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Workplace atmospheres — Ultrafine, nanoparticle and nano-structured aerosols — Inhalation exposure characterization and assessment

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Workplace atmospheres — Ultrafine, nanoparticle and nano-structured aerosols — Inhalation exposure characterization and assessment

Air des lieux de travail — Particules ultrafines, nanoparticules et aérosols nanostructurés — Caractérisation et évaluation de l'exposition par inhalation

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Foreword

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ISO/TR 27628 was prepared by Technical Committee ISO/TC 146, *Air quality*, Subcommittee SC 2, *Workplace atmospheres*.

Introduction

Aerosol exposure has historically been characterized by the mass concentration of airborne material, usually associated with specific size ranges corresponding to different deposition regions within the respiratory system. However, there are indications that mass concentration alone may not provide a suitable indication of the health risks associated with some aerosols. A number of toxicology studies have indicated that, on a mass for mass basis, some very small respirable insoluble particles may be more toxic than larger respirable particles with a similar composition [4 to 11]. Ambient aerosol epidemiology studies since the early 1990s have demonstrated an increase in health impact from particles smaller than 2,5 µm compared to those smaller than 10 µm on a mass for mass basis [12 to 22]. While there is very limited health impact data specific to inhaling very fine respirable particles from the occupational environment, there is evidence to suggest that health effects associated with inhaling such particles generated in hot processes, such as metal processing and welding, are greater than mass-based exposures would indicate [23][24]. Taken together, the evidence points towards a particle size-related health risk following inhalation exposure to some occupational aerosols that is not appropriately reflected by mass concentration alone. In recognition of the potential importance of particle size, the term "ultrafine aerosol*"* has gradually been adopted and loosely refers to particles "smaller than 100 nm in diameter". The term is now widely used to refer to incidental aerosols where there are potential particle size-dependent health effects. As research and development into nanotechnology has increased over recent years, concern has also been expressed over the potential health impact of purposely generated particles with nanometre diameters or nanoscale structures [25 to 28]. In this context, the terms "engineered nanoparticle*"* and "engineered nanoaerosol*"* have also been used loosely to describe particles and aerosols associated with engineered nanometre-structured materials. However, a generally accepted set of definitions for these terms is still under discussion. For clarity, in this report, the term "nanoparticle*"* is used to describe all aerosol particles with diameters smaller than approximately 100 nm that present a potential inhalation health hazard. Larger particles with a nanometre-scale structure that may also present a potential health hazard (such as agglomerates of nanoparticles and nanometre-diameter fibres) are referred to as "nanostructured*"* particles, and aerosols of nanoparticles and nanostructured particles are referred to as "nanoaerosols*"*.

With only limited toxicity data and negligible exposure data, it is currently unclear how exposure to nanoaerosols should be most appropriately monitored and regulated. There is strong toxicity-based evidence that aerosol surface area is an appropriate exposure metric for low solubility particles that removes the dependency on particle size [5][8][9][29]. However, there are also indications that in some instances particle number within specific particle size ranges may be important [23]. Recent studies on particle translocation within the body have further indicated a size-dependency on the likelihood of deposited particles moving from the respiratory system to other organs [30][31]. At the present time, there is insufficient information to determine which physical exposure metrics – size-selective number, surface area and mass concentration – are most relevant, or which are the most appropriate exposure characterization techniques to use. A first step to providing the necessary information is to establish the means by which exposure can be measured against different metrics. In the short term, this will provide a means to evaluate exposures where there is concern over the inadequacy of mass-based methods, particularly in emerging nanotechnologies where engineered nanoparticle exposure may be significant. It will also provide a basis for developing a deeper understanding of associations between aerosol exposure and health effects using a range of exposure metrics and will lay the foundation for future characterization standards.

In this context, the overall aim of this Technical Report is to provide generally accepted definitions and terms, as well as guidelines on measuring occupational nanoaerosol exposure against a range of metrics. By providing the means to undertake potentially more relevant exposure measurements where current methods and standards appear inadequate, it addresses an immediate need and will form a basis for extending knowledge on how occupational exposure to nanoaerosols should most appropriately be measured. The development and adoption of appropriate measurement approaches is an essential step toward developing and implementing future exposure measurement standards for nanoaerosols.

