental conditions, especially prevailing winds.

9.3.5.3 Closed containment of a process (enclosure and isolation)

Enclosure and isolation of the process can minimize the airborne release of particles into the work environment during production or use. This approach should be considered for processes with a high potential risk to the health and safety of workers. It allows more effective emission control than in open processes, while facilitating treatment of environmental emissions when necessary.

In the precautionary approach, it is desirable to avoid any exposure to nanomaterials, and a number of containment approaches might be considered. Operations can be performed by isolating the materials in separate, ventilated rooms equipped with a system that avoids any possibility of contaminating other workplaces[234]. Other examples of isolation are: the use of closed-circuit processes, use of robotics and equipment enclosure. In certain situations where the process is too polluting, workers can be isolated in a controlled atmosphere workstation to operate the entire process by remote control. The workers are located in booths or rooms where the air quality conditions are controlled to protect their health and safety[249].

Maximum protection for the environment and the worker is provided by use of a Class III Biological Safety Cabinet (BSC), which was designed for work with highly infectious microbiological agents and for the conduct of hazardous operations[275]. More details are provided in Annex D.

In general, source enclosure (i.e. isolating the generation source from the worker) should be effective for capturing airborne manufactured NOAAs, based on what is known of nanoscale particle motion and behaviour in air[172]. Cena and Peters evaluated the effectiveness of ventilated enclosures including a Class II, Type A2 BSC and a custom fume hood during the manual sanding of epoxy test samples reinforced with CNTs[263]. The authors suggested that the poor performance of the custom fume hood could have been due to its rudimentary design, which did not include a front sash or rear baffles. The lack of these common fume hood features along with the low average face velocity could have resulted in poor airflow distribution across the face and increased leakage.

A glove box isolator fully contains a small-scale process and is sometimes referred to as a primary protection device (Figure 12 in Reference [201])[276]. The design can be either the more typical hard unit or a soft, flexible containment unit (often referred to as a glove bag). Glove boxes provide a high degree of operator protection but at a cost of limited mobility and size of operation. In addition, cleaning the glove box can be difficult, and, to prevent exposures, operators should use caution when transferring materials and equipment into and out of the glove box. In general, glove boxes include a pass-through port, which allows the user to move equipment or supplies into and out of the enclosure.

Materials such as carbon black, silica fumes, nanoscale TiO_2, metals and nanoscale metal oxides are normally produced in closed circuit processes[234], and enclosure and isolation controls are used in practice for handling a variety of different NOAA types[238]. In the ICON review, a number of organizations working with NOAAs reported the use of cleanrooms (using pressure differentials) with separate and isolated heating, ventilation and air conditioning (HVAC) systems[238]. Organizations also reported the use of glove bags and glove boxes, and closed piping systems to segregate any materials deposited down a drain into a separate collection system. Further information on gloveboxes can be found in the *Guideline for Gloveboxes*[277]. One potential problem reported with glove bags is the build-up of static electricity charges, which can be problematic for flammable or potentially explosive NOAAs[238]. Further details of specific enclosure and isolation controls reported are shown in Annex D, Table D.1. An example of the use of an enclosing hood with HEPA exhaust is also shown in Figure 7 below[278].

Figure 7 — Enclosing hood with HEPA exhaust (from NIOSH Progress Toward Safe Nanotechnology in the Workplace[278])

In case of a leak from an enclosed process, primary nano-objects can escape and disperse through the plant. How much nano-object aerodynamic properties resemble those of gases is yet to be determined. But from known relationships[279], a 10 nm particle is expected to have a diffusion coefficient considerably lower than a nitrogen or oxygen molecule of around 0,3 nm in size.

As the dispersion process progresses, the particles agglomerate and airborne dispersion becomes more difficult. Nonetheless, inhalation exposure to these agglomerates is possible during NOAA recovery, bagging and maintenance and cleaning operations[234].

9.3.5.4 Source capture of pollutants, e.g. local exhaust ventilation (extraction)

If the use of closed containments is not possible, then it is even more important to avoid the formation of dusts or aerosols. However in some processes, it is impossible to avoid airborne release of dusts and aerosols. Source capture of these pollutants (e.g. by using local exhaust ventilation, LEV) is then the method of choice to prevent airborne propagation of these products in the work environment, contaminating all the work areas and being breathed in by workers[280].

LEV equipment performance is closely linked to the quality and efficiency of design and maintenance, and often of work methods. Ventilation systems should be designed, tested, and maintained using approaches such as those recommended by the American Conference of Governmental Industrial Hygienists (ACGIH)[250][274]. It is important for effective containment that the systems be checked daily and records of the checks kept. In-line monitoring should be fitted at key points in the system (e.g. behind the hood, across the filters). The efficiency of new ventilation systems should always be evaluated to ascertain their performance level.

Reduction by source capture with negative pressure is one of the most effective measures in operations not performed in closed circuits - such as for mixing, recovery, bagging or weighing of products. Source capture is commonly used in welding[281], cutting and spray metallization processes, among others. These processes, which have been used for many years, generate a significant number of particles of nanoscale dimensions. An example of the use of LEV in nano-operations is for controlling fugitive emissions during precursor mixing at a primary nanoscale oxide production facility[278], as shown in Figure 8 below.

Figure 8 — Local exhaust ventilation controlling fugitive emissions (from NIOSH Progress Toward Safe Nanotechnology in the Workplace[278])

Fume hoods were the most frequently used engineering control for the handling of NOAAs in the organizations participating in the ICON survey[238]. Fume hoods are used for a variety of different NOAA types — nanopowders, carbon nanotubes, colloidal dispersions, fullerenes, quantum dots, polymers, nanowires, nanocrystals and carbon black. Exhaust filtration systems are frequently used with the fume hoods by the organizations working with NOAAs. A number of different filter types are used in practice — HEPA filters, non-HEPA filters, wet scrubbers primarily for removing water soluble organic materials and submicrometer rated cartridge filters that block nanoparticles down to less than 10 nm in size. Biosafety cabinets are designed to protect personnel from potentially harmful agents, and are used in some nano-enabled organizations[238]. Class I and Class II biosafety cabinets both utilize extraction through HEPA filters. Characteristics of biosafety cabinets are shown in Annex D.

A laminar flow clean bench (also called a laminar flow hood) is not a biosafety cabinet. It is not designed to protect personnel or the environment from potentially harmful agents. It is designed to keep material clean. HEPA-filtered air is drawn across the materials and then flows out through the open front of the cabinet, where the worker will be located.

9.3.5.5 General ventilation

General ventilation by dilution in the work environment can draw the contaminants outwards, and if it is the only engineering control utilized, might allow significant exposure of workers to NOAAs. If the use of LEV for open processes is not practicable, then it might be preferable to use displacement ventilation to reduce background levels, where fume is extracted at roof or ceiling level.

9.3.5.6 Air recirculation and filtration

Filtration plays an important role in the control of exposure to airborne particles. High Efficiency Particulate Air filters might be used in engineering control systems to clean the air before returning it to the workplace, or before discharge into the atmosphere. These filters are usually classified as mechanical filters. Current knowledge indicates that a well-designed exhaust ventilation system with a HEPA filter should effectively remove NOAAs[172]. However, only a limited amount of work has been done to quantify the performance of filters against particles in the nanoscale size range.

NOTE An extension of analysis would be to examine the performance of filters for different types of NOAAs.

If HEPA filters are used in the dust collection system, they should be coupled to a well-designed filter housing. If the filter is improperly seated, particles have the potential to bypass the filter, leading to filter efficiencies much less than predicted[282].

To ensure a new air supply for some processes, a fraction of the air used in the collection and filtration systems is also evacuated[234]. This evacuated air can be treated by several filtration stages with, possibly, capture in wet scrubbers or electrostatic precipitators in the final stage. This capture principle, involving electrostatic attraction, is particularly effective for very fine particles. Periodic cleaning of the collection plates is usually accomplished by liquid jet spraying of these plates.

Filtration involves a number of mechanisms by which particles might be captured by filter fibres[283], giving efficient separation of particles from air. As an aerosol penetrates through a filter, the trajectories of particles might follow the streamline, or be deviated from the streamline (e.g. by diffusion). Mechanical capture of particles can occur by (see Figure 9):

- direct interception, where a particle follows the streamline and is captured if it comes into contact with a fibre;
- inertial impaction, where capture is effected by deviation of a particle from the streamline by its own inertia;
- diffusional deposition, where the combined effect of airflow and Brownian motion brings a particle into contact with a fibre;
- gravitational settling.

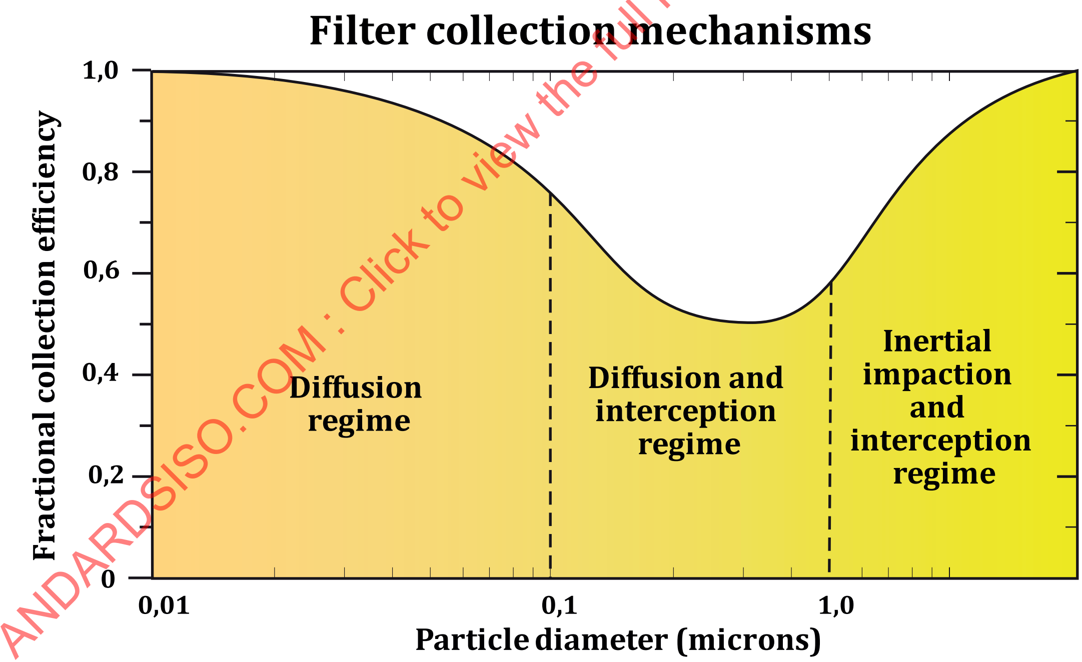


Figure 9 — Fractional collection efficiency versus particle diameter for a mechanical filter (from Guidance for Protecting Building Environments from Airborne Chemical, Biological, or Radiological Attacks[282])

For some filter types, electrostatic forces will effect capture.

According to single fibre filtration theory, particles larger than 300 nm are collected most efficiently by impaction, interception, and gravitational settling, while particles smaller than 300 nm are collected most efficiently by diffusion or electrostatic attraction[279]. Between 100 nm and 300 nm in size, particles are most likely to flow uncaptured through filters. This is the “Most Penetrating Particle Size” (MPPS), and collection efficiency is lowest[284][285][286]. The main mechanical filtration mechanisms involved around lowest collection efficiency are diffusion and interception, with the impaction mechanism playing only a minor role[279][287]. The MPPS range can vary due to a number of factors, for example the type of filter media employed and the flow rate.

For particles less than 300 nm, Brownian diffusion is the dominant mechanical mechanism causing particles to impact on filter fibres[279][288][289]. Filtration efficiency due to Brownian diffusion increases as particle size decreases. Brownian diffusion is caused by collisions between particles and the air molecules to create random paths which the particles follow. The random motion increases the probability of a particle contacting one of the filter elements. Once the particle is collected onto a surface it will adhere to it due to the Van der Waals forces.

Current methods for certification of HEPA filters do not routinely require testing at particle sizes below 100 nm. The US. Department of Energy's standard, *DOE HEPA Filter Test Program*[290], an internationally recognized standard, requires that the filter is tested at an aerosol diameter of 300 nm aerodynamic diameter and that the particle collection efficiency is greater than 99,97 %. Given the dimensions and physical properties of nano-objects, an approved HEPA filter should have filtration efficiency greater than 99,97 % for most nano-objects.

As described above, due to Brownian diffusion, filtration efficiency will increase as particle size decreases. Research has shown that penetration of nano-objects through filter media decreased down to 2,5 nm as expected by traditional filtration theory[286][291][292]. As particles approach molecular size (less than 2 nm), they might be less likely to adhere to filter fibres during diffusional collisions, and thermal rebound can occur[293][294][295].

The filtration efficiency was still found to increase as particle size decreases, but it does not increase as quickly as predicted by traditional filtration theory. In practice, thermal rebound might not be of any significance for the filtration of nano-objects, e.g. if filters are designed to ensure a sufficient number of collisions.

9.3.6 Administrative means for the control of workplace exposures

9.3.6.1 Administrative controls

Administrative means of control constitute an additional approach to supplement engineering approaches, but are not a substitute for engineering approaches.

NOTE Engineering control methods are always used according to standard practices.

Administrative controls can help guarantee industrial hygiene in the working environment, and if necessary, companies and research facilities should seek expert advice from occupational hygienists. In working with NOAAs, the administrative means to be applied will depend on the type of NOAAs and other materials involved, and the nature of the work to be performed.

Application of engineering measures might be limited in some situations because, for example, they are not sufficiently advanced technically or they cannot be implemented due to prohibitive costs. In these situations, administrative approaches for limiting the risks of occupational exposure to NOAAs include:

- modification of work practices;
- minimizing the number of exposed workers;
- limiting access to working areas and restricting access to authorized personnel;
- providing effective personal hygiene measures;
- housekeeping, including routine clean-up of work areas and clean-up of NOAA spills;
- use of preventative maintenance, which minimizes the risks of unscheduled interruption of production while assuring safer operations.

9.3.6.2 Recordkeeping

Effective recordkeeping is required to help establish and maintain safe and healthy workplaces. Recordkeeping requirements have been defined in national codes of practice for handling hazardous substances and dangerous goods (with consideration for minor quantities of dangerous goods)[296][57][58]. Records need to be kept of:

- induction and training programs;
- risk assessments;
- servicing of and testing of equipment (including fire-fighting equipment);
- workplace monitoring;
- health surveillance (records kept confidentially);
- dangerous occurrences and near misses;
- work-related injuries and illnesses;
- workplace engineering controls maintenance, daily checks and examinations;
- disposal records.

In general (except for confidential records e.g. health surveillance), records need to be clear, easily accessible and understandable so that managers, employees, employee representatives and public authorities can gain access to information to which they are entitled. Public authorities might also define the length of time records should be kept for, and what actions should be taken if company ownership changes or a company ceases to trade.

9.3.6.3 Training

Effective training and instruction for the workforce is critical to ensure workers' health and safety when handling NOAAs. A number of topics are covered in training undertaken by organizations working with NOAAs[238] e.g.:

- safe handling of NOAAs and Standard Operating Procedures (SOP);
- hazards and toxicity;
- personal protective equipment;
- engineering controls and equipment maintenance;
- emergency procedures;
- waste handling;
- definition of NOAAs;
- environmental release/shipping/customer protection;
- exposure monitoring;
- applicable regulation.

Sources of information and guidelines for training include government agencies (e.g. NIOSH, OSHA, and EPA in the United States the UK Health and Safety Executive, and the Industrial Technology Research Institute in Taiwan), scientific literature and toxicological studies, internet databases, internal expertise, conferences, external experts (e.g. consultants), industry associations and suppliers' Safety Data Sheets and International Chemical Safety Cards[297].

9.3.6.4 Reduction of work periods

Reduction of work periods might be applied beneficially under certain conditions, e.g. when working in a hot environment (to avoid heat stress), or in situations where risks cannot be controlled by engineering techniques. For the handling of NOAAs, this approach should not be widely applicable.

9.3.6.5 Personal hygiene

Effective personal hygiene is needed to help protect the health of workers[172][234]. Particular focus is required when people might be exposed to substances such as NOAAs which; (a) are known to be hazardous, or (b) might be hazardous. Even in facilities with very efficient engineering controls, some workers could still be exposed to NOAAs, e.g. during clean-up and maintenance work. Hygiene arrangements for working with NOAAs are suggested below. The effectiveness is yet to be evaluated, but a number of these approaches are currently used in organizations working with NOAAs[238]. A good description of procedures and cleaning instructions is helpful in preventing personal contamination.

Washbasins and showering facilities are needed in the workplace for decontamination of areas of the skin exposed to dust or liquids — for example, prior to leaving the worksite. If there is potential for toxic product spatters or spills, the emergency shower is an indispensable tool to respond as quickly as needed.

Smoking, drinking and eating in the workplace should be prohibited, except in clean areas reserved for this purpose (which are separated from the areas where NOAAs are handled). To help prevent dermal absorption of NOAAs, open wounds should be effectively covered.

Facilities for changing clothes should be provided, and clean working clothes should be provided and used. Working clothes and private clothes should be separated. In the presence of highly toxic products, double lockers (one for work clothes, one for home clothes) avoid any risk of contamination outside the work areas. To prevent transfer of contamination to the home environment, soiled clothing should not be taken home. Clothing must be cleaned safely — working clothes should not be cleaned by the employee, and the use of air jets for cleaning should be prohibited.

In order to prevent transfer of NOAAs via shoes, sticky mats can be placed at laboratory entrances. These can be sheets of sticky paper adhered to the floor that must be crossed when leaving the laboratory. It is intended that NOAAs attached to the shoes of employees will stick to the mats and not be transferred to the rest of the building.

Procedures should be in place for disposal of personal protective equipment (e.g. gloves and coveralls). Also a procedure should be in place indicating the frequency of changing and washing non-disposable personal protective equipment.

9.3.6.6 Routine clean-up of work areas and clean-up of NOAA spills

Until relevant information specific to NOAAs is available, it is suggested that it would be prudent to base strategies for dealing with NOAA spills and NOAA-contaminated surfaces on established good practices for larger particles[172]. However, use should also be made of available information on the possibility for NOAA exposure, and the relative significance of different exposure routes should be considered[172]. Organizations frequently reported using more than one clean-up method (e.g. wet wiping and vacuum cleaning), depending on the NOAA and its phase during handling[238]. The effectiveness of the approaches for NOAAs is yet to be evaluated. Many of the methods used for the routine clean-up of powders and liquids are also applicable for the clean-up of powder and liquid spills.

Work area clean-up, including removal of dust deposited on the floors, walls and work surfaces, should be performed regularly to avoid any (i) accumulation, (ii) risk of atmospheric re-suspension or (iii) explosion (should the dusts be explosive, as in the case of certain metallic powders)[234]. All equipment should be cleaned thoroughly and isolated, as needed, before it undergoes maintenance.

NOTE For example, the equipment might be ‘locked out' or electrically isolated for safety.

The most frequently used methods for NOAA clean-up are wet wiping, vacuum cleaning and dry wiping[238]. Regular cleaning of workplaces using wet wiping methods, HEPA-filtered vacuum cleaners, or a combination of both would be suitable for most NOAAs.

NOTE The cleaning frequency needed will vary from workplace to workplace, depending on operating conditions. Cleaning at the end of each work shift might be an appropriate frequency for a number of workplaces.

Damp cleaning methods with soaps or cleaning oils are preferred[172]. The use of commercially available wet or electrostatic microfibre cleaning cloths might also be effective in removing particles from surfaces with minimal dispersion into the air. Dry wipe is only used by organizations for cleaning up solutions. If vacuum cleaning is employed, care should be taken that HEPA filters are installed properly and bags and filters changed according to manufacturers' recommendations[172]. The performance of any HEPA-filtered vacuum cleaner should be regularly tested to ensure adequacy of seals etc.

Standard approaches to cleaning up powder and liquid spills, including the use of HEPA-filtered vacuum cleaners, wetting powders down, using dampened cloths to wipe up powders and applying absorbent materials/liquid traps can be used for NOAA spills.

Energetic cleaning methods such as dry sweeping or the use of compressed air should be avoided or only be used by workers using adequate respiratory protective equipment and with precautions that ensure that particles suspended by the cleaning action are trapped by HEPA filters[172]. Clean-up should be conducted in a manner that prevents worker contact with wastes, and the disposal of all waste material should comply with all applicable national and local regulations. A small number of organizations reported that they stored the spilled NOAAs in separate, sealed waste containers[238].