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Workplace atmospheres — Ultrafine, nanoparticle and nano-structured aerosols — Inhalation exposure characterization and assessment

1 Scope

This Technical Report has been prepared in response to

— increasing concern over the potential health risks associated with occupational exposure to nanometre-diameter and nanometre-structured aerosol particles (collectively referred to as nanoaerosols, including the subset of particles produced as a by-product of industrial processes and generally referred to as ultrafine aerosols),

— the lack of current guidelines and standards applicable to minimizing the health risks, and

— the need to establish valid sampling methodologies as part of the process of formulating appropriate exposure and exposure monitoring standards.

The principle aim is to provide the necessary background information and sampling guidelines to enable occupational hygienists and researchers to effectively characterize and monitor nanoaerosol exposures in the workplace in advance of specific exposure limits and standards being developed and implemented. Occupational nanoaerosols represent a class of airborne material dominated by particles smaller than typically 100 nm in diameter (either as discrete particles or as agglomerates).

This Technical Report contains guidelines on characterizing occupational nanoaerosol exposures and represents the current state-of-the-art, with an emphasis on nanometre-diameter particles. Background information is provided on the mechanisms of nanoaerosol formation and transportation within an occupational setting and on industrial processes associated with nanoaerosol exposure. Exposure metrics appropriate to nanoaerosols are discussed, and specific methods of characterizing exposures with reference to these metrics are covered. Specific information is provided on methods for bulk aerosol characterization and single particle analysis.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
accumulation aerosol
aerosol associated with particle growth from the nucleation range through coalescence, coagulation and/or condensation

NOTE Distribution modes typically extend from 50 nm to 1 μm, but are not confined to these limits.

2.2
aerodynamic diameter
diameter of a spherical particle with a density of 1 000 kg/m^3, that has the same settling velocity as the particle under consideration

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

2.3
aerosol
metastable suspension of solid or liquid particles in a gas

2.4
agglomerate
⟨aerosols⟩ group of particles held together by relatively weak forces, including van der Waals forces, electrostatic forces and surface tension

NOTE The term is frequently used interchangeably with “aggregate”.

2.5
aggregate
⟨aerosols⟩ heterogeneous particle in which the various components are held together by relatively strong forces and thus not easily broken apart

NOTE The term is frequently used interchangeably with “agglomerate”.

2.6
coagulation
formation of larger particles through the collision and subsequent adhesion of smaller particles

2.7
coalescence
formation of homogeneous particles through the collision of smaller particles and subsequent merging or mixing of constituent material

2.8
engineered nanoparticle
nanoparticle intentionally engineered and produced with specific properties

2.9
mobility
⟨aerosols⟩ propensity for an aerosol particle to move in response to an external influence, such as an electrostatic field, thermal field or by diffusion

2.10
mobility diameter
diameter of a spherical particle that has the same mobility as the particle under consideration

NOTE Mobility diameter is generally used to describe particles smaller than approximately 500 nm, and is independent of the density of the particle.

2.11
nanoaerosol
aerosol comprised of, or consisting of, nanoparticles and nanostructured particles

2.12
nanoparticle
particle with a nominal diameter (such as geometric, aerodynamic, mobility, projected-area or otherwise) smaller than about 100 nm

2.13
nanostructured particle
particle with structural features smaller than 100 nm, which may influence its physical, chemical and/or biological properties

NOTE A nanostructured particle may have a maximum dimension substantially larger than 100 nm.

EXAMPLE A 500 nm diameter agglomerate of nanoparticles would be considered a nanostructured particle.

2.14
nucleation aerosol
aerosol dominated by particle formation from the gas phase, such as through nucleation from a supersaturated vapour

NOTE Aerosol distributions typically extend from less than 1 nm to 50 nm, but are not confined to these limits.

2.15
particle
small discrete mass of solid or liquid matter

NOTE See Reference [32].

2.16
primary particle
particle not formed from a collection of smaller particles

NOTE The term typically refers to particles formed through nucleation from the vapour phase before coagulation occurs.