Given the limited knowledge on the hazards of NOAAs, cleaning wipes should be properly disposed of as hazardous waste. Drying and reuse of contaminated wipes can result in re-dispersion of particles — therefore, wipes should not be reused.

When developing procedures for cleaning up NOAA spills or contaminated surfaces, consideration should be given to the potential for exposure during clean-up[172]. Inhalation exposure and dermal exposure will likely present the most probable routes of exposure. Consideration will therefore need to be given to appropriate levels of personal protective equipment. For inhalation exposure, dusts are likely to present greater inhalation exposure potential than liquids, with liquids in turn presenting a greater potential risk than encapsulated or immobilized NOAAs. Exposure will be influenced by the likelihood of material re-aerosolization. The use of respirators while cleaning up nano-spills has been reported[238].

While vacuum cleaning might prove to be effective for many applications, electrostatic forces might make it difficult to remove particles from surfaces[172]. The electrostatic charge on particles will cause them to be attracted to oppositely charged surfaces and repelled by similarly charged surfaces. A similarly charged vacuum brush or tool might repel particles, making it difficult to capture the aerosol or even causing it to be further dispersed. Vigorous scrubbing with a vacuum brush or tool or even the friction from high flow rates of material or air on the vacuum hose can generate a charge. The vacuum cleaners recommended for cleaning copier and printer toners have electrostatic-charge-neutralization features to address these issues.

One organization has reported that it uses a vacuum hose rather than vacuum cleaner to clean up spills of NOAAs, because the electric motor of a vacuum cleaner has the potential to ignite flammable NOAAs[238]. Risk of ignition in vacuum lines should be also considered[298].

For NOAAs, other methods of equipment decontamination reported are solvent washing, burning, and dissolving using acid and plasma cleaning[238]. A small number of organizations reclaim NOAA spills, and two organizations report that they evacuate the area during spill clean-up. An example of a workplace clean-up and decontamination methodology for highly toxic dusts is provided in the *Summary of good clean-up and decontamination practices for workplaces with beryllium-containing dust*[299].

9.3.6.7 Waste disposal

Many organizations dispose of their waste NOAAs through waste management companies[238]. Some dispose of their NOAAs in separate disposal containers. Labelling of containers should comply with established labelling codes of practice, making appropriate use of available information for the contained NOAAs (e.g. hazard information from the literature, including research papers). For storage, glass containers, metal containers, and sealed metal drums are used by organizations[238].

Disposal methods used by organizations that do not dispose of their NOAA waste through an external company include[238]:

- treating NOAAs in-house before disposal;
- recycling all NOAAs;
- incinerating their waste NOAAs on-site (all carbonaceous material);
- returning NOAAs to suppliers.

9.3.6.8 Fire, explosion and catalysis prevention and control

Combustible dusts in the workplace present a risk of fire, deflagration and explosion if they are not managed effectively. A (NOAA) dust explosion or serious fire can cause catastrophic loss of life, injuries as well as significant damage to equipment and buildings. Controlling combustible dust hazards requires a systematic long-term approach contained in a combustible dust/OHS management program.

Different dust profiles present different hazards. A dust accumulation might present either a fire, deflagration and/or explosion hazard. An explosion or deflagration can start with a dust fire that ignites finer dust that is dispersed into the air by some other mechanism.

Evaluating the risk of fire, deflagration and explosion requires consideration of five elements of a combustible dust explosion — fuel (dust), dispersion, oxygen, ignition and confinement. Deflagration is a rapid combustion of fuel (e.g. dust) mixed with air. Fire requires fuel (dust), oxygen and ignition, and can be the source of ignition for subsequent deflagration and/or explosion events.

The same principles applying to the management of fine powders, dusts or dusty materials should be considered for NOAAs, with particular care taken in the case of easily oxidizable metallic dust[202]. However, the effectiveness of methods for NOAA fire, explosion and catalysis prevention and control is yet to be evaluated.

Explosion protection measures have been described for dust dispersions (e.g. by OSHA[300]) and for hazardous quantities of larger sized materials[301], and can be applied to the handling of potentially explosive NOAAs. For the handling of flammable NOAAs, following these types of measures has also been recommended[235]. For reactive or catalytically active NOAAs, contact with incompatible substances should be prevented[235].

Fire prevention must take into account pertinent fire codes, as well as existing regulations, especially electrical requirements[234]. Relevant standards [such as the National Fire Protection Association (NPFA) standards] should also be consulted for additional guidance to prevent/control the risk of fires, deflagration and explosions in the workplace. The design of electrical equipment protection should take into account the fine granulometry and very long settling time of NOAAs, with dust protection needed.

NOTE For some NOAAs, the type of protection used to protect equipment from vapors is needed.

In addition, further precautions should be taken regarding the operating temperature of electrical equipment, in regard to the risks of auto-ignition of NOAAs. The selection of an extinguishing agent should consider the compatibility or incompatibility of the material with water[234]. Some metallic dusts react with water to form, among other things, hydrogen, which ignites very easily and deflagrates. Chemical powders are available to extinguish burning metallic dust powders. In extinguishing burning metallic dust powders, care should be taken to avoid significant air movement, since this has the effect of putting the metallic dust in suspension, thereby increasing the risk of deflagration. To reduce the risks of fire and deflagration, it might prove necessary to use controlled-atmosphere production and storage processes, using carbon dioxide, nitrogen or inert gas. This might introduce further hazards into the system, notably the risk of asphyxiation.

When working with potentially explosive NOAAs, the following issues should be considered[238]:

- anti-static shoes and mats being used in areas where the materials are handled. The shoes reduce the build-up of static charge, which could potentially ignite the materials.
- a distillation system for evaporating solvent from a colloidal dispersion being housed within an explosion-proof enclosure. This enclosure was designed with concern for the potential for these particular NOAAs to be explosive.

9.3.6.9 Storage

Handling large amounts of NOAAs for packaging and storage might need to involve special protection to conserve the products and to ensure workplace health and safety. Suitable records should be kept of all materials stored on site. Storage containers for NOAAs should accommodate the different granulometric characteristics and reactivity of the particles[234]. The fine granulometry of the materials might result in long settling times and re-dispersion. Reservoirs should be tightly sealed to avoid leakage of the product or contamination of the premises during transport. Appropriate arrangements, some of which resemble those used for storing gases, should be considered.

The small size of the particles (which often tend to agglomerate), provides a very large surface area in contact with the surrounding air, thereby facilitating chemical reactivity. Depending on the product to be stored, a variety of preventive procedures can avert deterioration of the product, and the risk of fire or explosion. Possible solutions include storage in inert gas or in anhydrous conditions. To avoid oxidation, or even explosion in the case of certain metals, NOAAs often need to be protected from air. In other conditions, it might be possible to surround the NOAAs in a protective layer of salts or various polymers. These layers can be removed before using the product[234].

9.3.6.10 Other aspects of prevention

For nano-processes, preventative measures might also need to be developed and implemented to prevent asphyxiation and electrocution. In some current processes using or manufacturing NOAAs, the risk of asphyxiation is possible due to using large quantities of inert protective gases[234]. There is also risk of electrocution related to the generation of a plasma using high currents[298]. Procedures for emergency response, including the use of emergency protective equipment and for specialized first aid should also be developed.

9.3.7 Evaluating the work environment

9.3.7.1 General

The scientific framework and methods for assessing exposure to NOAAs are examined in detail in Clause 7. Monitoring and evaluating the work environment will determine the effectiveness of the control approaches described in 9.3.3 to 9.3.6. Evaluation findings will inform about whether personal protective equipment (PPE) is required.

Regarding the measurement of airborne NOAAs, the direct measurement of personal exposures proved to be difficult in the past because most of the measuring instruments were not designed to be attached to the person. However, currently commercially available instruments (both personal monitors and personal samplers) are technically mature and reliably usable in field measurements of NOAA in the personal breathing zone[179][174]. Data can be measured both with high time resolution or as time weighted (e.g. shift based) averages. This means that compliance testing using statistical techniques could be performed according to national standards or professional guidelines the same way as for non-NOAAs. However, due to the absence of non-regulatory binding OELs and the variety of exposure metrics the applicability to legislative standards is yet unclear.

In addition to direct personal exposure monitoring, there exists an alternative method to evaluate the workplace environment — by estimating statistically the personal exposure concentrations from the aerial concentration data measured in the workplace at regular or random intervals. The feature of the described method is to be able to estimate the arithmetical average concentration and the upper limiting exposure concentration[302][303].

9.3.7.2 Area sampling: statistics

Arithmetical average concentration (C1)

It indicates the exposure concentration of the person who acts averagely at the workplace. The value is estimated using the following formula[302][303]:

$$\log C1 = \log Mg + 1{,}151 \log 2\ SDg$$

where

Mg is the geometric mean of measured concentrations;

SDg is the geometric standard deviation of measured concentrations.

Upper limiting exposure concentration (C2)

The value of the upper limiting exposure concentration, which is defined as the level at which only 5 % of all airborne concentrations measured at any time and any place in the unit workplace exceed the exposure limit, is calculated from the following formula[302][303].

$$\log C2 = \log Mg + 1{,}645 \log SDg$$

NOTE Assuming a lognormal distribution of airborne concentrations in the unit workplace.

Using this method, the workplace environment can be evaluated without conducting the measurement of personal exposure concentrations.

9.3.7.3 Personal breathing zone sampling: statistics

National or supranational standards like the European standard EN 689 “Workplace atmospheres — Guidance for the assessment of exposure by inhalation to chemical agents for comparison with limit values and measurement strategy”[304] are available to guide occupational hygienists and other occupational health professionals on measurement strategies for determining compliance with occupational exposure limits. In addition guidance from professional societies like the BOHS/NVvA and AIHA can be used[305][306]. Although these standards or guidelines originally were developed for non-NOAAs, the underlying statistical techniques can be applied for NOAAs as well. Such techniques have been programmed in regularly used statistical Excel tools like IHSTAT+: v. 235[306] and BWStat, v2.1[305] for implementing the BOHS/NVvA Sampling Strategy.

9.3.8 Personal protective equipment (PPE)

9.3.8.1 General

Engineering and administrative protection measures should be supplemented by PPE, e.g. respirators, protective gloves, protection goggles and full protective clothing, when further protection for workers is needed. In practice, the majority of nanomaterial organizations surveyed in the ICON study recommended their employees or researchers use PPE[238]. Conventional laboratory wear was most often reported as the recommended means of protection.

9.3.8.2 Protection from inhalation exposure — Use of filter respirators and air-supplied respirators

The use of filter (air purifying) respirators or air-supplied respirators is required to supplement, but not to replace engineering and administrative controls when such controls do not adequately keep worker exposures to an airborne contaminant below a regulatory limit or an internal control target.

NOTE Air purifying respirators depend on filtration as a means of cleaning the air prior to it being breathed by the worker.

Respirators should be used as part of a complete respiratory protection program. Preliminary findings have shown that respirators will help provide workers with protection against NOAAs.

The decision to institute respiratory protection should be based on a risk assessment after all other controls are in place. In regard to risk assessment, there are only few specific exposure limits for airborne exposures to NOAAs (see ISO/TR 18637[222]). For some NOAAs, occupational exposure limits exist for larger particles of similar chemical composition, and this general toxicology information should be considered. However, current scientific evidence indicates that NOAAs might produce a larger biological reaction than larger particles of similar chemical composition for the same mass of material and thus might pose a greater health risk when inhaled[232].

The effectiveness of engineering, administrative and work practice controls can be evaluated using the measurement techniques described in Clause 7 (Exposure Assessment). If worker airborne exposure to NOAAs remains a concern after instituting these measures to control exposure, the use of respirators can further reduce exposures. A respiratory program should include the following elements as a minimum:

— an evaluation of the worker's ability to perform the work while wearing a respirator;

— regular training of personnel;

— periodic environmental monitoring;

— respirator fit testing;

— respirator maintenance, inspection, cleaning, and storage;

— selection of respirators made by a person knowledgeable about the workplace and the limitations associated with each type of respirator;

— detailed records of all these elements should be kept.

NOTE When respirators are required to be used in the workplace in the United States, OSHA's respiratory protection standard [29 CFR 1910.134] requires that a respiratory program be established[307].

Information for employees using respirators is provided in US. OSHA's respiratory protection standard 29 CFR 1910.134[307]. Several classes of respirators exist that can provide different levels of protection when properly fit tested on the worker. Assigned Protection Factors (APFs) for respirators have been recommended by a number of organizations (see Annex E, Table E.1)[308][309]. This includes information from US. OSHA's *Assigned Protection Factors; Final Rule* (published in 2006)[310]. Notes on the advantages and disadvantages of various types of respirators is shown in Annex F (Table F.1), using information in the *NIOSH Respirator Selection Logic 2004*[311].

NOTE IRSST has also published a complete guide to respirator selection and use[312].

The collection efficiency of particles by filters was examined in detail in the earlier subclause on *Air recirculation and filtration* (in 9.3.5.5). The most penetrating particle size (MPPS) for many filters is around 300 nm, but this can vary based on the type of filter media employed, flow rate, and the condition of the respirator. For example, the most penetrating particle size for HEPA class filters can range from 100 nm to 300 nm[284][286], while for N95 class air purifying respirators containing electrostatically charged filter media it has been found to be around 30 nm to 70 nm[313] and around

50 nm to 100 nm[172][314][315]. Below the MPPS, filtration efficiency will increase as particle size decreases due to particle diffusion.

Current methods for certification of respirator filters do not routinely require testing at particle sizes below 100 nm. For example, European Standards for respirator filter cartridges[316] and for filtering face pieces[317] require that these systems are tested against sodium chloride aerosols with a mass median diameter of 300 nm, again based on an expectation that this would be the most penetrating size.

Recent research indicates that respirators can offer considerable levels of protection against nano-objects, but not necessarily the expected levels of protection at high inhalation (or respiratory) flow rates. Manikin-based tests using sealed face-pieces showed that the penetration of ~30 nm to 70 nm monodisperse nanoparticles through some of NIOSH certified N95 filtering face-piece respirators could exceed the 5 % threshold at high inhalation flow rates[313]. Average penetrations of 5 % and 6 % (with standard deviation of 1 %) were measured at 1,4 × 10^{-3} m^3/s inhalation flow rate for the two types of N95 respirators examined. However, another report[318] compared the penetration of sodium chloride particles at 1,4 × 10^{-3} m^3/s flow rate through five NIOSH approved N95 filtering face-piece respirator models using two test methods: a monodisperse aerosol test (20 nm to 400 nm) and a polydisperse aerosol test similar to what is used for NIOSH certification testing. The average initial penetration levels from the polydisperse aerosol tests ranged from 0,61 % to 1,24 %. Monodisperse aerosol penetrations behaved according to single fibre filtration theory. The most penetrating particle size was found to be near 40 nm. The mean penetration level of 40 nm particles for the five models ranged from 1,4 % to 5,2 % and exceeded 5 % for only two of the respirator models. The rank ordering of the filtration performances of the five respirator models was consistent between the two test methods. The correlation coefficients between the average penetrations from the polydisperse aerosol tests and average penetrations of monodisperse particles of 40 nm, 100 nm, 200 nm and 300 nm were 0,945, 0,979, 0,996 and 0,994, respectively.

Over a range of particle sizes down to 20 nm, particle penetration through N95 and P100 filtering face-piece respirators and respirator cartridges (two models from each of four categories) was also examined in a study by the Battelle Memorial Institute[315], to assess the effect of particle size and flow condition on measured penetration. The following trends were observed:

- pnetrations varied within respirator groups, i.e. within the group of P100 filters tested, and within the group of N95 filters tested.
- the MPPS for P100 cartridges was generally between 100 nm to 200 nm and shifted toward the lower end of this range with increased flow. The MPPS for the N95 cartridges was generally 50 nm to 100 nm for all flow conditions, and the MPPS for both P100 and N95 filtering face-piece respirators was 50 nm to 100 nm.
- for 50 nm particles at flow rates of 85 l/min, mean particle penetrations ranged from < 0,000 1 % to 0,002 %, 0,7 % to 8,8 %, 0,01 % to 0,048 %, and 2,8 % to 9,7 % for P100 cartridges, N95 cartridges, P100 filtering face-piece respirators, and N95 filtering face-piece respirators, respectively.

The choice of respirator type will depend on the specific task and the materials being handled. It has been reported that a number of filter specifications are used by organizations working with NOAAs[238]. Cartridge respirators with either a full face mask or a half mask are used by a number of organizations. Some organizations indicated that disposable particulate respirators are recommended for employee use while working with NOAAs[238]. Some disposable particulate respirators are N/R/P/95/99/100 U.S. NIOSH-Certified filtering face-piece respirators, but some inexpensive masks might be largely untested and not certified by any recognized body. Masks and respirators that are not certified should not be relied upon for protection against NOAAs. Users cannot be ensured that they provide a certain level of protection. It has been recommended that respirators should be used when handling powders (particularly when working with larger amounts), and for maintenance work on production machines[234]. An example of the use of respirators for sampling and data collection during a mixing operation is shown in Figure 10 below[278]. Individual airway protection equipment used in locations where NOAAs are produced in the particulate form should be particularly efficient. Wearing a full-face mask with high-efficiency filters (over 99,97 % efficient) has been recommended by some[234].

Figure 10 — Use of respirators for sampling and data collection during a mixing operation (from NIOSH Progress Toward Safe Nanotechnology in the Workplace[278])

The frequency of cartridge change-out and/or face-piece respirator disposal should be carefully considered, and should occur before workers have difficulty breathing or can smell chemical vapors, or the filter is clogged. Change-out/disposal schedules should be more frequent at higher scales of production and among organizations that work with NOAAs in the dry powder form.

Often, the determining factor which governs the effectiveness of Respiratory Protection Equipment (RPE) against particulate challenges is not absolute penetration through the filter, but rather face-seal leakage which bypasses the device. Face seal leakage is dependent on many factors including the fit of the mask to the face, duration of wearing and work activity. User comfort and equipment maintenance are also issues with RPE. Since it is expected that nano-object aerosols will have high mobility, it is possible that some leakage will occur, although no more than might be expected for a gas[231], noting that aerosol particles attach firmly to any surface they contact[279]. Numerous studies on mask leakage have been conducted on larger particles and on gases/vapors. For example, work done by researchers at the US. Army RDECOM on a head-form showed that mask leakage (i.e. simulated respirator fit factor) measured using submicrometer aerosol challenges (720 nm polystyrene latex spheres) was representative of vapour challenges such as sulfur hexafluoride and isoamyl acetate[319].

A higher level of protection is ensured by using a Powered Air-Purifying Respirator (PAPR), which includes high-efficiency filtration and a pump supplying filtered air to a full-face mask[234]. The air current generated on the wearer's face might increase the level of protection by maintaining positive pressure inside the mask. This results in greater comfort for the worker and minimizes exposure when the mask seal is imperfect. In cases where the APF of the PAPR is insufficient, or where the concentration is Immediately Dangerous to Life or Health (IDLH), airline respirators or self-contained breathing apparatus are necessary.

In conclusion, several RPEs are available and should be used according to a particular workplace situation.

9.3.8.3 Dermal protection

Dermal exposure might occur during the manufacturing, use and handling of NOAAs. During NOAA manufacturing, exposure is most likely to occur in the product recovery and packaging stages, and from surface contamination e.g. during general maintenance of workplaces and equipment. Use of Skin Protective Equipment (SPE) is recommended where the possibility of dermal exposure cannot be excluded at all times. However, because of the small diameters of nano-objects, the various kinds of SPE might have limited effectiveness. For example, research conducted under EU Nanosafe2 project[320] showed that nano-objects might penetrate through commercially available gloves, and therefore, it is recommended to use at least two layers of gloves. Non-woven fabrics seem much more efficient against nano-object penetration. Thus, it is recommended that protective clothing made of cotton fabrics are not used[320].

Some soluble NOAA compounds can penetrate the skin by dissolution and absorption. However, penetration of NOAA through intact skin is highly unlikely[321]. Currently, there is a very limited state of knowledge in regard to potential health risks, and no dermal exposure standards have been proposed. As a precaution, it is preferable to introduce controls to exclude or limit the level of dermal exposure likely to occur. As for inhalation exposure, the UK's *Control of Substances Hazardous to Health Regulations 2002 (COSHH) (as amended)* provide a framework by which a strategy to prevent or control dermal exposure can be developed[244]. As with control of exposure by inhalation, the first approach is enclosure of the process, and powder handling processes can be enclosed successfully. However, in practice, particularly with products or processes which are in development, the main emphasis might be on investment in the design of safeguards during product synthesis. This might reduce the expenditure on sophisticated control and automation processes to deal with the relatively mundane tasks such as harvesting and packing of NOAAs. Even where such processes are in place, the requirements for attention to equipment breakdowns and maintenance means that the possibility of dermal exposure cannot be excluded at all times. In these and other instances, protection against dermal exposure typically consists of the use of SPE, i.e. suits, gloves and other items of protective clothing[231].

Clothing used by employees or researchers in organizations working with NOAAs is described in reference [238]. Lab coat materials include cotton, nylon and disposable material. Building suits ("bunny suit," "coveralls", "working suit") are frequently disposable, and are used for working in areas with a higher exposures instead of lab coats[238]. A range of glove materials are utilized, most often nitrile, latex, and rubber, with other materials including PVC, polyethylene, polychloroprene, and leather[238]. Long gloves that cover the wrists, double gloves, wrist barriers and gloves with cuffs are all used. Most organizations report their choice of gloves is based on the solvents being used, with choices based specifically on chemical compatibility. A number indicated that the use of glove types was application specific. Other forms of PPE used are hair bonnets and anti-static shoes in areas where there is concern about the explosive properties of the NOAAs.

Maintenance of skin protection equipment is an important issue to consider. If in practice it proves difficult to maintain and clean SPE, then there is the option to use disposable clothing, e.g. hooded coveralls, aprons and shoe covers which normally provide excellent skin protection.

NOTE 1 SPE made from breathable laminates might offer the possibility of washing and re-use for some application.

NOTE 2 From an environmental viewpoint, use of disposable clothing will probably result in clothing and NOAAs ending up in landfill, as opposed to NOAAs ending up in waste water from washing processes.

The same principle applies to gloves, which are available in a wide range of sizes and resistances to various chemicals, cuts and perforations. In general, deterioration of protective gloves occurs during use, and they need to be changed on a regular basis.

Procedures should be in place for disposal of personal protective equipment (e.g. gloves and coveralls). In addition, a procedure should be in place indicating the frequency of changing and washing non disposable personal protective equipment. Considering the effectiveness of SPE, mechanisms whereby skin contamination might occur when using SPE have been described[322]. These mechanisms are: (i) penetration or permeation through the materials making up the SPE, (ii) the transfer of substances through direct contact between the SPE surfaces and the skin, (iv) transfer of substances by direct contact of skin with contaminated surfaces and (iv) redistribution of substances by skin to skin contact e.g. as a result of touching the face with contaminated fingers.

NOTE Actions such as flexing of the material can also impact ~~on~~ particle penetration.

Transport of contaminants through permeable clothing occurs by aerosol penetration and liquid transport. External air pressure and the "bellows effect" (i.e. the expulsion and entering of air during movement) can be considered to be the driving force for penetration of aerosols through fabric, whereas the mechanisms of liquid transport are capillary penetration, pressure penetration, impact penetration and evaporation-condensation. Repeated deformations and contact with the liquid carrier of the nanoparticle were shown to increase the potential of nanoparticle penetration through gloves[323][324][325]. Mass transport through non-permeable clothing is a diffusion process driven by concentration.

Current European testing for certification of PPE against dermal exposure only takes account of permeation or penetration of the material. However, recently, new tests have been proposed which take account of human factors[326], involving real workplace SPE performance tests and/or workplace simulations. Even for powders above 100 nm in size, it is recognized that SPE is limited in its effectiveness to reduce or control dermal exposure[172]. The penetration efficiency (i.e. % penetration) for 10 widely different fabrics (including woven, non-woven, and laminated fabrics) against an aerosol of polystyrene latex spheres with a mean diameter of 477 nm has been examined[327]. Particle penetration measurement results ranged from 0 % to 54 %. The three fabrics exhibiting significant pressure drop (i.e. the least air permeable) all had penetration levels less than 1 %. No information on either the efficacy of SPE against direct penetration of nano-objects, or the impact of nano-objects on the probability of failure of SPE due to human factors has been identified. However, some existing clothing standards already incorporate testing with nanoscale particles, and therefore provide some indication of the effectiveness of protective clothing to nano-objects. For instance, ASTM standard F1671–03 specifies the use of a 27 nm bacteriophage to evaluate the resistance of materials used in protective clothing from the penetration of bloodborne pathogens[328]. New test methods have been developed to assess the penetration of protective clothing in conditions simulating use, e.g. with pressure applied to the material, repeated deformation, contact with a liquid simulating sweat[329][323][324]. It has been suggested that penetration of SPE by nano-objects is likely to be even greater than by larger particles[234]. It has also been proposed that, since it is likely that nano-objects which escape into the workplace will become widely dispersed, it is likely that the human factor element will be even more critical than for micrometer-scale particles[231], further contributing to SPE being less effective against nano-objects than against micrometer-scale particles.

9.3.8.4 Eye protection

Eye protection is recommended where there is potential for exposure to NOAAs. Goggles, safety glasses and full-face shields are all used in practice[238], though the use of a full-face shield was not always recommended for reasons related to the handling of NOAAs specifically (e.g. it was also recommended when there is increased exposure to solvents or hot material). One organization does not allow the use of contact lenses in the laboratory. Full face-piece respirators offer eye protection in addition to respiratory protection. They also allow the use of corrective lenses or contact lenses.

9.3.8.5 Preventing ingestion exposure

It is considered that ingestion exposure in the workplace results primarily from hand-to-mouth contact, but might also occur via the mucociliary escalator after inhalation[232]. Ingestion could also occur from swallowing particles trapped in the head airways region. It follows that strategies which tend to reduce dermal exposure to NOAAs in the workplace will also tend to reduce exposure by ingestion.

9.4 Health surveillance

Health surveillance should be considered for all workers where there is risk of exposure to NOAAs, and where it has been demonstrated that there is a relationship between exposure to the substance and a measurable biological indicator. It is strongly recommended that a health surveillance program is established for workers if NOAAs contain chemicals or components for which current guidelines recommend health surveillance.

Given that exposure to very low concentrations of NOAAs might be widespread, measurable changes in biological indicators from baseline levels, rather than comparison of body burden with the Biological Exposure Index (BEI), might be the most appropriate parameter to examine. The use of health surveillance in this context is as an indicator of whether exposure is occurring, rather than in determining that levels of exposure are safe. Due to the currently limited capability for measuring airborne concentrations of NOAAs, the use of biological indicators might be a very useful approach in evaluating the effectiveness of control measures introduced.

At this stage, where the impact of NOAAs on human health is unclear, continuous health checks for workers are particularly important to detect any adverse effects from NOAAs. Health check records are important evidence in identifying adverse health effects.

It is recommended that a basic worker health monitoring program is established. Such a program should include at a minimum:

a) identifying staff (NOAA workers) exposed to manufactured NOAAs of unknown health effects;

b) conducting workplace characterization and worker exposure assessments;

c) providing NOAA workers with "baseline" medical evaluations and including them in a nonspecific routine health monitoring program.

It is recommended to ensure that NOAA workers are offered periodic medical evaluations that might include routine tests such as pulmonary, renal, liver, and haematopoietic function testing.

A recent survey has indicated that there is insufficient evidence to recommend a targeted nano-specific workplace medical screening or surveillance programme for most nanomaterials. However it is recommended that both exposure surveillance and routine medical screening are strengthened and, where feasible, a registry is established[202].

9.5 Product stewardship

Product Stewardship is a business process for the responsible and ethical management of the environmental, health and safety (EHS) aspects of products throughout their total life cycle. This encompasses strategies and processes necessary to make EHS an integral part of research and development, manufacturing, marketing, distributing, using, recycling and disposing of products. Those who supply nanomaterials should implement stewardship practices that promote the responsible development and use of these materials. Generally, the stewardship practices for non-nanoscale materials are also appropriate for nanomaterials. Examples of stewardship practices include those of the chemical industry's Responsible Care® programs in place in many countries and includes codes of management practices for EHS, Process Safety, Pollution Prevention, Product Stewardship, Security, Emergency Response and Community Awareness.

The life cycle assessment is directly linked, and vice versa, to the risk assessment of nanomaterials. The development of techniques for the monitoring and diagnosis of nanomaterials and for determining safe, appropriate and sustainable forms of production (including waste management) is essential so that the benefits of nanotechnologies can be safely enjoyed and aimed at protecting the environment for the present and future generations.

An objective of a stewardship program and/or required by laws/regulations is to establish, maintain and provide information on EHS hazards and reasonably foreseeable exposure from new and existing products. This information is necessary for risk characterization and risk management and the following are examples of various communication formats:

- Safety Data Sheets — Safety Data Sheets (also known as Material Safety Data Sheets) are often legally required for materials that meet the legal definition of "hazardous". Some nanomaterials are hazardous. For non-hazardous materials and nanomaterials SDS's are not required but providing them is considered good product stewardship. SDS's should address the known and reasonably foreseeable hazards of the materials and provide advice to mitigate these hazards, such as identifying appropriate engineering controls and/or personal protective equipment. Those who develop SDS for nanomaterials should follow the guidance of the UN Globally Harmonized System (GHS) and the legal requirements adopted by some countries in their implementation of the GHS. If a non-nano form of the nanomaterial exists it should not be presumed that the hazards for the nanomaterial are the same as the non-nano form. The potential contributions to hazard from the nanoscale nature must be taken into account.

- Labels — Like SDS's, labels are generally required for "hazardous" materials and labelling is considered a good stewardship practice even if not legally required. The considerations noted for SDS's should also be considered for labels.
- Product Information Sheets/Technical Data Sheets — These documents are often provided as marketing and/or technical documents that feature important use or benefit information. Often, EHS information is also provided. If so, the information should be accurate, clear, and consistent with the SDS and label information.
- Technical instructions — For some products information on how to use the product in certain applications might need to be very specific. EHS considerations should also be included to ensure that the product is used as intended and that the recommended steps are conducted in a safe manner.
- Personal interaction — Those who supply nanomaterials might have personal contact with buyers and users of the materials. Any information provided to buyers and users of nanomaterials should consider the safe handling, use and disposal of the materials. They should also consider if the users can do so safely and, if there is a concern that they cannot, they should not provide the nanomaterial to them.
- Accompanying letter — If additional letters such as cover letters accompany EHS and product stewardship information they must convey information consistent with product stewardship and other EHS information.
- Specification sheets/Certificates of analysis — These documents can include information that is particularly important for nanomaterials such as particle size and particle size distribution. It should be clear if the reported particle size pertains to primary particle size, aggregate size or agglomerate size and the relevance of the size. Primary particle size can be important for use in intended applications but aggregate particle size might be relevant for EHS purposes.
- Operation manuals — Operation manuals should ensure that the practices described take into account safe use. The properties of some nanomaterials might introduce factors that are different or magnified compared to non-nanoscale materials. For example, the increased surface area of nanomaterials can increase combustibility. The practices described in the manual should consider these properties and practices should be modified as needed to ensure potential environmental, personal and process safety impacts are addressed.

Annex A
(informative)

Primary chemical composition of nanomaterials

A.1 Carbon nano-objects and related carbon nanoscale materials

A.1.1 Fullerene

Fullerene is a molecule composed solely of an even number of carbon atoms, which form a closed cage-like fused-ring polycyclic system with 12 five-membered rings and the rest six-membered rings. Fullerene derivative is a compound that has been formed from fullerene by substitution of carbon and/or covalent attachment of a moiety[330]. Fullerenes are chemical entities which can be envisioned as primarily spherical cages built from carbon atoms chemically bonded to three nearest neighbours. The best known example is a soccer-ball shaped C_{60} fullerene, also known as buckminsterfullerene. Fullerene molecules can contain from 28 C atoms to more than 100 C atoms with some experimental studies reporting molecules containing up to 1 500 atoms with 8,2 nm diameter[331]. Existence of even larger fullerene molecules has been postulated from theoretical considerations[332]. Multi-shell fullerene-like nanoparticles referred to as carbon nano-onions, can range in size between 4 nm and 36 nm[333]. Fullerenes are actively investigated for a wide range of potential applications including: lithium-ion batteries, solar cells, fuel cells, oxygen, hydrogen and methane storage materials, additives to plastics, oil and rubber, imaging contrast agents, cancer and AIDS treatments.

A.1.2 Carbon black

Carbon black is an engineered material, primarily composed of elemental carbon, obtained from the partial combustion or thermal decomposition of hydrocarbons, existing as aggregates of aciniform morphology which are composed of spheroidal primary particles which exhibit uniformity of primary particle sizes within a given aggregate and turbostratic layering within the primary particles[334].

Carbon black exhibits a hierarchy of morphological features: particles (that is, primary particles), aggregates, and agglomerates. While the fundamental building block of carbon black is the primary particle, they almost never exist in isolation, but are strongly fused by covalent bonds into aggregates.

NOTE The one exception to this general characteristic of manufactured carbon black is thermal black, in which primary particles can exist in isolation and the primary particle sizes within an aggregate are not necessarily uniform.

The primary particles are conceptual in nature, in that once the aggregate is formed the primary particle no longer exists, they are no longer discrete and have no physical boundaries amongst them. Once produced, individual aggregates join together by van der Waals forces to form agglomerates. Agglomerates do not break down into smaller components unless adequate force is applied (e.g. shear force). Primary particle and aggregate sizes are distributional properties and vary depending on the carbon black grade. Transmission electron micrographs demonstrate that while primary particle and aggregate sizes vary greatly within a given grade of carbon black, the primary particle size is essentially uniform within an individual aggregate.

Regarding the morphological attributes, carbon black is placed on the market typically in an agglomerated state. Agglomerate size typically range from 1 µm to 100+ µm. The carbon black's smallest dispersible unit is the aggregate. Aggregates typically range from 85 nm to 500 nm.

Approximately 90 % of carbon black is used in rubber applications, with the remainder used as an essential ingredient in hundreds of diverse applications, such as plastics, pigments, and coatings[335].

A.1.3 Carbon nanofibres and carbon scrolls

Carbon nanofibre (CNF) is a nanofibre composed of carbon[330]. Carbon nanofibres (CNFs) are cylindrical or conical structures that have diameters ranging from a few to one hundred nanometers and lengths ranging from under micrometer to several millimetres. The internal structure comprises stacked curved graphite layers (or graphene sheets, see also A.1.5) that form cones (herringbone structure), cups (bamboo structure), rods (solid structure), or tubes (hollow structure)[336]. The main distinguishing characteristic of nanofibres from nanotubes is the stacking of graphene sheets which make a non-zero angle with the fibre axis. When graphene sheets are parallel to the fibre axis, they form carbon nanotubes (see next subclause). Since there are “in-plane” and “interplane” components of transport and mechanical properties along the fibre axis, as well as presence of unsaturated bonds similar to graphite, carbon nanofibre characteristics differ from those of carbon nanotubes.

Carbon nanofibres are produced during chemical vapour deposition processes from carbon rich gases such as hydrocarbons over metal catalysts[337]. A greater control over carbon nanofibre structure and composition can be achieved with catalytic plasma-enhanced chemical vapour deposition[338]. Carbon nanofibres are produced on an industrial scale and find applications as polymer additives, gas storage materials and catalyst supports[339].

Carbon scrolls are formed structurally by rolling up a single layer of graphene and exhibit distinct properties from those of graphene and carbon nanotubes[340]. They have been produced using various methods, such as arc-discharge, high-energy ball milling of graphite, extended sonication of intercalated graphite, microwave-assisted exfoliation of graphite in liquid nitrogen, chemical vapour deposition, Langmuir-Blodget method and molecular combing[341]. Potential applications in materials storage and transport[342] and nano-mechanical devices[343] have been reported.

A.1.4 Carbon nanotubes

Carbon nanotube (CNT) is a nanotube composed of carbon (usually consisting of curved graphene layers), including single-wall carbon nanotubes and multiwall carbon nanotubes[330]. Carbon nanotubes (CNTs) represent a diverse family of carbon-based materials based on a graphene sheet rolled up in the form of a tube. CNTs can be made up of one sheet (Single-Wall) or several concentric sheets (MultiWall). Single-wall CNT can be open- or closed-ended depending on whether they are capped with fullerene halves at each end. Carbon nanotubes can have a diameter as small 0,4 nm and reach several centimeters in length[344][345]. Multiwall form can exceed 100 nm in diameter[346].

Single-wall carbon nanotubes display metallic or semiconductive properties depending on how the graphene sheet is rolled up, and their electronic response can be tuned using elemental substitution[347]. Carbon nanotubes have been predicted to be as much as 60 times stronger than steel and six times lighter[348]. They are considered excellent heat and electric conductors, have a great capacity for molecular absorption and are chemically and thermally very stable[349].

Applications which are currently being investigated include; polymer and inorganic material composites, electromagnetic shielding, electron emitters, super capacitors, batteries, hydrogen storage, energy conversion, catalysis, sensors and structural composites. Main synthesis methods for carbon nanotubes fall into two classes: those in which elemental carbon is vaporized typically by a laser or an electric arc and those in which the carbon is derived at lower temperature from a carbon source usually assisted by a catalyst or plasma[5][350].

Commercial manufacturing and supply of carbon nanotubes at a large-scale production rate appears to be taking place in a number of countries. Though tremendous progress has been made in growing CNTs, there are still many challenges in growing CNTs with desired properties especially the control of chirality.

A.1.5 Graphene nanosheet

Graphene is a single layer of carbon atoms with each atom bound to three neighbours in a honeycomb structure. It is an important building block of many carbon nano-objects[330]. Graphenes are a family of materials that can have differing numbers of layers. For example, there are bilayer, trilayer, and few layer graphenes. Microscopic roughening through out-of-plane deformations makes graphene sheet effective thickness of about 1 nm. Graphene was shown to possess unique electronic, magnetic, optical and mechanic properties and might find applications in flat flexible electronic devices and coatings[351]. Since graphene was first isolated from graphite by a mechanical exfoliation method, a number of synthesis approaches have been explored to prepare graphene thin films, such as liquid-phase exfoliation, reduction of graphene oxide, epitaxial growth on silicon carbide, molecular assembly and chemical vapour deposition[352]. Graphene sheets can be derivatized by carboxylic acid at the edges, and phenol, hydroxyl and epoxide groups mainly at the basal plane, resulting in a material, broadly classified as graphene oxide[353].

A.2 Oxides

New oxide nanomaterials with a variety of physicochemical features and morphologies are continually being reported. Synthetic methods of oxide nanomaterials include hydro- and solvothermal methods, microwave-assisted strategies, sonochemical methods, using ionic liquids, as well as combined synthetic approaches. Applications for oxide nanomaterials can range from nanomedicine (e.g. targeting of tumours through magnetic oxide nanoparticles); cosmetics; food packaging; nanosensors; and catalysis[355].

Nanostructured metal oxides have been found to exhibit interesting morphological, functional, biocompatible, non-toxic and catalytic properties. Many nanostructured metal oxide materials have potential applications in biosensors due to their unique properties to interface with biomolecules (e.g. optical, electrical, surface charges, morphology, particle size, surface area, functionality, adsorption capability, electro-transfer properties). In addition, their optical, electrical and magnetic properties can be enhanced through the incorporation of other nanomaterials such as carbon nanotubes, graphene, gold, silver and quantum dots[356].

Metal oxide nanostructured materials in the form of agglomerated and aggregated nanoparticles are often coated to achieve desired properties (see Figure A.1). Main production methods are spray pyrolysis, laser ablation and solution phase synthesis.

Metal oxide nano-objects can be grown with a variety of simple shapes such as nanorods, nanotubes[357], nanoflakes, and more complex structures such as nanobrushes, nanosprings, and nanobelts[358]. These nanostructures exhibit unique electronic properties and can find novel applications in optoelectronics, sensors, transducers, and medicines.

Synthetic amorphous silica can be manufactured as a nanostructured material via gas-phase synthesis or wet chemical processes, such as precipitation or sol-gel process. The nanostructured material consists of primary particles within a range of 5 nm to 10 nm forming hard aggregates (~0,1 µm to 1 µm). Primary particles do not exist as individual units; aggregation and agglomeration are predominant in particle formation and growth. Synthetic amorphous silica is currently used in a wide variety of industrial applications. Most of them are related to the reinforcement of various elastomers, the thickening of various liquid systems, the free-flow of powders or as a constituent of matting, absorbents and heat insulation material[359][360].