2.17
secondary particle
particle formed through chemical reactions in the gas phase (gas to particle conversion)

2.18
surface area, active
surface area of a particle that is directly involved in interactions with surrounding gas molecules

NOTE Active surface area varies with the square of particle diameter when particles are smaller than the gas mean free path, and is proportional to particle diameter for particles very much larger than the gas mean free path.

2.19
surface area, specific
surface area per unit mass of a particle or material

2.20
ultrafine aerosol
aerosol consisting predominantly of ultrafine particles

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

2.21
ultrafine particle
particle with a nominal diameter (such as geometric, aerodynamic, mobility, projected-area or otherwise) of 100 nm or less

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

3 Symbols and abbreviated terms

AFM	Atomic Force Microscopy
BET	Brunauer, Emmett and Teller method of measuring surface area [33]
CPC	Condensation Particle Counter
DMA	Differential Mobility Analyser
EDX	Energy Dispersive X-ray analysis
EELS	Electron Energy Loss Spectroscopy
ELPI	Electrical Low-Pressure Impactor
ESEM	Environmental Scanning Electron Microscope
FEG-SEM	Field Emission Gun Scanning Electron Microscope
GSD	Geometric Standard Deviation
HEPA	High-Efficiency Particulate Air filter
ICRP	International Commission on Radiological Protection
MMAD	Mass Median Aerodynamic Diameter
NSOM	Near-field Scanning Optical Microscopy
OPC	Optical Particle Counter
SEM	Scanning Electron Microscope
SMPS	Scanning Mobility Particle Sizer, Stepped Mobility Particle Sizer
SPM	Scanning Probe Microscopy
STEM	Scanning Transmission Electron Microscope
STM	Scanning Tunnelling Microscopy
TEM	Transmission Electron Microscope
TEOM®	Tapered Element Oscillating Microbalance[1)]
A_d	minimum acceptable fractional projected area of a particle with diameter d in a microscope field of view (see Annex A)
A_f	area of the field of view in a microscope, in square metres (m^2) (see Annex A)
A_s	effective area of a collection substrate, in square metres (m^2) (see Annex A)

1) TEOM® is an example of a suitable product available commercially. This information is given for the convenience of users of this Technical Report and does not constitute an endorsement by ISO of this product.

C_d particle number concentration as a function of particle diameter, in number of particles per cubic metre (particles/m^3) (see Annex A)

d particle diameter, in metres (m)

E_d sampling efficiency as a function of particle diameter (see Annex A)

N_d minimum acceptable number of particles with diameter d per field of view in a microscope (see Annex A)

n_s minimum acceptable particle density on a microscope sample, in number of particles per square metre (particles/m^2) (see Annex A)

q sampling flow rate, in cubic metres per second (m^3/s) (see Annex A)

t sampling time, in seconds (s) (see Annex A)

λ wavelength of illuminating light, in metres (m)

4 Background

4.1 Nanoaerosols (including ultrafine aerosols) and potential health effects

Since the late 1980s, toxicological evidence has been emerging indicating that the health effects associated with inhaling nanoaerosols may not be closely associated with particle mass. Early studies with polytetrafluoroethene (PTFE) particles around 20 nm in diameter showed that airborne concentrations of a supposedly inert insoluble material lower than 50 µg/m^3 could be fatal to rats [4][5][34]. Since then, a number of studies have indicated that the toxicity of insoluble materials increases with decreasing particle size on a mass for mass basis. The precise mechanisms by which these materials exhibit higher levels of toxicity at smaller particle sizes have yet to be elucidated, although there are many hypotheses. A number of studies indicate that biological response depends on the surface area of particles deposited in the lungs [8][9][35 to 37]. It has also been suggested that due to their small diameter, nanoparticles are capable of penetrating epithelial cells, entering the bloodstream from the lungs [31][38 to 41], and even translocating to the brain via the olfactory nerves [30]. Health effects associated with such particle activity would be closely associated with particle size, and also possibly particle number. Particles in the nanometre size range have a high percentage of surface atoms, and are known to show unique physico-chemical properties. One would expect particles within this size range to demonstrate biological behaviour closely associated with particle diameter, surface area and surface activity.