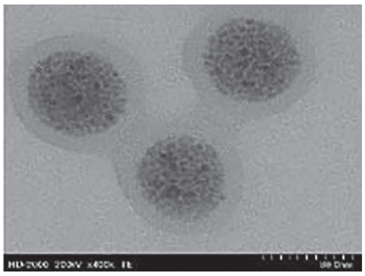


Figure A.1 — Example of oxide nanomaterials – Transmission electron micrograph of aggregated iron oxide particles (based on Figure A.5 from Reference [354]) Image courtesy of National Research Council Canada

A.3 Metals

Gold nano-objects are one of the most extensively studied metal nano-objects. Gold and silver nano-objects are characterized by a prominent optical resonance in the visible range (see Figure A.2(a)), which is sensitive to environmental changes, size, and shape of the particles as well as to local optical interactions in resonant systems. These unique properties are utilized in a number of applications such as optical markers and as hyperthermal cancer treatment agent in medicine. Silver nano-objects are produced in largest volumes among metal nano-objects and used in numerous applications ranging from wound dressings to washing-machine disinfectant for its antimicrobial activity[361].

Metal nano-objects with well-defined size and shape can be synthesized using metal reduction from a solution phase[362]. Among the various solution-phase methods, polyol reduction is the best established for generating silver and gold nano-objects with controllable shapes and optical properties[363].

Metal nanowires such as cobalt, gold and copper-based can be conductive or semiconductive and could be used as interconnectors for the transport of electrons in nanoelectronic devices (see Figure A.2(b)) [5]. Nanowires are typically manufactured by involving a template followed by the deposition of a vapour to fill the template and grow the nanowire[5]. Deposition processes currently include Electrochemical Deposition and Chemical Vapor Deposition. The template might be formed by various processes including etching, or the use of other nanomaterials such as nanotubes[5]. A recent focus has been on the combining two or more nanomaterials to achieve increased multifunctionality as well as creating opportunities for next generation materials with enhanced performance[364].

(a) Solutions of gold-silver alloy particles (per-cent of gold content)

(b) Scanning electron micrograph of barcode nanowires (multi-segmented nanowires)

Figure A.2 — Examples of metal nanomaterials (based on Figure A.6 from Reference [354]). Image courtesy of National Research Council Canada

A.4 Quantum dots

Quantum dots are crystalline nanoparticles that exhibit size-dependent properties due to quantum confinement effects on the electronic states[363]. Spherical nanocrystals from 1 nm to 14 nm in diameter composed of group II and VI (e.g. CdSe), group III and V (e.g. InP) and group IV (e.g. Si) semiconductor materials often possess unique electronic properties due to quantum confinement effects, when exciton Bohr radius becomes comparable with particle size, hence they are often called quantum dots (see Figure A.3)[365]. The electronic band gap of the quantum dots is inversely proportional to their crystal size and thus the emitted light can be adjusted throughout the whole UV-VIS-NIR range[231][366]. Negligible photobleaching, broad excitation, and narrow size-tunable emission spectra are some of their attractive properties[367].

Quantum dots are used, among other purposes, as fluorescent probes in diagnostic medical imaging and in therapeutics; they are used for these purposes due to their optical properties and our ability to coat and modify their surfaces with peptides, antibodies, nucleic acids and other biologically important molecules[365][368].

Currently, chemistry, physics and material science have provided methods for the production of quantum dots and are allowing tighter control on factors such as particle size and shape, toxicity and emission properties. The most common method to produce quantum dots is by wet chemical colloidal processes[5].

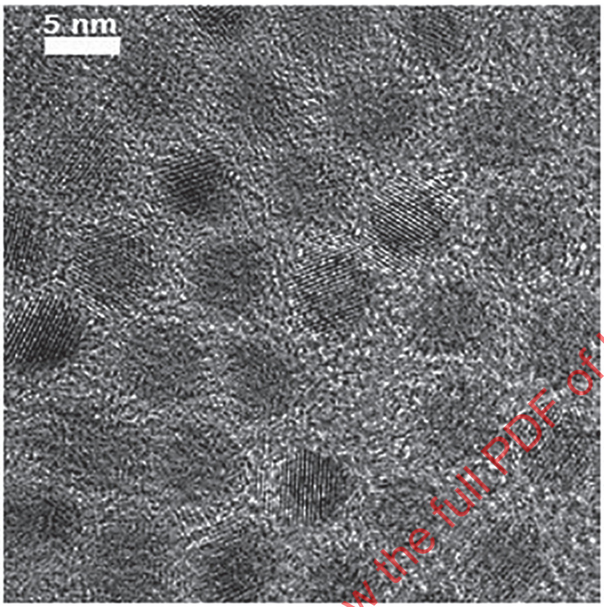


Figure A.3 — Examples of quantum dots –Transmission electron micrograph (TEM) of PbSe quantum dots (based on Figure A.7 from Reference [354]). Courtesy of National Research Council Canada

A.5 Organic polymer nanomaterials

A.5.1 Dendrimers

Dendrimers are a new class of controlled-structure multi-branched polymers with nanoscale dimensions. Dendrimers are molecules comprising a multifunctional molecule with a dendritic wedge of highly branched monomers regularly attached to each functional site, leading to a monodisperse, tree-like or generational structure[369]. They allow precise, atomic-level control of the synthesis of nanostructures according to the desired dimensions, shape and surface chemistry. They can display both hydrophilic and hydrophobic characteristics and can accommodate a wide variety of functional groups for medical applications. They are finding an increasing use in the medical and biomedical field, in particular, as a drug delivery platform[370][371][372].

Dendrimer nanostructures are of particular interest in the field of drug delivery due to their physical, chemical and structural properties as well as their ability to overcome biological issues to reach the intended target(s)[371]. Dendrimers are core-shell nanostructures, From their core central structure, branches of other atoms called ‘dendrons’ grow through a variety of chemical reactions[373]. The ability to tailor dendrimer properties makes them ideal carriers for biomolecules and small molecule drugs. Main properties of dendrimers include

(i) nanoscale container properties (i.e. encapsulation of a biomolecule/drug),

(ii) nano-scaffolding properties (i.e. surface adsorption or attachment of a biomolecule/drug), and

(iii) biocompatibility[374].

Most syntheses of dendrimers involve the repetitious alternation of a growth reaction and an activation reaction such as the more traditional Michael reaction, or the Williamson ether synthesis, and more modern solid-phase synthesis, organo-metallic chemistry, organo-silicon chemistry, and organo-phosphorus chemistry[375][376].

The properties of dendrimer-nanoparticle composites are primarily determined by the properties of the nanoparticles. However, the dendrimer, functional groups or dendrimer-nanoparticle arrangement can create new properties. Applications for dendrimer-nanoparticle composite include catalysis, biomedical research and electronic devices[377].

A.5.2 Polymer fibres

Nanofibres can be made of a wide variety of polymeric materials. The main manufacturing techniques are electrospinning and gas-blowing. These techniques allow for great flexibility in controlling chemical composition and physical parameters such as fibre diameter and length. Nanofibre scaffolds can be used in a number of applications such as sensors and ultrafiltration devices for liquid and gas phase[378]. Biodegradable polymer nanofibres can find numerous applications in medicine as scaffolds for tissue engineering, in controlled drug release, wound dressings, molecular separation, and bone restoration[379].

A.6 Bio-inspired materials

Bio-inspired nanotechnology (sometimes known as “biomimetic nanotechnology”) uses principles found in biology for the design and/or fabrication of nanomaterials, nanoscale devices or nanoscale systems[380]. The unique properties and functions of bio-inspired materials are partly due to the fact that living biological systems are made up of nanoscale self-assembly of biological molecules.

The dimensional similarities between nanomaterials and biomolecules (or DNA) suggest that the integration of biomolecules with various nanomaterials might generate hybrid systems with novel properties. Such systems can include biomolecule-nano-objects (e.g. Quantum Dots, nanotubes, etc.) hybrids with new tailored functionalities[381].

One class of bio-inspired nanomaterials is biological templates. Biological templates are structures that behave like containers (e.g. viral capsids, protein cages). They act as carriers for substances such as biological materials, biomolecules and drugs. Another class of bio-inspired materials is biomimics. Biomimic nanomaterials are inspired by engineering principles in nature and involve designing nanomaterials to mimic biological processes and properties[382].

Emerging applications for bio-inspired nanomaterials include improved adhesion, superhydrophobicity, antireflection and other optical properties, hardening surfaces, antibiofouling, changing colours, self-healing, novel sensory systems, sensors, as well as nanoscale/bioelectronic devices[381][383][384][385]. More recently, bio-inspired nanomaterials can be potentially incorporated into energy, environment and medicinal applications[382][386].

Bio-inspired nanomaterials include materials in which biological substances can be trapped, encapsulated or adsorbed on the surface. They include a wide range of engineered assemblies of biological building blocks such as lipids, peptides, polysaccharides and lipoproteins[387] utilized as carriers for drugs, receptors, nucleic acids and imaging agents. Examples are polymeric micelles, protein cage architectures, viral-derived capsid nanoparticles, polyplexes, and liposomes[376] used in transport and optimal targeting of drugs. A number of formulations are under development for drug delivery via gastrointestinal and inhalation routes and skin applications.

Micelles are formed in solution as aggregates in which amphiphilic molecules are arranged in a spheroidal structure with hydrophobic cores shielded from the water by a mantle of hydrophilic groups. These dynamic systems, which are usually below 50 nm in diameter, are used for the systemic delivery of water- insoluble drugs. Drugs or contrast agents might be trapped physically within the hydrophobic cores or can be linked covalently to component molecules of the micelle[388].

Liposomes are closed lipid bilayer vesicles that form by hydration of dry phospholipids. Drug molecules can be either entrapped in the aqueous space or intercalated into the lipid bilayer of liposomes, depending on the physicochemical characteristics of the drug. The liposome surface is amenable to modification with targeting ligands and polymers[388].

Polyplexes are assemblies, which form spontaneously between nucleic acids and polycations or cationic liposomes (or polycations conjugated to targeting ligands or hydrophilic polymers), and are used in transfection protocols. The shape, size distribution, and transfection capability of these complexes depends on their composition and charge ratio of nucleic acid to that of cationic lipid/polymer. Examples of polycations that have been used in gene transfer/therapy protocols include poly-L-lysine, linear- and branchedpoly(ethylenimine), poly(amidoamine), poly-ß-amino esters, and cationic cyclodextrin[388].

Protein cage architectures and viral-derived capsid nanoparticles are formed by self-assembly of certain proteins[388].

Building blocks of bio-inspired nanomaterials can be obtained from natural materials and using synthetic techniques[389]. Supramolecular self-assembly is another approach that can be used to develop bio- inspired nanomaterials that involved small organic molecules, peptides or proteins, and viruses. Potential applications include drug or gene delivery, and tissue engineering[390].

A.7 Cellulose nanomaterials

Cellulose nanomaterials (CNMs) are a family of nanomaterials (nano-objects and nanostructured materials) derived from nanostructures present in natural cellulose. Cellulose nanomaterials extracted from cellulose sources are renewable, biodegradable, sustainable, and retain the biocompatibility of cellulose. Such nanomaterials are either extracted by breaking down the cellulosic feedstocks such as wood, annual plants, or other cellulose sources including algae and tunicates, or produced directly by living organisms such as bacteria. For example, cellulose nanomaterials can be manufactured by conversion of wood pulp through chemical, biological or mechanical processes [see Figure A.4][391][392][393][394][395][396]. Potential applications for cellulose nanomaterials include, but are not limited to, paper, building materials, composites, optical films and devices, catalysts, pharmaceuticals, healthcare, defence, electronics, automobiles and aircraft[391][392][393][394][395][396].

The family of cellulose nanomaterials can be subdivided into different categories, such as (but are not limited to) the following (based on ISO/TS 20477:2017)[392]:

a) Nano-objects

  i) cellulose nanofibril is a cellulose nanofibre composed of at least one elementary fibril, containing crystalline, paracrystalline and amorphous regions, with aspect ratio usually greater than 10, which can contain longitudinal splits, entanglement between particles, or network-like structures.

  ii) cellulose nanocrystal (CNC) is a nanocrystal predominantly composed of cellulose with at least one elementary fibril, containing predominantly crystalline and paracrystalline regions, with aspect ratio of usually less than 50 but usually greater than 5, not exhibiting longitudinal splits, inter-particle entanglement, or network-like structures.

b) Nanostructured materials

  i) cellulose nanostructured material is a nanosctructured material of which the internal or surface nanostructure is predominantly composed of cellulose.

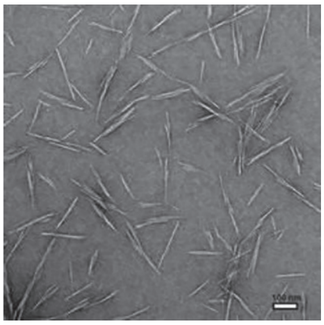


Figure A.4 — Example of cellulose nanomaterials — TEM image of cellulose nanocrystal (CNC) derived from wood pulp (based on Figure 1, from Reference [391]). Image courtesy of National Research Council Canada

Annex B

(informative)

Nanomaterial-specific animal and cell culture toxicity studies

B.1 Carbon nano-objects and related carbon nanoscale materials

B.1.1 Fullerene

Fullerene is defined as molecule composed solely of an even number of carbon atoms, which form a closed cage-like fused-ring polycyclic system with 12 five-membered rings and the rest six-membered rings[330]. Acute oral and inhalation toxicity of fullerene has been studied in experimental animals[34]. An acute oral study conducted for fullerene based on OECD TG 474[397] showed no mortality at maximum experiment concentration (88 mg/kg bw)[34]. Dermal irritation/corrosion of studies based on OECD TG 404[397] of fullerene did not cause irritation and corrosion to skin. Eye irritation and corrosion studies conducted for based on OECD TG 405[397] did not cause eye irritation and corrosion[34]. Skin sensitization study based on OECD TG 406[397] for fullerene showed slightly irritating effect[34]. Fullerene has been tested subacutely based on OECD TG 407[397]. Although there was decrease in albumin in males and increase of total protein in females, there was no histopathoglogical change. NOAEL of repeated oral dose toxicity of the fullerene was considered to be 1 000 mg/kg bw/day. Fullerene was tested for repeated dose toxicity through inhalation subacutely. There was only slight inflammatory response in the lungs at test level (0,12 mg/m^3) of fullerene. Fullerene tested for *in vitro* genotoxicity based on OECD TG 471[397] was negative for bacterial reverse mutation assay with or without presence of metabolic system.

NOTE For NOAAs, studies with the Ames test (TG 471) and *in vitro* bacteria might not be appropriate for identifying genotoxicity as uptake of the NOAAs by the bacteria is doubtful. So, a negative outcome in studies with the Ames test and *in vitro* bacteria has no meaning as DNA exposure to the NOAA can be lacking, only a positive outcome can be interpreted.

Chromosomal aberration tests conducted for fullerene was negative. *in vivo* genotoxic studies for fullerene based on OECD TG 474 Mammalian Erythrocyte Micronucleus Test[397] were negative. *in vivo* comet assay, fullerene showed negative response (summarized from Reference [34], dossier fullerene).

B.1.2 Carbon nanotubes

B.1.2.1 Summary

A number of toxicological studies of CNTs have been performed in recent years (see references cited in References [32], [398], [399]). These studies have suggested that the biological response to CNTs in cell-culture and animal studies can vary widely depending on the test material and the test method applied. The nature of the test material depends on the method of production and post-production treatment resulting in different levels of impurities (metals, organic molecules, other forms of carbon, support material etc.) and different structures (atomic structure, number of walls, agglomeration state, etc.) and geometries (diameter, length, deformations) of carbon nanotubes. Therefore it is essential that certain physical characteristics of a test material are determined and reported in conjunction with any investigation of their hazards[400].

Biomarkers of oxidative stress were elevated in an *in vitro* assay after incubation with single-wall carbon nanotubes[58]. SWCNTs purified by acid treatment have been shown to produce adverse effects, including acute inflammation with early onset, yet progressive, fibrosis and granulomas in the lungs of mice after a single pharyngeal aspiration. Although these results, obtained by pharyngeal aspiration, suggest some hazardous potential of SWCNTs by inhalation, the actual human inhalation toxicity of SWCNTs has not been determined yet.

In October 2014, the International Agency for Research on Cancer (IARC) classified MWCNT-7 (length, 1 µm to 19 µm; diameter, 40 nm to 170 nm) as being possibly carcinogenic to humans (Group 2B); and SWCNTs and MWCNTs excluding MWCNT-7 were categorised as being not classifiable as to their carcinogenicity to humans (Group 3)[401].

B.1.2.2 SWCNTs

Acute oral and inhalation toxicity of three SWCNTs have been studied in experimental animals. Acute oral study conducted for SWCNT based on OECD TG 423[397] showed no mortality with LD 50 > 50 mg/kg/bw[402]. Maximum concentration could not be used due to high volume per mass. Other acute oral toxicity tests were done together with *in vivo* micronucleus test based on OECD TG 474[397]. In these case SWCNTs were dosed once per day for two days. SWCNTs did not show any mortality up to 20 mg/kg/day and LD 50 > 200 mg/kg bw, respectively[403]. Only one study on acute inhalation toxicity was conducted to C57BL female mice with one concentration at 5,52 ± 1,37 mg/m^3 for four days (5/d). No mortality was observed. They reported that SWCNT inhalation was more effective than aspiration in causing inflammatory response, oxidative stress, collagen deposition, and fibrosis as well as mutations of K-*ras* gene locus in the lung of mice[404]. Since SWCNTs were very difficult to produce large amount as produced as MWCNTs, it is very difficult to conduct acute toxicity test based on the test guideline as described as maximum concentration 5 g/m^3.

Dermal irritation/corrosion of studies based on OECD TG 404[397] of SWCNTs did not cause irritation and corrosion to skin[405]. Another study using immune-competent hairless SKH-1 mice caused dermal toxicity with induction of free radical generation, oxidative stress, and inflammation[406]. Eye irritation and corrosion studies conducted for SWCNTs based on OECD TG 405[397] did not cause eye irritation and corrosion. Two SWCNTs were studied for sensitization based on OECD TGs 406 and 429[397] and had no sensitizing effect[405].

There have also been studies on the effects of exposure to carbon nanotubes on the skin and eyes. An *in vitro* study reported that unpurified single-wall carbon nanotubes caused a significant decrease in cellular viability and biomarkers of oxidative stress with a dose-response relationship, as well as a significant increase in lipid peroxides on human epidermal keratinocytes[407]. This study concluded that dermal exposure to unrefined single-wall carbon nanotubes might lead to dermal toxicity in exposed workers. Another *in vitro* study using human fibroblasts and keratinocytes indicates that single-walled CNTs, functionalized with peptides, are capable of penetrating the cell membrane[408]. However, the application of a filter saturated with a solution containing fullerene soot with a high content of single-walled CNTs during a patch test did not cause irritation or allergies in volunteers[409]. Ocular instillation of an aqueous suspension of nanotubes in a modified Draize test with rabbits did not cause irritation[409].

SWCNTs have been tested subacutely based on OECD TG 407[397] for MWCNT with compliance to GLP[402]. They found no treatment related changes of body weight, behavioural and blood biochemical parameters were observed. NOAEL of repeated oral dose toxicity of the SWCNT was considered to be 12,5 mg/kg bw/day. SWCNT exposed up to 0,4 mg/m^3 induced increased neutrophil cells in blood at 3 mo after the administration in the high concentration group. Neither the low concentration exposure group nor the high concentration exposure group showed increase of the pulmonary wet weight, the infiltration of the inflammatory cell and increase of the HO-1 gene. SWCNT exposed up to 0,13 mg/m^3 did not induce neutrophil inflammation in the lung under the conditions in the present study. NOAEC 0,13 mg/m^3 were suggested.

Several SWCNTs were tested for *in vitro* genotoxicity based on OECD TG 471[397].

NOTE For NOAAs, studies with the Ames test (TG 471) and *in vitro* bacteria might not be appropriate for identifying genotoxicity as uptake of the NOAAs by the bacteria is doubtful. So, a negative outcome in studies with the Ames test and *in vitro* bacteria has no meaning as DNA exposure to the NOAA can be lacking, only a positive outcome can be interpreted.

All SWCNTs were negative for bacterial reverse mutation assay with or without presence of metabolic system[403][410]. Chromosomal aberration tests conducted for several SWCNTs were negative[403][410]. *in vitro* mammalian cell micronucleus tests conducted for some SWCNTs were positive for two SWCNTs[411][412]. *in vitro* comet assay conducted for several SWCNTs resulted in contradicting positive[410][412][413]

[414][415][416][417] and negative results[413]. *in vivo* genotoxic studies for SWCNT (Nikkiso) and SWCNT (Super growth C100) based on OECD TG 474 Mammalian Erythrocyte Micronucleus Test[397] were negative for both SWCNTs[403]. *in vivo* DNA damage and/or repair, mouse spot tests were positive for SWCNT (CNI) and SWCNT (Elicarb)[418][419]. Interestingly SWCNT (Elicarb) were only positive for intratracheal administration which is comparable to SWNCTs exposure route and negative for oral administration[420]. Although SWCNT (CNI) were negative in *in vitro* standard tests such as bacterial reverse mutation assay and chromosomal aberration test, they were positive for mitochondrial DNA damage[418], increased K-*ras* mutation[404] and induced mitotic spindle disruption[421].

Developmental toxicity and teratogenicity study was conducted in CD-1 mice. SWCNTs were injected intravenously at 0,01, 0,1, 0,3, 3,3 µg/mouse in the retorbulbar venous plexus of pregnant females at postcoital day 5,5. AT 155 DPC, animals were sacrificed and foetus and placentas were analysed. At 30 µg/mouse dose, SWCNTs but not nCB (carbon black) were able to induce gross fetal morphological abnormalities. In addition, a substantial percentage of SWCNTs exposed mice (ranging from 19 % to 31 %) presented swollen uteri (at least twice the diameter of a nonpregnant normal uterus) with no developed embryos a finding never observed in control female mice. A wide variety of malformations was found, but no differences in their type and severity were observed among the three groups of CNTs and among different concentrations within each group. In some cases the foetuses appeared morphologically normal, but significantly growth retarded; more often, foetuses with abdominal wall or head deformities or limb hypoplasia were observed. In more abnormal foetuses, a severe retardation in the development of several organs and tissues was associated with an abnormal torsion of the trunk or the body plan was profoundly affected and the outlines of the foetus could be barely identified. They suggested that exposure to SWCNTs can represent a potential risk for pregnant women, especially in the occupational setting, where the risk of accidental exposure might become real in light of the foreseen increased production of ENM in the near future[422].

Carcinogenicity of SWCNT (CNI) were tested in male SD rat with single intratracheal injection of SWCNTs. Eight-week old SD rats were intratracheally instilled with SWCNT (AIST) and sacrificed at 24 h, 3 d, 1 wk, 1 mo, and 3 mos. In a second experiment, rats were administered a single dose of 1 mL/kg bw of a 0 (vehicle), 0,04, 0,2, or 1,0 mg/mL solution of SWCNT in Tween 80 (doses corresponding to 0,0, 0,4, 0,2 and 1,0 mg/kg bw), and sacrificed at 3 d, 1 wk, 1 mo, 3 mos, and 6 mos. No tumours were found in any group[423]. The experiment was too short to observe any tumours. Groups of six F344 was injected a single dose of SWCNT at 10 mg/rat intraperitoneally using the “Kertai fold” model, a capsular structure constructed by the ligament of minor omentum attached to the concave-shaped inner surface of the liver lobe, then observed for 12 mos. As a negative control, zinc oxide was used. Mesotheliomas were not found but foreign body granulomatous lesions were observed in the “Kertai fold”[424]. IARC concluded that two studies with SWCNTs in rats were inconclusive and categorized as to their carcinogenicity to human as “Group 3”[401].

For SWCNTs false positive toxicity outcomes might be observed based on interaction of the NOAAs with the read out system (e.g. MTT assay)[425][426], or absorption of nutrients from the tissue culture medium.

B.1.2.3 MWCNTs

Acute oral, dermal and inhalation toxicity have been studied in experimental animals. Acute oral and dermal conducted based on OECD TGs 420, 423 and 474[397] for oral, 402[397] for dermal, and 403[397] for inhalation using various MWCNTs did not show any mortality up to 2 000 mg/kg/bw. Many oral acute toxicity study could not make maximum dose 2 000 mg/kg/bw due to large volume of MWCNTs per mass. Acute inhalation toxicity of MWCNT study based on OECD TG 403[397] did not show any mortality up to 241 mg/m^3 (the highest concentration that can be generated for Baytube).

Dermal irritation/corrosion of studies based on OECD TGs 404 and 431[397] of MWCNTs did not cause irritation and corrosion to skin. However some MWCNTs tested based on OECD TG 405[397] were irritants[405]. Several MWCNTs including MWCNT (Baytube), MWCNT (Arkema Graphistrength C100), and MWCNT (Mitsui MWCNT-7), were studied for sensitization based on OECD TGs 406 and 429[397]. The MWCNTs had no sensitizing effect.

Purified (“purified” in this context means treated with acid to remove metal contaminants) ground and intact multiwall carbon nanotubes administered intratracheally to rats were found to be biopersistent (still present in the lung after 60 d) and induced inflammatory and fibrotic reactions[72]. Pulmonary lesions induced by CNTs characterized by the formation of collagen-rich granulomas were caused by the accumulation of large CNT agglomerates in the airways. Ground CNTs were better dispersed in the lung parenchyma and also induced inflammatory and fibrotic responses. Chrysotile asbestos and carbon black were included as reference materials. As expected, asbestos induced inflammatory and fibrotic reactions while carbon black only showed inflammatory reaction[72].

MWCNTs have been tested based on OECD TG 407 and 420[397] (it is a fixed dose method for acute toxicity study but was modified for repeated dose) for 28 d oral toxicity and showed no toxic effect up to 0,5 mg/kg bw/day for MWCNT (Nanocyl NC 7000) and 50 mg/kg bw/day for MWCNT (Nikkiso)[402].

Six kinds of MWCNTs were tested for repeated dose toxicity through inhalation toxicity subacutely and subchronically. MWCNT (Baytube) exposed up to 6 mg/m^3 induced lung damages including upper respiratory tract (goblet cell hyper- and/or metaplasia, eosinophilic globules, focal turbinate remodelling) and the lower respiratory tract (inflammatory changes in the bronchiolo-alveolar region, increased interstitial collagen staining). Granulomatous changes and a time-dependent increase of a bronchiolio-alveolar hyperplasia occurred at 6 mg/m^3. The suggested NOAEL was 0,1 mg/m^3. MWCNT (Archema graphistrength C100) were used to expose animals up to 1,3 mg/m^3 for 5 d and allowed to recover for 28 d. There were slight increase in neutrophil count was observed at 1,30 mg/m^3. Protein levels were increased middle and high dose after exposure and four week recovery and macrophage infiltration was observed at high dose after five day exposure and recovery. Hypertrophy of the bronchial and bronchiolar cells was observed at the high dose after exposure and recovery. Although it is difficult to categorize hazard data from short-term inhalation study, relative low concentration of MWCNTs caused lung inflammation. MWCNT (Nanocyl NC 7000) were used to expose animals up to 2,5 mg/m^3 for 90 d. MWCNT induced, lung weight increase, pronounced multifocal granulomatous inflammation, diffuse histiocytic and neutrophilic inflammation, and intra-alveolar lipoproteinosis even at 0,1 mg/m^3, there was still minimal granulomatous inflammation in the lung and in lung-associated lymph nodes. NOAEL would be lower than 0,1 mg/m^3. MWCNT (Nikkiso) were used to exposed animals only 0,37 mg/m^3 for 90 d. Although less pulmonary inflammation responses were observed comparing with intratracheal instillation, it is very difficult to make any judgement from one dose inhalation study. MWCNT (Hanwha CM-100) were tested for short-term and subacute inhalation toxicity. The 5 d short-term inhalation exposure to MWCNT increased H_2O_2 in the BAL fluid even after one month post exposure. In the 28 d exposure study with 90 d recovery study resulted that the inflammatory cytokine levels in the bronchoalveolar lavage (BAL) fluid did not show any statistically significant difference. The H_2O_2 levels in the BAL were significantly higher in the middle (0 d post-exposure) and high- (0 d and 28 d post-exposure) dose groups. The short-length MWCNTs deposited in the lung cells were persistent at 90 d post-exposure. Although there were some increases in H_2O_2 in the BAL in the both studies, distinct increases in other inflammatory biomarkers and histopathological legions were not observed. Subacute inhalation at 10 mg/m^3 exposure of MWCNT (Mitsui MWCNT-7) induced dose-dependent pulmonary inflammation and damage with rapid development of pulmonary fibrosis, and also demonstrated that MWCNT can reach the pleura after[427]. Subchronic exposure to MWCNT-7 increased lung weights increased 1,2-fold with 1 mg/m^3 and 1,3-fold with 5 mg/m^3 in both sexes compared to the controls. In the bronchoalveolar lavage fluid (BALF) analyses, inflammatory parameters were increased concentration-dependently in both sexes from 0,2 mg/m^3. Granulomatous changes in the lung were induced at 1 mg/m^3 and 5 mg/m^3 in females and even at 0,2 mg/m^3 in males. Focal fibrosis of the alveolar wall was observed in both sexes at 1 mg/m^3 or higher. Inflammatory infiltration in the visceral pleural and subpleural areas was induced only at 5 mg/m^3. The lowest-observed-adverse-effect level for respiratory tract toxicity in the present inhalation exposure study of rats was found to be 0,2 mg/m^3. Therefore NOAEL would be much lower than the lowest-observed-adverse-effect level of 0,2 mg/m^3 (summarized from Reference [34], dossier MWCNT).

Genotoxicity of MWCNTs were extensively studied in *in vitro* bacterial reverse mutation assay (based on TG 471)[428][429][430] and chromosomal aberration assay (based on TG 473)[428][429][431], and *in vitro* mammalian cell gene mutation test based on OECD TG 476[431], or non-guideline based test. MWCNTs were also tested *in vivo* micronucleus assays (TG 474)[429][430] and comet assay (OECD TG 489)[432][433]. Most studies were conducted in compliance to GLP and MWCNT were exposed intraperitoneally[430], orally[429] or inhalation[432][433]. All MWCNTs tested by *in vitro* bacterial reverse mutation assay were negative for genotoxicity.

NOTE For NOAAs, studies with the Ames test (TG 471) and *in vitro* bacteria might not be appropriate for identifying genotoxicity as uptake of the NOAAs by the bacteria is doubtful. So, a negative outcome in studies with the Ames test and *in vitro* bacteria has no meaning as DNA exposure to the NOAA can be lacking, only a positive outcome can be interpreted.

Baytubes, graphistrength, and Hanwha CM-100 evaluated by *in vitro* chromosomal aberration test were negative and graphistrength and Hanwha-CM-100 evaluated by *in vitro* mammalian cell gene mutation test were negative. Nikkiso MWCNT, and MWCNT-7 evaluated by chromosomal aberration were positive and MWCNT-7 evaluated by *in vitro* mammalian cell gene mutation test negative but positive with *in vitro* mammalian cell micronucleus test. MWCNT (Nikkiso) exposed orally[429], MWCNT (Hanhwa CM-100) exposed intraperitoneally[430] and MWCNT-7 administered orally[429] evaluated by *in vivo* mammalian erythrocyte micronucleus test were negative but MWCNT (Hanhwa CM-100) exposed by inhalation acutely or subacutely were positive to lung cells with comet assay[432][433].

Reproductive and developmental toxicity was studied in ICR mouse and SD rat based on OECD TG 414[397]. The study conducted by Fujitani et al. (2012)[434] with two different routes exposure, intraperitoneal and intratracheal instillation, showed that the number of litters having foetuses with external malformations and that of litters having foetuses with skeletal malformations were both increased in proportion to the doses of MWCNT. Although Authors suggested that MWCNT has a potency of teratogenicity at least under the present experimental conditions, the doses used in this study in consideration of large volume per mass were so extreme that it could cause the teratogenicity. Other study conducted according to OECD TG 414[397] (prenatal development toxicity study) showed that the repeated oral dose of MWCNTs during pregnancy induced minimal maternal toxicity and no embryo-fetal developmental toxicity at 1 000 mg/kg bw/d in rats. The NOEL of MWCNTs is considered to be 200 mg/kg bw/d for dams and 1 000 mg/kg bw/d for embryo-fetal development (summarized from Reference [34], dossier MWCNT).

There have been a few toxicity studies of functionalized carbon nanotubes. Numerous effects have been reported and the toxicity seems to vary greatly with the nature of the functional groups. An *in vitro* study with lung tumour cells showed that the toxicity of unpurified multiwall carbon nanotubes and nanostructured carbon black obtained by grinding of graphite increases after their surfaces are chemically functionalized with carbonyl, carboxyl and hydroxyl groups[435]. On the other hand, another *in vitro* study using cultured human dermal fibroblasts showed that cytotoxicity of purified single-wall carbon nanotubes functionalized with phenyl-SO_3H and phenyl-$(COOH)_2$, decreases as the degree of functionalization increases[84]. *in vivo* mice studies showed that water-soluble, single-walled CNTs functionalized with diethylenetriaminepentaacetate and labelled with indium (^{111}In) for imaging purposes, were not retained in either liver or spleen and were rapidly cleared from systemic blood circulation through the renal excretion route after intravenous administration[436].

Especially for the various forms of carbon nanotubes there has been the concern for the induction of mesothelioma similar to brown/blue asbestos fibres. This concern also would be proper for any other fibre-like nanomaterial. Certain types of MWCNT, with characteristics like length > 20 µm, rigid and non-degradable and thus persistent, induced in an animal model similar inflammatory responses as asbestos while more curled/tangled MWCNT did not[68]. Also in *in vivo* animal studies MWCNT were found to induce tumours in mice and rats[66][67]. In contrast in a two year follow up study MWCNT did not induce tumours after a single intraperitoneal injection[69]. These latter MWCNT were not rigid and had a more tangled/curled appearance.

Carcinogenicity of MWCNT (Nanocyl) were tested in male Wistar rat with single injection of MWCNTs with defect and without defect at two doses. They found that the incidence of tumours other than mesothelioma was not significantly increased across the groups[69]. MWCNT-7 were extensively studied using intraperitoneal, intrascrotal and inhalation routes of exposure. Takagi et al. (2008; 2012) studied carcinogenicity of MWCNT (MWCNT-7) initially relatively high doses and low doses later in p53 (±) heterozygous mice[66][437]. After the high dose intraperitoneal injection of the MWCNTs, the MWCNTs induced mesothelioma in the p53 (±) mice. Later lower dose intraperitoneal administration also induced mesotheliomas with incidences 19/20, 17/20 and 5/20, respectively. They suggested that the severity of peritoneal adhesion and granuloma formation were dose dependent and minimal in the lowest dose group. However, the time of tumour onset was apparently independent of the dose. Another study administered MWCNTs to Fischer 344 rat intrascrotal also showed similar results with mesothelioma induction[67]. Nagai et al. (2011) studied carcinogenicity using aggregated and non-aggregated, and thin and thick and tangle MWCNTs[438]. They found that thin MWCNTs (diameter ~50 nm) with high crystallinity showed mesothelial cell membrane piercing and cytotoxicity *in vitro* and subsequent inflammogenicity and mesothelioma-genicity *in vivo*. In contrast, thick (diameter ~150 nm) or tangled (diameter ~2 nm to 20 nm) MWCNTs were less toxic, inflammogenic, and carcinogenic. Thin and thick MWCNTs similarly affected macrophages. The tangled MWCNTs hardly caused any mesothelioma. Pretreatment of methylcholanthrene (MCA) to male B6C3F1 mouse prior to inhalation exposure of MWCNT-7 for 15 d at 5 mg/m^3 induced lung bronchiolo-alveolar adenomas and lung adenocarcinomas. Thus this result suggested that MWCNT exposures promote the growth and neoplastic progression of initiated lung cells in B6C3F1 mice. Rittinghausen et al. (2014) administered tailor made MWCNTs that manufactured from various carbon sources such as benzene, cyclohexane, acetonitrile with length longer than 8 µm and diameter larger than 37 nm were injected to Wistar rat intraperitoneally[439]. All tested MWCNT types caused mesotheliomas with highest frequencies and earliest appearances after treatment with the rather straight MWCNT types A and B. In the MWCNT C groups, first appearances of morbid mesothelioma-bearing rats were only slightly later. Later during the two-year study, they found mesotheliomas also in rats treated with MWCNT D that are the most curved type of nanotubes, indicating shape might not be the critical factor in induction of mesothelioma.

In 2014, International Agency for Cancer Research (IARC) reviewed carcinogenicity of CNTs. The IARC Working Group concluded that there was sufficient evidence for MWCNT-7, limited evidence for the two other types of MWCNTs with dimensions similar to MWCNT-7, and inadequate evidence for SWCNTs. Mechanistic and other data in rodents provided evidence of translocation of three types of MWCNTs (including MWCNT-7) to the pleura. Additionally, inhalation of some MWCNTs or SWCNTs induced acute or persistent pulmonary inflammation, granuloma formation, fibrosis, and bronchiolar or bronchiolo-alveolar hyperplasia in rodents. Studies in rodents and in cultured human lung or mesothelial cells showed that MWCNTs, SWCNTs, or both induce genetic lesions such as DNA strand breaks, oxidized DNA bases, mutations, micronucleus formation, and chromosomal aberrations. SWCNTs and MWCNTs also perturb the cellular mitotic apparatus, including microtubules and centrosomes, in human lung epithelial cells. As a whole, the Working Group acknowledged that the above mechanisms are all relevant to humans. However, a majority did not consider the mechanistic Evidence for carcinogenicity — especially concerning chronic end points — to be strong for any specific CNT. Furthermore, the lack of coherent evidence across the various distinct CNTs precluded generalization to other types of CNTs. Thus, MWCNT-7 was classified as possibly carcinogenic to humans (Group 2B); and SWCNTs and MWCNTs excluding MWCNT-7 were categorized as not classifiable as to their carcinogenicity to humans (Group 3)[401].

B.1.3 Graphenes

Graphene, defined as single layer of carbon atoms with each atom bound to three neighbours in a honeycomb structure[330] is a recently developed nanomaterial that possesses unique physicochemical properties, and thus a number of potential applications are expected in many areas of industry and science. The toxicity of graphene has also been demonstrated in *in vivo* and *in vitro* experimental settings, where graphene nanoplates were found to induce the expression of inflammatory cytokines in mouse lungs after a single instillation[440] and cause reduced cell viability, mitochondrial damage, and elevated autophage-related proteins in a human bronchial epithelial cell line, BEAS-2B[440]. Plus, graphene oxide has also been shown to increase cytotoxicity and apoptosis in BEAS-2B cells[441]. Other *in vitro* data have shown that low doses of graphene oxide did not induce cytotoxicity in lung epithelial cells, A549[442]. The five day exposure to graphene did not change the body weights or organ weights of the rats after the exposure and during the recovery period. No statistically significant difference was observed in the levels of lactate dehydrogenase, protein, and albumin between the exposed and control groups. However, graphene ingestion by alveolar macrophages was observed in the exposed groups. The results suggest that the five day repeated exposure to graphene only had a minimal toxic effect at the concentrations and time points used in this study[443]. A subacute inhalation study (28 d) with 90-d post-exposure period study up to 1,88 mg/m^3 indicated that the inhaled graphenes were mostly ingested by macrophages. No distinct lung pathology was observed at the 1 d, 28 d and 90 d post-exposure. The inhaled graphene was translocated to lung lymph nodes. This study suggests low toxicity and a NOAEL of no less than 1,88 mg/m^3[444].

B.2 Oxides

B.2.1 General

Experimental studies in animals have shown that at equivalent mass doses, poorly soluble nanostructured metal oxides in the form of agglomerated or aggregated nano-objects (e.g. titanium dioxide, aluminium oxide, and manganese dioxide) are more potent than larger particles of similar composition, in causing pulmonary inflammation, tissue damage, and lung tumours in animals[21] to [25][73][75][76]. For these and other poorly soluble particles, a consistent dose-response relationship is observed when dose is expressed as particle surface area[22][23][29][74][77]. These animal studies suggest that for nanostructured materials and larger particles with similar chemical properties, the toxicity of a given mass dose will increase with decreasing particle size due to the increasing surface area. In addition to particle size and surface area, other physical and chemical properties of particles are known to influence toxicity, including solubility, shape, surface reactive sites, charge, and crystal structure[6][31][79][80][445]. For poorly soluble particles of relatively low toxicity, some animal studies have identified doses that were not associated with observed adverse responses. For example, a recent animal study reported mass doses of either fine or nanostructured TiO_2 in rats at which the lung responses did not significantly differ from controls, while crystalline silica caused more severe lung responses at the same mass dose[446][447].