Although further research is needed on the physical attributes of nanoaerosols which are most closely associated with potential health risk, it is apparent that measuring exposures against mass alone is not sufficient. Of the three primary physical exposure metrics (mass, surface area and number), there is strong evidence to suggest that occupational nanoaerosols should be monitored with respect to surface area. In this context, aerosol surface area is not well defined. Surface area is dependent on the characterization approach 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 [33]. 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 may 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 addition, some studies have shown there may be critical particle sizes influencing the fate and toxicity of respirable particles in the lungs [41][42]. For each of these exposure metrics, but particularly in the case of mass concentration, size-selective sampling will need to be employed to ensure that only particles within the relevant size range are sampled [43].

4.2 Lung deposition of nanoparticles

Lung deposition probability refers to the mean probability for an inhaled particle with a specific diameter to deposit somewhere in respiratory system. Total deposition probability is composed of the sum of probabilities within distinct regions of the respiratory tract. Three major anatomical regions are usually considered:

— the extra-thoracic region, which refers to deposition in the nasal passages, mouth, larynx and pharynx;

— the tracheo-bronchiolar region, consisting of the trachea and bronchi from which deposited particles are cleared by ciliary action;

— the alveolar region, consisting of the respiratory bronchioles, alveolar ducts and alveoli.

These regions have been adopted by the International Commission on Radiological Protection (ICRP) [1] and the UK National Council on Radiation Protection and Measurements (NCRP) [44] in human respiratory tract models used to calculate radiation doses to the respiratory tract of workers resulting from the intake of radionuclides.

The ICRP deposition model [1] characterizes the distribution of inhaled particulate material within the different anatomical regions specific to the age and gender of the subject and various physiological parameters. The deposition model is one of the six elements of the overall human respiratory tract model for radiological protection, together with morphometry, respiratory physiology, radiation biology, clearance and dosimetry.

The sites and magnitude of particle deposition in the human respiratory tract are determined by physical mechanisms, together with respiratory tract morphological and physiological parameters of the subject inhaling the particles.

The five main distinct mechanisms of deposition of inhaled particles are

a) sedimentation, which is due to gravitational force acting on particles,

b) inertial impaction, which characterizes the airborne behaviour of massive particles,

c) interception, which occurs when the edges of a particle contact the surface of the respiratory tract, leading to deposition,

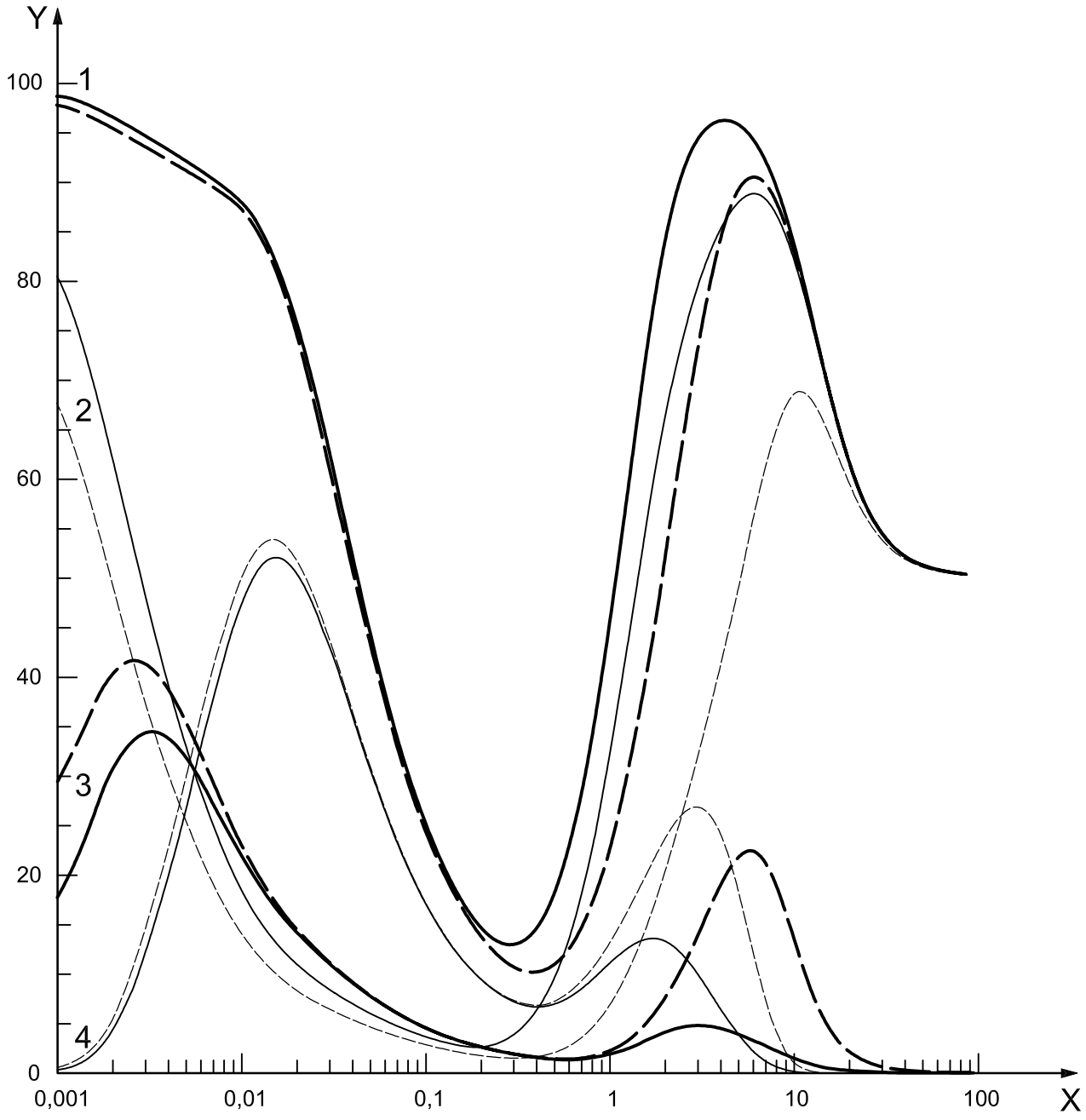
d) diffusion, due to the random (Brownian) motion of small particles, and