B.2.2 Silicon dioxide

Acute oral and inhalation toxicity of silicon dioxide have been studied in experimental animals. All acute oral study conducted for silicon dioxide based on OECD TG 401[397] showed no mortality at maximum experiment concentration and no acute toxicity hazard was assigned for silicon dioxide. Acute inhalation toxicity conducted for silicon dioxide based on OECD TG 403[397] showed no mortality at test concentrations.

Dermal irritation/corrosion of studies based on OECD TG 404[397] or US. EPA method of five types of silicon dioxide did not cause irritation and corrosion to skin. Eye irritation and corrosion studies conducted for silicon dioxides based on OECD TG 405[397] or Draize test did not cause eye irritation and corrosion. Thus, silicon dioxide might not cause dermal and eye irritation/corrosion.

Silicon dioxide (NM-200) has been tested subacutely based on OECD TG 407[397] with compliance to GLP. No significant adverse effects were observed. NOEL of repeated oral dose toxicity of the silicon dioxide was considered to be 1 000 mg/kg bw/d. silicon dioxides were tested for repeated dose toxicity through inhalation subacutely based on OECD TG 408[397]. All silicon dioxide exposed up to 4 500 mg/kg bw/d during 13 wk. No clinical symptoms or other findings including haematological, blood-chemical and urinary parameters were observed.

Precipitated silicon dioxide has been tested subacutely based on OECD TG 413[397] with compliance to GLP. Test level (35 mg/m^3) induced generally mild changes, which quickly recovered during the exposure period. NOAEC were not identified and assigned as none. Precipitated silicon dioxide has been tested subacutely based on OECD TG 412[397] with compliance to GLP. The high exposure concentration (25,2 mg/m^3) induced substance-related effects which reflect an inflammatory response of the lung tissue. These tend to disappear during recovery, but apparently not completely, but show clear sings of reversibility. Effects in the mild exposure concentration (5,39 mg/m^3) were confined to a very slight increase in the relative neutrophil count with concomitant decrease in the relative macrophage count at the day after exposure, but only statistically significant in males. NOAEC could be defined as 5,39 mg/m^3. Silicon dioxide (NM-201) has been tested subacutely based on OECD TG 413[397] with compliance to GLP. Test level (35 mg/m^3) induced generally mild changes, which quickly recovered during the exposure period. NOAEC were not identified and assigned as none. Pyrogenic silicon dioxide has been tested subacutely based on OECD TG 413[397] with compliance to GLP. Inhaled silica provokes an inflammatory response in the respiratory tract of rats, in particular the lung, at low concentration. All silica was completely cleared from the lung. The clinical sign noted was increased respiration rate.

NOAEC could be defined as 1,3 mg/m^3.. In another study silicon dioxide was tested subacutely based on OECD TG 412[397] with compliance to GLP. The mild and high exposure concentration (5 mg/m^3 and 25,2 mg/m^3) induced substance-related effects and dose-related effects which reflect an inflammatory response of the lung tissue. These tend to disappear during recovery. The lymph nodes were also affected. NOAEC could be defined as 1,39 mg/m^3.

SiO_2 was tested for *in vitro* genotoxicity based on OECD TG 471[397] and were negative for bacterial reverse mutation assay with or without presence of metabolic system.

NOTE For NOAAs, studies with the Ames test (TG 471) and *in vitro* bacteria might not be appropriate for identifying genotoxicity as uptake of the NOAAs by the bacteria is doubtful. So, a negative outcome in studies with the Ames test and *in vitro* bacteria has no meaning as DNA exposure to the NOAA can be lacking, only a positive outcome can be interpreted.

Chromosomal aberration tests conducted for NM-200, NM-201 and NM-203 were negative. *in vivo* genotoxic studies for three kinds of SiO_2 (NM-200, NM-201, NM-203) based on OECD TG 474[397]. *in vivo* DNA damage and/or repair, mouse spot tests were all negative for SiO_2.

Two generation study for was conducted for SiO_2 (NM-200) and was fed by oral to 1 000 mg/kg bw/d. It had no adverse effect on the reproductive performance of rats or on the growth and development of the offspring into adulthood, examined over two consecutive generations. One generation study for was conducted for SiO_2 and fed by oral. Clinical symptoms were not observed for parents and during lactation, behavioural or developmental or structural abnormalities were not observed (summarized from Reference [34], dossier SiO_2).

IARC evaluated amorphous silica as Group 3 (the agent is not classified as to its carcinogenicity to humans) in 1998. Among amorphous silica, synthetic amorphous silica is known as fumed silica and is a nanomaterial. Synthetic amorphous silica has inadequate evidence in animal studies to make a determination about its carcinogenic potential.

B.2.3 Titanium dioxide

Acute oral and dermal toxicities are recommended to be conducted based on OECD TGs 420, 423, and 474 for oral, 402 for dermal, and 403 for inhalation[397]. However, it was not clear whether these studies were conducted according the test guidelines. Acute oral and dermal did not show any mortality up to 10 000 mg/kg/bw. Acute inhalation toxicity study did not show any mortality up to 2,29 mg/m^3.

Titanium dioxide NOAAs did not cause irritation and corrosion to the skin[448]. Titanium dioxide NOAAs also did not cause eye irritation and corrosion based on test conducted[449].

There was no toxicological report for classifying respiratory sensitization. According to human and guinea-pig data, titanium dioxide NOAAs had no sensitizing effect on the skin.

Titanium dioxide NOAAs did not show any significant effects in 14 d repeated exposure study at 6 250, 12 500, 25 000, 50 000 or 100 000 mg/kg of oral feed[450].

Many studies indicated that titanium dioxide nanoparticles could be target organ (respiratory system) toxicity repeated exposure. First P25, including NM-105 showed the inflammatory effect and histopathological findings on the lung[22][23][27][451][452][453][454][455]. NM-104 was tested for repeated dose toxicity through inhalation sub acutely according to OECD TG 412[397] exposed up to 48 mg/m^3 affected on the hematology, clinical, organ weights, gross pathology, and non-neoplastic histopathology[453]. Exposure to 3,2 mg/m^3 approximately 20 mg/m^3 of unknown titanium dioxide nanoparticles also caused focal septal thickening due to hypertrophy and hyperplasia of epithelial cells and to mononuclear cell infiltration by eight months exposure[456]. Aerosols of titanium dioxide nanoparticle also caused desquamation of the lung tissue, interstitial coniosis, bronochitis, and emphysema by one to two month exposure[457]. In contrast, Trochimowicz et al. (1987)[458] reported that NOAEL for four weeks repeated titanium dioxide nanoparticles was 1 120 mg/m^3. Lee et al. (1985)[73] also reported that no abnormal clinical signs, body weight changes, or excess mortality, unchanged incidence and severity of neoplastic and non-neoplastic lesions.

Genotoxicity of TiO_2 (P25) *in vitro* was not clear. Genotoxicity of TiO_2 was positive in the alkaline comet assay[459] and comet assays[460][461], and genotoxicity was also positive in the single cell gel/comet assay in the in Wistar rat[462]. However, negative results were also reported. Namely, genotoxicity of TiO_2 was negative in the bacterial reverse mutation assay[463]. The effects were also negative in the comet and micronucleus assay[464]. Though genotoxicity of TiO_2 was positive in the colon and spleen of rats[462], those of TiO_2 were negative in the micronucleus assay and *in vivo* single cell gel/comet assay[462]. Genotoxicity of TiO_2 (Hombikat UV 100) was positive in the *in vitro* comet assay and micronucleus assay[465]. However, TiO_2 did not cause positive effect in the single cell gene/comet assay and micronucleus assay in the Sprague Dawley rats administrated intratracheally[462]. Seven genotoxicity studies of TiO_2 (PC105) in the single cell gel/comet assay were positive from 8 experiments, but one was positive in the micronucleus test from seven studies in the *in vitro* system[466]. Genotoxicities were negative in gene mutation. In the *in vivo* system, genotoxicity was positive only one cases in the single cell gel/comet assay from three cases. Genotoxicity were negative in the micronucleus assay and transgenic animal mutagenicity assay. Though two positive results were reported from six *in vitro* micronucleus tests, genotoxicity of TiO_2 (UV TITAN M262) were negative in the *in vivo* system[466]. There were some positive genotoxic reports on titanium dioxide. However, more negative results were reported.

Scuri et al. (2010)[467] reported that TiO_2 caused age-dependent up regulation in the expression of lung neurotrophins associated with increased airway responsiveness in neonates and weanlings but not in adults (summarized from Reference [34], dossier TiO_2).

IARC conducted carcinogenicity evaluation for pigmentary and ultrafine titanium dioxide in 2006. The carcinogenicity study by mice, by intratracheal administration in hamsters and female rats and mice, by subcutaneous injection in rats and by intraperitoneal administration in male mice and female rats. In one inhalation study, the incidence of benign and malignant lung tumours was increased in female rats. In another inhalation study, the incidence of benign lung tumours was increased in the high-dose groups of male and female rats. Cystic keratinizing lesions that were diagnosed as squamous-cell carcinomas but re-evaluated as non-neoplastic pulmonary keratinizing cysts were also observed in the high-dose groups of female rats. Two inhalation studies in rats and one in female mice gave negative results. Intratracheally instilled female rats showed an increased incidence of both benign and malignant lung tumours following treatment with two types of titanium dioxide. Tumour incidence was not increased in intratracheally instilled hamsters and female mice. Oral, subcutaneous and intraperitoneal administration did not produce a significant increase in the frequency of any type of tumour in mice or rats[468].

B.2.4 Cerium dioxide

Acute inhalation toxicity of cerium dioxide has been studied in experimental animals. There was no mortality in the study of acute inhalation toxicity of cerium dioxide. CeO_2 increased neutrophil content and biochemical parameters in broncho alveolar lavage fluid by 28 d of inhalation and NOAEL was suggested to be 1,2 mg/m^3 (summarized from Reference [34], dossier CeO_2).

B.3 Metals

B.3.1 Summary

in vitro studies indicate that some metal nano-objects can exhibit acute inflammatory effects in animals related to the ability of metal ions to generate reactive oxygen species. The dose-response relationships differ for different metals, which might relate to the physicochemical properties and mechanisms of toxicity. For example, recent studies using rat liver derived cell lines *in vitro* indicate that silver nanoparticles (15 nm and 100 nm) at 5 µg/ml to 50 µg/ml showed a significant cytotoxicity to the cells, whereas other particles such as aluminium nanoparticles (30 nm and 103 nm) and micrometer scale tungsten particles (27 µm), had no measurable effects at the same mass doses[469]. *in vitro* studies of mouse spermatogonia cell lines reported cytotoxic effects of silver nanoparticles (15 nm)[470]. Peters *et al.* studying the behaviour and viability of human endothelial cells *in vitro*, observed that cobalt and nickel nanoparticles were incorporated into the vacuoles of the cells[471]. Cobalt nanoparticles were found to cause inflammation and to be cytotoxic, while nickel nanoparticles did not produce these effects. In contrast, an *in vivo* study found nickel nanoparticles was more toxic than cobalt nanoparticles, and that the toxicity correlated with the free radical activity[472]. In other *in vivo* studies, nickel nanoparticles was found to be more toxic to the lungs than the same mass dose of micrometer scale nickel particles[473], and cobalt nanoparticles were more toxic than micrometer scale cobalt particles[474]. In an oral gavage study, copper nanoparticles caused severe toxicity and injury to the kidney, liver, and spleen, while microscale copper particles did not[475]. Colloidal gold nanoparticles, which have been developed for therapeutic and diagnostic uses, did not generate any toxicity in mice by an intravenous injection at a therapeutic dose level[476]. However, the size of the Au nanoparticles does have an influence of the organs exposed after intravenous exposure as smaller Au nanoparticles showed a more widespread organ distribution[477].

B.3.2 Silver

Acute oral, dermal and inhalation toxicity have been studied in experimental animals. Acute oral and dermal conducted based on OECD TGs 423, 401, and 402[397] using 10 nm AgNPs did not show any mortality up to 2 000 mg/kg/bw[478]. Acute inhalation toxicity study based on 403 did show any mortality up to 750 $\mu g/m^3$ (the highest concentration that can be generated) did show any mortality[479]. Skin sensitization study conducted based on OECD TG 406[397] indicated that silver nanoparticles are a weak sensitizer[478].

Silver nanoparticles have been tested based on TGs 407 and 408[397] showed wide dose responses. Ten nm AgNPs did not show significant effects in subacute and subchronic studies, showing NOAEL 250 mg/kg/bw[480]. On the contrary, 58 nm to 60 nm AgNPs GLP compliant subacute and subchronic study showed consistent hepatic toxicity with increases in serum alkaline phosphatase and cholesterol accompanying bile duct hyperplasia[481][482]. AgNPs can be a powerful intestinal secretagogue and induce an abnormal mucin composition in the intestinal mucosa[483]. NOAELs for 28 d and 90 d study as 30 mg/mg/bw were suggested.

Inhalation toxicity of silver nanoparticles were extensively studied subacutely and subchronically. Although no significant effects were observed in the subacute inhalation study based on OECD TG 412[397][484], subchronic silver nanoparticle exposure study conducted based on OECD TG 413[397][485] in compliance to GLP indicated that exposure to silver nanoparticle induced bile duct hyperplasia dose-dependently in both male and female rats and also induced mixed inflammatory infiltrate, chronic alveolar inflammation and small granulomatous lesions in lungs with decreases in the lung function parameters such as tidal volume and minute volume. The target organs for silver nanoparticles were liver and lungs and NOAEL of 133 $\mu g/m^3$. Another subchronic study exposed silver nanoparticles for 12 week and studied clearance of silver from organs and recovery of lung function parameters[486]. Male rats showed consistent lung function (tidal volume) decrease during exposure and recover period. In contrast, the female rats did not show a consistent lung function decrease either during the exposure period or following the exposure cessation. The histopathology showed a gradual recovery from the lung inflammation in the female rats, whereas the male rats in the high-dose group exhibited persistent inflammation throughout the 12 week recovery period. NOAEL 117 $\mu g/m^3$ was suggested in this study[486]. Gender difference accumulation of silver in organs including kidneys and adrenals have been several oral and inhalation repeated dose studies after silver nanoparticle repeated exposure. Female kidneys and adrenals accumulated two to three times more silver than those of male[481][482][485][486].

Genotoxicity of silver nanoparticles were studied in *in vitro* bacterial reverse mutation assay (based on OECD TG 471[397])[478] and chromosomal assay (OECD TG 473[397])[478] and *in vivo* micronucleus assays (OECD TG 474[397])[481] and comet assay (OECD TG 489[397])[487].

NOTE For NOAAs, studies with the Ames test (TG 471) and *in vitro* bacteria might not be appropriate for identifying genotoxicity as uptake of the NOAAs by the bacteria is doubtful. So, a negative outcome in studies with the Ames test and *in vitro* bacteria has no meaning as DNA exposure to the NOAA can be lacking, only a positive outcome can be interpreted.

Most studies were conducted in compliance to GLP and silver nanoparticles were exposed to orally[481] and inhalationally[487][488] in *in vivo* genotoxicity studies. All *in vitro* and *in vivo* genotoxicity studies indicated that silver nanoparticles were negative for genotoxicity and one 12-week inhalation exposure study showed a positive result at the high concentration exposure that would not be expected to be exposed to workers[487].

Reproductive and developmental toxicity was studied in Sprague-Dawley rats based on OECD TG 422[397] with GLP compliance. No reproductive toxicity to parental animals as well as offspring was found (summarized from Reference [34], dossier silver). In an *in vitro* assay nanoAg was found to inhibit differentiation of murine embryonic stem cells (embryonic stem cell assay) into contraction myocytes[489]. The 20 nm Ag nanoparticles were most potent compared to 80 nm and 113 nm Ag particles, but not as potent as ionic Ag (used as $AgNO_3$). The effect on the embryonic stem cell differentiation was observed at higher concentrations than those associated with a decrease in metabolic activity in the cells.

B.3.3 Gold

No study results were available for acute, dermal, eye irritation, and sensitization. Two studies on inhalation and one intravenous injection studies on gold nanoparticles (Mintek) were available. Two sizes of gold nanoparticles (14 nm and 95 nm) were exposed to rats 5 d inhalationally at concentration of 12,58 µg/m^3 for 14 nm and 13,7 µg/m^3 for 96 nm gold nanoparticles. Then the rats were allowed to recover for 28 d to evaluate clearance from the lungs. The results showed that size dependent clearance of gold nanoparticles from the lung, showing faster clearance of 14 nm than 95 nm. Gold nanoparticles showed biopersistence in the lung tissue and old nanoparticles did not translocate extrapulmonary to other tissues such as kidney, brain and testis. Small number of gold nanoparticle translocated from lung to liver and spleen[490].

Subchronic 90 d inhalation toxicity study on gold nanoparticles (4 nm to 5 nm) based on OECD TG 413[397] in compliance to GLP was conducted in male and female Sprague-Dawley rats with concentrations 1,85 × 10^6 particles/cm^3 (20,02 µg/m^3); 2,36 × 10^5 particles/cm^3 (0,38 µg/m^3), and 2,36 × 10^4 particles/cm^3 (0,04 µg/m^3). Among the pulmonary function test parameters, there were significant changes in tidal volume and minute volume during the 90 d of gold nanoparticle exposure ($P < 0{,}01$ to 0,05). Dosed dependent tidal volume decreases in male rats led to minute volume decreases in the high-dose animals. A tendency towards a dose-dependent decrease in the tidal volume appeared to be present in female rats, but was not statistically significant. Blood clotting time: No significant differences were found between the control and any treated animals in APPT or PT for blood clotting time. Tissue distribution of gold nanoparticles to lung, kidney, liver, blood, brain and olfactory nerve was prominent and concentration dependent. No Observed Adverse Effect Level (NOAEL) of gold nanoparticles was considered to be 2,36 × 10^5 particles/cm^3 (0,38 µg/m^3) in rats[491].

Gold nanoparticles (14 nm) were injected into tail vein of SD rats for 28 d and the rats were allowed to clear their tissue gold nanoparticles for 1 month, 2 months, and 4 months. No mortality or clinical signs were observed. No significant difference in body weight, food and water consumption was observed in any of the dose groups. No significant difference was observed in hematology and clinical chemistry in any of the dose groups. No significant gross effects were observed in any of the dose groups. No significant difference was observed in organ weight in any of the dose groups. No significant difference among dose group was observed for histopathology. Under the test conditions, the No Observed Adverse Effect Level (NOAEL) of gold nanoparticles 14 nm was considered to be 100 µg/kg bw/d in male rats (summarized from Reference [34], dossier gold).

B.4 Semiconductors

Toxicity of quantum dots depend on a host of factors arising from their chemical structure and environmental conditions. Size, charge, concentration, bioactivity of the surface coating, and oxidative, photolytic, and mechanical stability contribute to their toxicity[492]. The long-term stability of the complexes and their complete degradation before elimination needs to be further evaluated given that some of their constituents, such as Pb, As, Cd, and Tl, are potentially highly toxic[493]. Coatings can inhibit quantum dot degradation which might result in the release of toxic constituents, as well as loss of quantum dot fluorescence *in vivo*[494]. *in vitro* studies suggest that some quantum dots can be cytotoxic, with dose-response relationship. For example, CdTe quantum dots caused rat pheochromocytoma cell death *in vitro* indicated by chromatin condensation and membrane blebbing[495]. Smaller positively charged quantum dots were more toxic than larger, equally charged, quantum dots with the former localized in the nuclear compartment and the latter in the cytosol. Authors explained this by the presence of Cd^{2+} ions, free radical formation, or interaction with intracellular components leading to loss of function. Other, *in vitro* studies, reported that CdSe quantum dots were cytotoxic to liver cells and that surface oxidation of the quantum dots produced Cd^{2+} ions, recognized as carcinogenic[85][496]. Encapsulation of the quantum dots with ZnS tended to reduce this effect and it decreased to almost zero with encapsulation by bovine serum albumin. The *in vitro* cytotoxicities of realgar (As_2S_2, a semiconducting material which is also used in some traditional medical formulations) with small sizes (100 nm to 150 nm) to human umbilical vein endothelial cells were stronger than those of larger particles (200 nm to 500 nm)[497]. The surface area-dependent cytotoxicity of realgar particles can be explained by the amount of released active ingredients in the incubation medium from the particles, which might be highly dependent on the amount of surface area.