e) electrostatic attraction (when particles carry a charge).

In most studies, this last mechanism is neglected, although it could influence deposition if a subject is exposed to highly charged particles. Particle hygroscopicity may also affect deposition through enhancing deposition by impaction and sedimentation.

Respiratory tract morphology and other physiological parameters can vary greatly, depending on the individual as well as on the type of activity undertaken. Several factors may alter the normal structure and function of the respiratory tract, including age, respiratory illness and gender. Predictions of lung deposition probability are generally based on average parameters, and thus may not represent the range of aerosol doses that occur in a diverse population. However, lung dosimetry models do account for some factors contributing to group differences in particle deposition and clearance (e.g. age- and gender-specific anatomical and physiological parameters for particle deposition and modifying factors for conditions, such as pre-existing disease or a smoking habit, that influence particle clearance from the respiratory tract) [1].

Figure 1 shows the total and regional aerosol deposition as a function of particle size between 1 nm and 100 µm using the ICRP model. The curves are for a “reference person” either breathing through the nose or mouth, with 1/3 of the time spent sitting and 2/3 of the time spent undertaking light exercise (a standard workload [1]).



Key

X particle diameter, in micrometres (μm)

Y deposition fraction relative to ambient aerosol concentration, in percent (%)

1 total

2 extra-thoracic

3 tracheo-bronchiolar

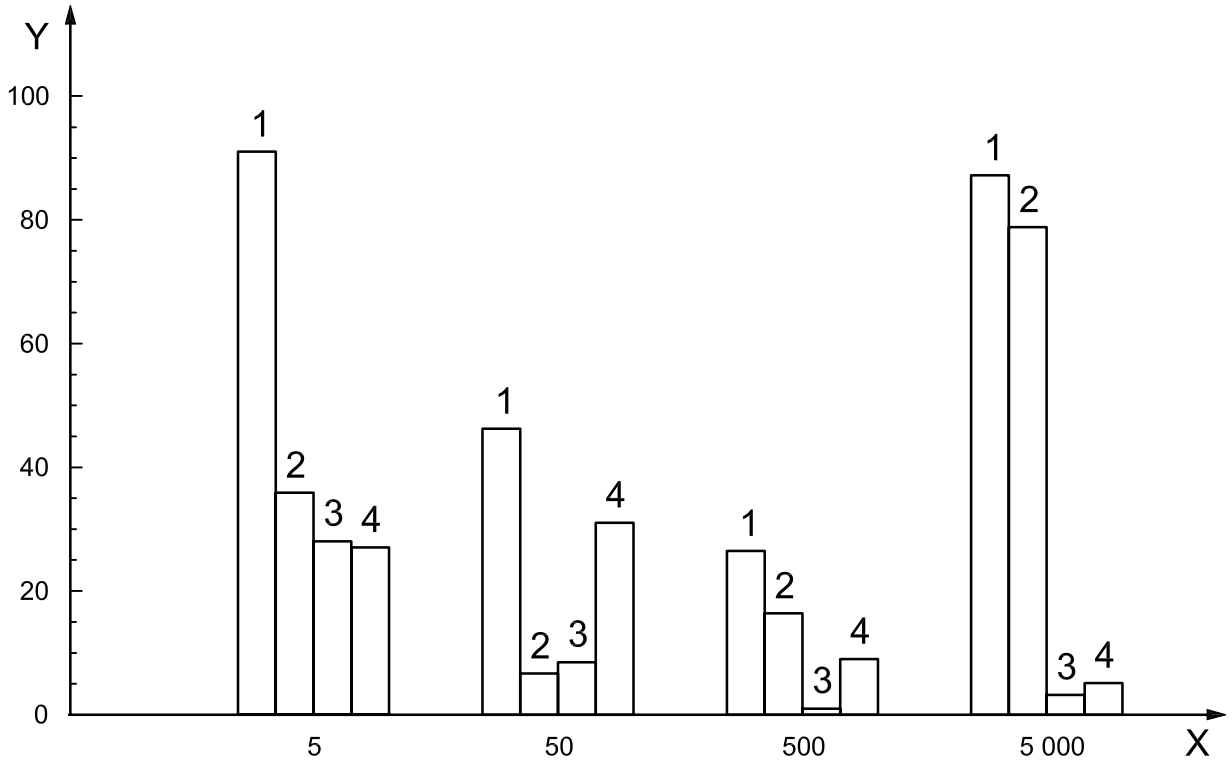
4 alveolar

Deposition fraction includes the probability of particles being inhaled (inhalability). The subject is considered to be either a nose breather (solid lines) or a mouth breather (dotted lines) and to be performing standard work [1]. Calculations were made using the LUDEP program [2].