Some *in vivo* studies showed no observed adverse effects: mice injected with amphiphilic polyacrylic acid polymercoated quantum dots and with polyethyleneglycol amine conjugated quantum dots[498]; mice injected with CdSe/ZnS quantum dots[499].

B.5 Organic polymeric nanomaterials

Certain incidental organic NOAAs have been shown to pose acute toxic hazard upon human inhalation exposure. Among nanoscale particles, freshly-generated PTFE fume (generated at temperatures of more than 425 °C) is known to be highly toxic to the lungs. Freshly-generated PTFE fume (15 nm) caused haemorrhagic pulmonary oedema and death in rats exposed to less than 60 $\mu g/m^3$[78]. In contrast, aged PTFE fume was much less toxic and did not result in mortality, which was attributed to changes in surface chemistry as well as increase in particle size from accumulation (>100 nm)[29][500]. While PTFE fume differs from manufactured NOAAs, these studies illustrate properties of incidental NOAAs that have been associated with an acute toxic hazard.

Toxicity of nanoscale dendrimers is related to the nature of monomers and dendrimer synthesis[501]. Specifically, *in vitro* and *in vivo* animal studies showed that nanoscale dendrimers with positively charged surface groups similar to other biological macromolecules can destabilize cell membranes and cause cell lysis[502]. Thus, biological response to nanoscale dendrimers can be tailored through functionalization of the surface dendrimer sites. Nature of the dendrimer core also is also thought to affect its biological activity. For example, it has been suggested that dendrimers with aromatic interior can cause haemolysis through hydrophobic membrane contact[503]. Higher generation dendrimers (larger in size) have been found to be more cytotoxic[504].

B.6 Bio-inspired nano-objects

Engineered bio-inspired nano-objects can potentially produce the full range of health responses observed with naturally occurring nano-objects, from the benign and beneficial (such as insulin and growth hormone) to adverse and even lethal (such as protein biotoxins "designed" by nature to be toxic). It was shown that delivery of biologically active bio-inspired nano-objects into the blood circulation through oral administration can be facilitated by bile acids and proteinase inhibitors[505]. Recently, transdermal delivery of intact, biologically active protein medications, such as insulin, has been shown to be possible in the presence of phage peptide chaperones[506]. It appears that the mechanism of penetration is not specific to insulin and involves interactions between the phage peptide and the skin, facilitating a transfollicular route of insulin transport through the skin. These drug applications could potentially result in accidental exposures of workers during drug production and administration in health care settings.

Annex C
(informative)

Characteristics of selected instruments and techniques for monitoring nano-aerosol exposure

C.1 Mass concentration

C.1.1 General

Mass concentration can be determined by a number of methods collecting particles on filters (size selective aerosol samplers, single or multi stages) and some direct reading instruments (e.g. mass sensitive oscillating elements, absorption of radioactive emission). It is also possible to derive estimates of mass by calculation using a tandem of instruments such as Electrical Low Pressure Impactor (ELPI®) and Differential Mobility Analysing System (DMAS) (see C.4). Direct reading instruments are convenient to identify nano-object releases and to conduct risk management, because they show monitoring results after short duration of sampling and calculation. However, they cannot distinguish amongst different types and sources of particles. Off-line characterization methods are usually required for this purpose. Furthermore occupational exposure levels are often suggested as a (chemical) mass concentration. Novel samplers have just been developed in order to measure chemical mass and morphology of particles *in situ* (e.g. NANOBADGE).

C.1.2 Filter sampling

There are only few commercially available workplace aerosol samplers with a 100 nm size selection cut point. There is very little data on expected mass concentrations of nano-objects in workplaces but it is expected that high flow rates of about 10 l min^{-1} will be required to collect sufficient mass of nano-objects in an 8 h shift to be above the limit of quantification for weighing or mass analytical techniques such as ICP-MS, XRF and EC/OC. At these high flow rates it can be expected in principle to operate existing devices such as impactors and cyclones to provide a cut point at 100 nm.

Filter-based methods for collecting airborne nano-objects and their agglomerates and aggregates (NOAA) often yield very small particle masses (typically less than 0,1 mg). There are numerous challenges associated with both gravimetric and elemental analysis of lightly loaded filters. Longer sampling times and/or higher flow rates might be required to maximize the mass of samples collected. Errors caused by static electricity, vibration, particles outside the selected size range and particulate contamination during handling of the filter cassette should be eliminated in order to obtain reliable mass measurements. It is recommended that existing US. EPA[507] or European protocols[508] for conditioning and weighing filters loaded with $PM_{2.5}$ should be employed until further guidance specific to weighing NOAAs becomes available. These protocols are designed to control and/or account for changes in air temperatures, humidity and air density from pre-weigh-in to post-weigh-in. Strategies for improving gravimetric data quality have been described[509]. Mass measurements of lightly loaded MOUDI (micro-orifice uniform deposit impactor) filters loaded with NOAAs were also discussed[510].

Analytical techniques other than gravimetric such as ICP-MS, XRF and EC/OC could be also used for bulk characterization of elemental composition of collected NOAAs. Examples include NIOSH 7300 method for metals utilizing inductively-coupled argon plasma combined with atomic emission spectroscopy analysis and NIOSH 7500 method for silica utilizing X-ray diffraction analysis[151].

Precautions should be taken to avoid contamination filter samples with particles or metals while loading and unloading the filter cassette, as well as throughout all stages of handling and analysis. For lightly loaded filters, the contribution of metals from sources of inadvertent contamination could be greater than the contribution from the particles being samples[511]. Examples of precautions include using appropriate nitrile gloves while handling filters and loading/unloading filter cassettes in a clean laminar flow hood.

Selecting an appropriate filter materials will depend on the type of subsequent analyses required. The US. EPA protocol for $PM_{2.5}$[507] prescribes PTFE filters, while the European protocol[508] lists glass fibre, quartz, PTFE and PTFE-bonded glass fibre as alternatives. Blank values of elements under investigation should be determined from the filter material in use. Note that interferences caused by static electricity during gravimetric analysis could preclude the use of cellulose membrane filters.

Buoyancy-corrected gravimetric analysis of lightly loaded filters have also been investigated for their effectiveness over experimentally adjusted conditions of temperature and relative humidity[509]. By maintaining strict controls on humidity throughout pre- and post-weighing, it was possible to quantify error due to water absorption. Studies have recommended that protocols for weighing low-mass nanomaterial samples should include buoyancy corrections and tight temperature/humidity controls[510].

C.1.3 Cascade impactors

An alternative approach that has been used in both workplace[512][513] and environmental[514] studies is to use a low-pressure cascade impactor [e.g. Berner-type or micro-orifice cascade impactors (MOUDI) uniform deposit cascade impactors]. Both of these devices use inertial impaction to separate particles into discrete fractions according to their aerodynamic diameters and have two or more stages in the nanomaterial size range[515]. In both devices, the masses of NOAAs can be assessed by weighing the collection substrates before and after sampling, plotting the full size distribution and making a cut at 100 nm or whatever particle size is considered to be relevant for particles of nanostructured materials. These will be described in C.4.2. Where necessary to increase sample mass to exceed limits of quantification for subsequent analyses, one or more stages could be removed from the MOUDI to maximize the mass of the particles collected in the desired cut-size ranges.

C.1.4 Oscillating and piezoelectric microbalances

An alternative is to use the Tapered Element Oscillating Microbalance (TEOM)[516]. The TEOM principle (developed initially for measuring the mass of particles in space) involves the use of a small filter which is located on the tip of a tapered glass tube which forms part of an oscillation microbalance. The oscillation frequency of the microbalance changes with the mass of particles collected on the filter. The devices are widely used to continuously monitor ambient levels of PM_{10} and $PM_{2.5}$ aerosols in national air quality networks and have proved to give reliable information on particle levels for compliance with national air quality directives. Consequently, with a mass detection limit of 0,01 µg, they were considered to possibly have adequate measurement precision (±5 µg/m^3 for 10 min averaging times and ± 1,5 µg/m^3 for 1 h averaging times) for the measurement of nano-objects in workplaces. In using this instrument careful consideration needs to be given to select a pre-separator for the TEOM to match the size of nano-objects being studied and to change the collection filter to one that has high efficiency for nano-objects[275]. Personal versions of the TEOM instrument have been developed, for example for sampling respirable dust in coal mines. Recent developments of micro electro mechanical systems (MEMS) showed the ability to determine small masses attached to oscillating cantilevers. The change of frequency is used for detection. A realized sensor with a detection limit of 6,5 µg/m^3 was reported, further improvements are expected[517][518][519][520].

Operation of piezoelectric microbalance (or piezobalance) is based on changes in the resonance frequency of piezoelectric crystal as a function of its mass. By monitoring resonance frequency against a second crystal, mass deposited on the crystal can be continuously measured thus providing information about mass concentration of particles[521][522]. Airborne particles can be deposited on the crystal surface by either electrostatic precipitation or by impaction[523]. Collection efficiencies of either of these mechanisms are a function of particle size and particle properties and should be determined to achieve quantitative measurements. Quartz crystals have sensitivities of several hundred hertz per microgram which translates into the ability to measure the aerosol mass concentration of 100 µg/m^3 to within a few percent under one minute[523].

C.2 Number concentration

C.2.1 General

When selecting an instrument to determine the number concentration, the user should be aware that a specific instrument is only able to measure a certain interval of the particle size range and particle concentration emerging at workplaces. This can lead to a combination of instruments with different ranges of application. The recommendations of the manufacturers should be considered.

The total number concentration of ultrafine particles found at workplaces varies between 10^3 particles per cubic centimetre in clean air environment and 10^8 particles per cubic centimetre in welding plumes[524].

C.2.2 Condensation particle counters

The most widely used instrument for determining the number concentration of nano-objects is the Condensation Particle Counter (CPC). This device exploits vapour condensation on nanometer size (and larger) particles in order to grow the particles to a size range that can be detected optically.

The convective cooling laminar flow CPC is the most widely used and is also commercially available from a number of manufacturers in models with different lower particle size cut-offs. Particle laden air is drawn into the instrument at constant air flow, which is saturated using warm vapour (typically butanol, isopropanol or water). The saturated flow is then taken to a cool condenser tube in which the vapour is depleted onto the tube surface. However, as the flow cools, there will be regions in the flowwhere the vapour becomes supersaturated and condenses onto particles, which grow to large droplets. The detection limit at small particle diameters depends on vapour properties, operating temperatures (which determine the super-saturation), flows and geometries of the instrument. Devices using butanol are available with detection limits down to 3 nm, while isopropanol has successfully been used in portable instruments with a lower detection limit of 10 nm, and water is used in a commercially available instrument with a similar lower detection limit.

The upper size limit is approximately a few micrometers and mainly determined by the instrument's ability for aerodynamic transfer of larger particles.

At low concentrations the CPC counts individual particles and allows a direct determination of particle number concentration (single particle count mode). At higher concentrations some instruments include an evaluation of the total scattered light intensity without single particle counting and thus estimate the number concentration, based on assumptions of final particle size and optical properties (photometric mode).

The application of CPCs for workplace measurements is described in EN 16897, *Workplace exposure — Characterization of ultrafine aerosols/nano-aerosols — Determination of number concentration using condensation particle counters*[165].

Information on CPC calibration is given in Reference [164].

C.2.3 Electrometers

A second instrument type that is sensitive to nano-objects is an electrometer. This instrument detects the charge carried by aerosol particles and therefore its use depends on knowing the charge on individual particles in an aerosol flow. Known charge distributions are possible to obtain using chargers or neutralizers with known characteristics. However, as charging efficiency is strongly a function of particle size, accurate information of the concentration of nano-objects is difficult to obtain using an electrometer alone. An electrometer in series with a mobility analyser enables the determination of the size distribution of nano-objects. In practice, the electrometer is often used to calibrate other instruments, especially CPCs due to good detection efficiency at nanoscale size range.

C.3 Surface area concentration

Measurements of particle surface area have been possible for some time using the BET method[525]. However, it requires the collection of relatively large amounts of particles (up to 50 mg have to be collected for BET analysis)[526], and measurements are influenced by particle porosity (which might or might not be important) and collection/support substrate – particularly where the quantity of material analysed is small.

The diffusion charger measures the Fuchs or active surface area of the aerosols from the attachment rate of positive unipolar ions to particles, from which the aerosol active surface-area can be inferred[527]. However, particle losses affect measurements and, therefore, the instruments show a size dependency, which has to be determined experimentally and compared with the needed response[528]. The sampled aerosol passes through a weak plasma created by a corona discharge device where it mixes with the unipolar air ions produced by the corona. The air ions diffuse and attach to the exposed surface of the particles. The excess unattached ions are removed by a collecting electrode and the particles with attached charges, are collected on a HEPA filter within a Faraday cup electrometer. The current produced by the charged particles is measured by a sensitive electrometer and related to the surface area of the sampled particles. Diffusion charging surface area monitors are available from a number of companies and typically have quoted ranges of 0 $\mu m^2/cm^3$ to 2 000 $\mu m^2/cm^3$ and sensitivities of 1 $\mu m^2/cm^3$.

Two real-time surface area instruments critically evaluated recently[528] do show active surface area response to particle diameter in the range of 20 nm to 100 nm.

Also, below approximately 100 nm for open fractal-like particles and spherical particles, active surface area measured by diffusion chargers has been found to correlate well with geometric surface area as measured by Differential Mobility Analysing System (see C.4.1) and with projected surface area as measured by Transmission Electron Microscopy[528]. The challenge with the diffusion charging method is that for particles larger than 100 nm mobility diameter, the diffusion chargers increasingly underestimate the aerosol surface area with increasing particle size[528], as is anticipated from theory. Research is needed to establish whether this degree of underestimation is significant in relation to manufactured NOAAs' exposure and health effects.

Stationary and personal instruments are available which use a particular configuration of an aerosol charger to simulate the amount of material expressed as surface area deposited into the tracheobronchial and alveolar regions of the lung. A calculation of the particle number concentration and a mean particle diameter is sometimes added. This is in addition to other surface area instruments (described above) that measure the total active surface area. These instruments draw the aerosol through a size selector with a cut point of 1 µm or below and then into the mixing chamber to mix with the ion stream. Some personal monitors using electrometers are available and allow the determination of the exposure in the breathing zone. The charged aerosol is passed through an ion trap. Certain instruments can change the voltage of the ion trap such that it acts as a particle size selector to collect both the excess ions and particles that are not of an electrical mobility state (surface area size) corresponding to either the tracheobronchial or respirable aerosol fractions. The electric charges on the penetrating particles are then measured by the electrometer[529]. This new method has the potential to provide a measurement that correlates with deposited aerosol surface area in the lungs. The calibration of these instruments is done only for one breathing condition (nose only and activity level of light exercise) of workers, i.e. reference condition[530]. The calibration to reference conditions does not account for factors such as level of worker activity, worker's age and sex or pre-existing lung disease, which might markedly influence particle deposition. It might also not represent real exposures of different people performing different activities in the workplace. Thus the correlation of data provided by this instrument to actual deposited particle surface area in workers' lungs needs to proceed with caution. Comparisons with other instruments to assess the instrument performance can, for example, be made by calculating the deposited surface area, based on similar lung deposition models, from size distribution measurements with electrical mobility spectrometers. Recently performed comparisons for dioctyl sebacate, sodium chloride and diesel soot particles have shown good agreement[531]. It was shown that neither the level of activity nor gender has a major impact on the deposition curves and thus the exposure[532]. Only the dose is a function of breathing frequency and tidal volume. The dose, however, can be inferred from the measured exposure data based on the breathing pattern. This might also allow for a more personalized dose analysis. At present, research is underway indicating that calibration factors might be used to adapt the instrument to breathing behaviour, gender and age other than the reference condition.

C.4 Nano-aerosol size distribution

C.4.1 Measuring size distribution using particle mobility analysis

The most common instrument used for measuring size distributions of aerosols of nano-objects is the Differential Mobility Analysing System (DMAS). The DMAS is capable of measuring aerosol size distribution in terms of particle mobility diameter from approximately 3 nm up to around 800 nm, although multiple instruments typically need to be operated in parallel to span this range. However, there is a challenge in measuring size distributions of some manufactured nano-aerosols, such as single-wall carbon nanotubes. A recent study has reported anomalous instrument responses above certain voltages when characterizing aggregates of airborne carbon nanotubes using an electrical mobility analyser[533]. Problems were reported, when measuring nano-object penetration in filter media. If the system was not operated with a special cleaning procedure, phantom particles below approximately 50 nm were recorded[534]. Thus, care needs to be exercised in order to obtain correct size distributions when measuring nanometer-diameter conducting fibrous material, high particle concentrations and aggregates/agglomerates by electrical mobility analysis. DMAS comprises a Differential Electrical Mobility Sizer (DEMS) to separate the particles according to the electrical mobility diameter followed by a CPC or an electrometer to count the particles. Particles enter a pre-selector with a cut-point at 1 µm, then enter into a region where they are charged to Boltzmann equilibrium by passing them through a bipolar ion cloud formed from a radioactive source. They then pass through a well-defined electric field in the DEMS. The charged particles move between the electrodes, and those with a specific mobility are sampled from a small outlet at the exit of the electrodes, from where they are counted by a CPC or electrometer. By scanning or stepping the voltage between the electrodes, particles with electrical mobilities corresponding to a range of particle diameters can be counted sequentially, allowing the aerosol size distribution to be determined.

The sequential scanning or stepping of the voltage takes a significant time, with the fastest conventional scan speeds being about one minute, which is suitable provided that the process being monitored does not change within this timescale. However, there are many situations within the workplace environments where this might not be the case. If rapid fluctuations of the nano-aerosols appear, the use of a buffer vessel of a few litre in volume is recommended to keep the concentration stable for one scan. In case of periodical processes shorter than the scan time, multiple, sequential scans can be averaged in order to get a stable size distribution.

For applications requiring rapid analysis due to temporal variation, fast mobility-based particle spectrometers have been developed which use a parallel array of electrometer-based sensors to count the size segregated particles. Measurements might be made with a time resolution of one second or less, and operation at ambient pressures reduces evaporation of volatile particles. The instruments might be limited to measurements at relatively high aerosol number concentrations and some instruments are available with on-board dilution for very high concentration measurements. The lack of a radioactive source might make them a viable alternative to the DMAS in many workplaces. Research is currently being carried out to develop more compact and therefore cheaper aerosol mobility classifiers relying on particle migration across an opposing air flow[535].

The DMAS was limited in its widespread application in the workplace due to its size, expense, complexity of operation, the need for two or even three instruments operating in parallel to measure wide aerosol size distributions, and the use of a radioactive source to bring the aerosol to charge equilibrium. Some later instrument developments revealed smaller mobile devices and an X-ray source or a high-voltage charger source can be applied for creating a known charge distribution of the aerosols. If nano-objects are the only airborne particulate of interest, a single instrument might be sufficient.

C.4.2 Measuring particle size distribution using inertial impaction

Cascade impactors are widely available in a number of configurations, allowing either personal or static sampling with a range of particle size cut points. Static cascade impactors are available with lower cut points in the nanometer size region, low pressure impactors or multi-orifice impactors.

A number of low pressure cascade impactors are available. These instruments require vacuum pumps to provide the necessary air flow and so are not suitable for personal sampling. Personal cascade impactors are available with cut points of 250 nm and above[536], and thus are only able to provide very limited information on size distribution in the nanometer size range. Portable impactors having cut-point at 100 nm have been developed[537][538] and have been evaluated with size-controlled generated nanomaterial particles.

Determination of aerosol size distribution from cascade impactor data requires the application of data inversion routines. The simplest approach is to calculate cumulative mass concentration with particle diameter, and use the data to estimate the Mass Median Aerodynamic Diameter (MMAD) and the Geometric Standard deviation (GSD) of the size distribution. This approach assumes no losses between collection stages, ideal impactor behaviour, and a unimodal aerosol with a lognormal size distribution. Cascade impactors are usually used to measure the mass-weighted aerosol size distribution, and so assumptions of particle shape and density need to be made in order to estimate the number or surface-area weighted distribution. As these parameters are rarely quantified, great care needs to be taken in interpreting cascade impactor data in terms of aerosol number or surface-area.

C.4.3 Electrical Low Pressure Impactor (ELPI®) measurements

The Electrical Low Pressure Impactor (ELPI®) combines inertial collection with electrical particle detection to provide near-real-time aerosol size distributions for particles larger than 7 nm in diameter[539]. Aerosol particles are charged in a unipolar ion charger before being sampled by a low pressure cascade impactor discussed in C.1.3 and C.4.2. Each impactor stage is electrically isolated, and connected to a multi-channel electrometer, allowing a measurement of charge accumulation with time. As in the case of the diffusion charger (C.3), particle charge is directly related to active surface-area. Thus the integrated electrometer signal from all stages is directly related to aerosol active surface-area. The electrometer signal from a single stage is related to the active surface-area of particles within a narrow range of aerodynamic diameters, allowing limited interpretation of the shape of sampled particles. If the particle charging efficiency as a function of aerodynamic diameter is known or can be assumed, real-time data from the ELPI® can be interpreted in terms of the aerosol number-weighted size distribution. In practice, particle-charging efficiency is determined experimentally. Interpretation of measurements in terms of particle mass concentration or mass-weighted size distribution can also be carried out, although it requires knowledge of the effective particle density as a function of size and correction for particle losses.

As well as allowing online measurements of particle concentration and size distribution, aerosol samples collected by the ELPI® are available for off-line analysis, including electron microscopy and chemical speciation.

C.4.4 Calculations of nano-object concentrations from size distribution measurements

As well as providing information about the particle size characteristics of the aerosols in workplaces where nano-objects are being produced or handled, size distribution measurements can be used to calculate integrated nano-object exposure levels. For example, frequency distributions, combined with sample volume can be used to calculate number concentrations. With the assumption that the particles are nearly spherical and that their physical diameters are equivalent to their mobility diameters (for DMAS, see below) or aerodynamic diameters (for ELPI®, see below), the aerosol surface concentration can be calculated. A method has been also developed to calculate aggregate surface area and volume distributions using the electrical mobility diameter for nano-aerosols[540]. Similarly, with knowledge of particle density, the aerosol mass concentrations can be determined. The accuracy of these estimations is dependent upon the assumptions made about the physical characteristics of the particles.

Ku and Maynard[528] showed that for monodisperse aerosol particles smaller than 100 nm, particle geometric surface areas calculated by DMAS size distributions agree to within ± 20 % of surface area determined by a diffusion charger surface area instrument. However, the relationship diverged for larger particles because the Diffusion Charger (DC) instrument tends to underestimate surface area of larger particles compared to the DMAS. A similar relationship was found by Shi et al.[541] for polydisperse aerosols present in the ambient atmosphere. From comparative measurements at two outdoor sites they found good agreement between geometric surface area measurements using the epiphaniometer and the DMAS. It is therefore reasonable to suggest that reliable measurements of geometric surface area can be obtained with DC instruments, provided that suitable pre-selectors are used.

C.5 Sample collection for material characterization

Determination of the physical and chemical properties of airborne NOAAs relevant to their potential effect on human health is often required. Parameters such as particle size, shape, surface area, composition, agglomeration state, crystallinity, solubility and bio-persistence provide the basic information for the exposure and toxicological evaluation of new nanomaterials. The surface coating on the particles and their electrical charge will also have a significant impact on their state of agglomeration, which will in turn influence their physical behaviour and subsequent biological responses. Because particle nanoscale structure affects transport and locations of deposition within the respiratory system and might affect toxicology, it is important to characterize nanoscale structures of airborne materials used for toxicology studies. A new technique for characterizing particle size-dependent nanoscale structure in airborne single-walled carbon nanotube agglomerates utilizing a tandem mobility-mass analysis has been developed[542][543].

The main analytical techniques routinely available for determining the particle size, shape and composition are high resolution electron microscopies such as scanning electron microscopy (SEM), field emission gun SEM, transmission electron microscopy (TEM), and scanning transmission electron microscopy (STEM), combined with X-ray microanalysis, Electron Energy Loss Spectroscopy (EELS) and electron diffraction. Both SEM and TEM require samples of particles that are uniform in deposit and have minimal particle overlap[179]. This requirement rules out collection by impaction where particles are concentrated in small regions below the impaction jets. For the SEM, particles down to typically 20 nm in diameter can be sampled directly onto SEM supports using electrostatic precipitation. (An upper limit of electrostatic precipitation is given by the geometry of the instrument and can be assumed around 200 nm.) Point to plane electrostatic precipitators combine a charging and deposition field by using a sharp corona needle as one electrode, and a planar collection surface as the second electrode. Sampling efficiency approaching 100 % can be achieved for particles larger than 20 nm. For smaller particles, rapidly decreasing charging efficiency leads to a lower sampling efficiency. Deposits from electrostatic precipitators are generally uniform across the collection substrate, enabling discrete particle analysis in the SEM. A number of electrostatic precipitators are available from instrument manufacturers. Some studies also indicate that passive aerosol samplers can be potentially used to collect NOAAs for SEM analysis[544].

For the TEM, it is generally preferable to sample directly onto a TEM support grid, thus avoiding a secondary sample preparation stage. The deposition onto the coated grid can be achieved by thermal precipitation, electrostatic precipitation or direct airflow through the grid. Thermal precipitation is the most suitable collection mechanism as it relies on aerosol particles migrating from a hot region to a cold region, and is particularly effective for particles between 1 nm and 100 nm in diameter. Thermal precipitation can be used to sample aerosols at ambient temperatures by establishing a temperature gradient above the collection surface, and passing the aerosol across the surface. A number of suitable designs have been published[545][546] and they can be built by a reasonable laboratory workshop. The method for fibrous substances like asbestos or refractory ceramic fibres, NIOSH NMAM 7400 (Asbestos and other fibres by phase contrast microscopy) or 7402 (Asbestos by TEM)[547], has been often applied to observe morphology of carbon nanotubes.

C.6 Measurement of high length-to-width aspect ratio particles of nanomaterials

There is a wide variety of occupationally relevant particles of nanomaterials with high length-to-width aspect ratio. Among those, one can distinguish elongated particles of nanostructured materials (such as a chain of aggregated nanoparticles) and elongated nano-objects, also referred to as "nanofibres," which are characterized by distinct chemical compositions, structure and geometry (see Clause 5).

An example of nanofibres, which are produced on industrial scale, is carbon nanotubes. Single-wall carbon nanotubes essentially comprise a single layer of carbon atoms arranged in cylindrical structures of diameter about 1 nm and length up to about 1 mm. Carbon nanotubes might also form as multiple concentric tubes of diameters significantly greater than SWCNTs. The extreme aspect ratio of individual nanotubes, together with their potentially low solubility in the lungs might lead to toxic mechanisms analogous to those observed with other fibrous particles such as asbestos and synthetic vitreous fibres. The question might be asked therefore if they should be considered, for exposure measurement purposes, like asbestos fibres and be analysed by counting with the TEM.

However, SWCNTs are very rarely found as single fibres. They are generally produced as convoluted bundles of nanotubes (nanoropes) of diameters from 20 nm to 50 nm and then form complex clumps and agglomerates, of size between 100 µm and 1 mm, with other nanoropes and other carbonaceous and catalyst materials that are present. Laboratory and field studies by Maynard et al.[548] have shown that it is extremely difficult to break these clumps and generate aerosols of nanotubes. Normal procedures of transferring SWCNT powder from production vessel to storage bucket and then tipping into a second bucket showed no significant increase in nano-object numbers. It was only by using a single component vortex shaker fluidized bed, operating at over 50 % agitation that any significant increase in particle numbers was produced. Although aerosol generation rates of SWCNTs have been shown to be low during handling[548], published data to date indicate that inhaled airborne SWCNTs might present a pulmonary hazard. Regarding measurement and characterization of SWCNTs, it is noticeable that physicochemical properties of SWCNT aerosol particles released while handling unprocessed SWCNTs might vary significantly by particle size and production batch, and that evaluation of potential health hazard needs to account for this[542]. However, for certain applications, manufacturers are currently trying to prevent nano-objects from agglomerating by using some form of surface coating or other techniques. In addition, there is no information on the size distributions of particles released from the cutting, sanding or abrading of products that incorporate nanotubes bound into the matrix of the material (composites, tires, etc.). Therefore at the moment there is no reason to suggest that nanotubes should be treated like asbestos fibres for exposure assessments, but it would be wise when monitoring levels of NOAAs in workplaces where carbon nanotubes are being produced or handled to investigate samples collected for TEM analysis for discrete nanotubes. Finally, a careful watch should be kept on developments in nanotube production, and knowledge shared of any evidence of discrete airborne nanotubes found in workplace air.

One of the first reports on exposure assessment at a carbon nanofibre handling facility was published in 2006[205]. Measurements made with real-time instruments (CPC, diffusion charger, aerosol photometer and ELPI®) indicate that most processes did not release substantial quantities of carbon nanofibres when compared to background particle measurements. However, some processes (wet sawing of composite material and the transferring of carbon nanofibres to a mixing vessel) did elevate area airborne particle (from 300 nm to 2 500 nm diameter) mass concentrations up to 0,16 mg/m^3 shown by aerosol photometer. In addition, air and surface samples were collected with a vacuum sampling method on high purity quartz-fibre filters and analysed for total carbon using a thermal-optical analysis technique, which indicated up to 1,1 mg/m^3 in total carbon for inhalable fraction. A point-to-plane electrostatic precipitator was used to collect sample for TEM examination for particle size and shape. A few samples exhibited fibre bundles of varying diameters (some larger than 100 nm) and lengths. The majority of fibres appeared as loosely bundled agglomerates, rather than as single fibres[205].

The exposure concentration of MWCNT in a small scale production area was measured as elemental carbon (EC)[549], which was analysed by another analytical method for elemental carbon[550]. The exposure to MWCNTs was determined during packaging and furnace maintenance by the modified carbon analysis method[551]. During manual packaging and automated packaging, the concentration of CNT[552] was lower than 0,063 mg/m^3 and 0,009 mg/m^3 at the personal breathing zone (PBZ), respectively. In this case, SEM observation showed that CNTs were aerosolised as both aggregates/

agglomerates and free single CNTs. The same analytical method was applied to measure CNTs in an arc discharge production facility and CNT concentration up to 0,55 mg/m^3 were observed for sieving, mechanical work-up, pouring, weighing, and packaging[553]. Powder handling tasks can show exposure to CNTs[552][553][173][554][555][556]. Although no consistent trends were observed for similar processes at the various sites, measurements using direct-reading instruments are useful for detecting CNT generation, and evaluating control technology and other air contaminants. Measurements with direct-reading instruments are nonspecific and subject to interference from aerosols generated by other sources.

According to Tantra and Cumpson (2007)[557], Raman spectroscopy shows the most promise of the spectroscopic methods, but sensitivity is not enough for exposure assessment. SEM is more suitable than TEM, and Atomic Force Microscopy is more suitable than Scanning Tunnelling Microscope. If the OEL is set at very low level and the sensitivity of chemical mass analysis does not enough to measure the concentration, counting method for CNT exposure assessment should be established to improve quantifying ability and representativeness of electron microscopy analysis by coordination of sampling method and counting procedure.

Several OELs for different types of CNTs have been recommended with different end points. The level of the OEL is from 0,001 to 0,050 mg/m^3 and the level is low in comparison to other nanomaterials or bulk metal materials. Because it is difficult to measure this low levels of mass concentration by gravimetry, analysis of carbon mass concentration and fibre counting by electron microscopy methods are usually applied, EC or TC can be taken as an index of CNTs if contamination from outside air or other organic carbon source like vacuum pumps[549]. Although NMAM 5040[547] has been used to assess the CNTs exposure, contamination from outside air containing diesel exhaust particles, carbon black or other carbonaceous substances as impurity in CNTs can increase the value of the measured exposure to CNTs. Recently, modified method of carbon analysis[551] has been often applied for CNT exposure monitoring[553]. As oxidation speed of CNTs can be affected mainly by crystallinity and metal impurities of CNTs, more sophisticated research is needed to improve credibility of this method for carbon analysis. Recently analysis of metal impurity in CNTs is developed to monitor MWCNTs by taking metal contents as a surrogate of CNTs[135].

An emerging strategy that has been studied to identify process-related emissions of carbon nanotubes is to monitor metal impurities (i.e. elemental airborne tracers of carbon nanotubes) for workplace monitoring using wet electrostatic precipitation, filters cassettes and/or surface wipes; this is followed by elemental analysis of the collected samples by ICP-MS[135][558]. Selection of appropriate tracers requires an initial analysis of:

1) the bulk parent materials as different metal elements (e.g. Co, Cu, Fe, Mo, W, Ta, Y) are used as catalysts and these are unique to each manufacturing process.

2) the background environment to ensure that the proposed tracers do not arise from local sources). Elemental ratios assist in distinguishing CNT from ambient aerosols[135][558].

Annex D
(informative)

Characteristics of biosafety cabinets

Class III Biological Safety Cabinet is a gas-tight enclosure with a non-opening view window. Access for passage of materials into the cabinet is through a dunk tank, that is accessible through the cabinet floor, or double-door pass-through box (e.g. an autoclave) that can be decontaminated between uses. Reversing that process allows materials to be removed from the Class III BSC safely. Both supply and exhaust air are HEPA filtered on a Class III cabinet. Exhaust air should pass through two HEPA filters, or a HEPA filter and an air incinerator, before discharge to the outdoors (see Table D.1). Airflow is maintained by a dedicated, independent exhaust system exterior to the cabinet, which keeps the cabinet under negative pressure. Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet and allow direct manipulation of the materials isolated inside. Although these gloves restrict movement, they prevent the user's direct contact with the hazardous materials. The trade-off is clearly on the side of maximizing personal safety.

Table D.1 — Comparison of biosafety cabinet (BSC) characteristics (from biosafety in microbiological and biomedical laboratories (BMBL) 5th Edition, Appendix A)[275]

BSC Class	Face Velocity m/s	Airflow Pattern	Applications	
			Nonvolatile Toxic Chemicals and Radionuclides	Volatile Toxic Chemicals and Radionuclides
I	0,381	In at front through HEPA to the outside or into the room through HEPA	yes	When exhausted outdoors[a,b]
II, A1	0,381	70 % recirculated to the cabinet work area through HEPA; 30 % balance can be exhausted through HEPA back into the room or to outside through a canopy unit	Yes (minute amounts)	No
II, B1	0,508	30 % recirculated, 70 % exhausted. Exhaust cabinet air should pass through a dedicated duct to the outside through a HEPA filter	Yes	Yes (minute amounts) [a,b]

[a] Installation could require a special duct to the outside, an in-line charcoal filter, and a spark proof (explosion proof) motor and other electrical components in the cabinet. Discharge of a Class I or Class II, Type A2 cabinet into a room should not occur if volatile chemicals are used.

[b] In no instance should the chemical concentration approach the lower explosion limits of the compounds.

Table D.1 *(continued)*

BSC Class	Face Velocity m/s	Airflow Pattern	Applications	
			Nonvolatile Toxic Chemicals and Radionuclides	Volatile Toxic Chemicals and Radionuclides
II, B2	0,508	No recirculation; total exhaust tothe outside through a HEPA filter	Yes	Yes (small amounts)[a,b]
II, A2	0,508	Similar to II, A1, but has 0,508 m/s intake air velocity and plenums are under negative pressure to room; exhaust air can be ducted to outside through a canopy unit	Yes	When exhausted O utdoors (Formerly "B3") (minute amounts)[a,b]
III	N/A	Supply air is HEPA filtered. Exhaust air passes through two HEPA filters in series and is exhausted to the outside via a hard connection	Yes	Yes (small amounts)[a,b]

[a] Installation could require a special duct to the outside, an in-line charcoal filter, and a spark proof (explosion proof) motor and other electrical components in the cabinet. Discharge of a Class I or Class II, Type A2 cabinet into a room should not occur if volatile chemicals are used.

[b] In no instance should the chemical concentration approach the lower explosion limits of the compounds.

Annex E

(informative)

Assigned protection factors for respirators

Table E.1 — A comparison of past and present assigned protection factors (APFs) (based on Reference [308])

Type of Respirator	OSHA 29 CFR 1910.134 (2006) [310]	NIOSH Decision Logic (2004)[311]	ANSI Z88.2 (1992)[559],[b]
APR - quarter mask	5	5	10
APR - filtering facepeice	10	10	10
APR - tight fitting half mask	10	10	10
APR-tight fitting full face (if part. filter ≠ N-P-R 100)	50	10	100
APR-tight fitting full face (if part. filter = N-P-R 100)	50	50	100
PAPR - tight fitting half mask	50	50	50
PAPR - tight fitting full facepeice	1 000	50	1 000[c]
PAPR - helmet/hood	25/1 000[a]	25	1 000[c]
PAPR - loose fitting	25	25	25
SAR - demand mode - half mask	10	10	10
SAR - demand mode - full facepiece	50	50	100
SAR - continuous flow - half mask	50	50	50
SAR - continuous flow - full facepiece	1 000	50	1 000
SAR - continuous flow - helmet/hood	25/1 000[a]	25	1 000
SAR - continuous flow - loose fitting	25	25	25
SAR - pressure demand - half mask	50	1 000	50
SAR - pressure demand - full facepiece	1 000	2 000	1 000
Combo SAR/SCBA - pressure demand – full facepiece	—	10 000	—
SCBA - demand mode - half mask	10	—	10
SCBA - demand mode - full facepiece	50	50	1000
SCBA - demand mode - helmet/hood	50	—	—
SCBA - pressure demand - full facepiece	10 000	10 000	10 000[d]
SCBA - pressure demand - helmet/hood	10 000	—	—

[a] Employer should have evidence provided by manufacturer that testing these devices demonstrates performance at a level of protection of 1 000 or greater.

[b] Rescinded in 2003.

[c] For HEPA filter if used for particulate protection; if less than HEPA, APF = 100.

[d] For emergency planning purposes only.

Annex F
(informative)

Advantages and disadvantages of different types of air-purifying particulate respirators

Table F.1 — U.S. NIOSH respirator selection logic[311]

Respirator type	Advantages	Disadvantages
Filtering facepiece (disposable)	— Lightweight — No maintenance or cleaning needed — No effect on mobility	— Provides no eye protection — Can add to heat burden — Inward leakage at gaps in face seal — Some do not have adjustable head straps — Difficult for a user to do a seal check — Level of protection varies greatly among models — Communication might be difficult — Fit testing required to select proper facepiece size — Some eyewear might interfere with the fit — Respirator should be replaced whenever it is soiled, damaged or has noticeably increased breathing resistance
Elastomeric half-facepiece	— Low maintenance — Reusable facepiece and replaceable filters and cartridges — No effect on mobility	— Provides no eye protection — Can add to heat burden — Inward leakage at gaps in face seal — Communication might be difficult — Fit testing required to select proper facepiece size — Some eyewear might interfere with the fitc

Table F.1 *(continued)*

Respirator type	Advantages	Disadvantages
Powered with loose-fitting facepiece	— Provides eye protection — Protection for people with beards, missing dentures or facial scars — Low breathing resistance — Flowing air creates cooling effect — Face seal leakage is generally outward — Fit testing is not required — Prescription glasses can be worn — Communication less difficult than with elastomeric half-facepiece or full-facepiece respirators — Reusable components and replaceable filters	— Added weight of battery and blower — Awkward for some tasks — Battery requires charging — Air flow should be tested with flow device before use
Elastomeric full facepiece With N-100, R-100, or P-100 filters	— Provides eye protection — Low maintenance — Reusable facepiece and replaceable filters and cartridges — No effect on mobility — More effective face seal than that of filtering facepiece or elastomeric half-facepiece respirators	— Can add to heat burden — Diminished field-of-vision compared to half-facepiece — Inward leakage at gaps in face seal — Fit testing required to select proper facepiece size — Facepiece lens can fog without nose cup or lens treatment — Spectacle kit needed for people who wear corrective glasses

Table F.1 *(continued)*

Respirator type	Advantages	Disadvantages
Powered with tight-fitting half-facepiece or full facepiece	— Provides eye protection with full-facepiece — Low breathing resistance — Face seal leakage is generally outward — Flowing air creates cooling effect — Reusable components and replaceable filters	— Added weight of battery and blower — Awkward for some tasks — No eye protection with half-facepiece — Fit testing required to select proper facepiece size — Battery requires charging — Communication might be difficult — Spectacle kit needed for people who wear corrective glasses with full face-piece respirators — Air flow should be tested with flow device before use

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