Figure 1 — Predicted total and regional deposition of particles in the human respiratory tract related to particle size

As can be seen from Figure 1, total deposition reaches a minimum within the lung for airborne particles with an aerodynamic diameter of around 300 nm. At this size, particles are too large for diffusion to be effective and too small for impaction or interception to be effective. Below this minimum in deposition probability, predicted deposition increases as diffusional forces increase with decreasing particle diameter. Nanoparticles above 10 nm are deposited primarily in the alveolar region, while particles less than 10 nm have significant deposition in the head airways, and to a lesser extent in the tracheo-bronchiolar region, due to their very high diffusional mobility.

Figure 2 shows the predicted total and regional deposition for four polydisperse log-normally distributed aerosols [each having a geometric standard deviation (GSD) of 2] for a “reference” nose breather undertaking a “standard” workload.



Key

X count median particle diameter, in nanometres (nm)

Y deposition fraction, in percent (%)

1 total

2 extra-thoracic

3 tracheo-bronchiolar

4 alveolar

Deposition fraction includes the probability of particles being inhaled (inhalability). The subject is considered to be a nose breather and to be performing under “standard” working conditions [1]. Calculations were made using the LUDEP program [2].

Figure 2 — Predicted total and regional deposition of particles in the human respiratory tract for polydisperse aerosol with a given count median geometric standard deviation of 2

From Figures 1 and 2, it is clear that a substantial fraction of inhaled nanoparticles will deposit within the respiratory tract. For particles larger than 5 nm, deposition is predominantly in the alveolar region of the lungs. However, there is a significant predicted deposition probability for nanoparticles in the extra-thoracic and tracheo-bronchiolar regions, particularly for particles smaller than 5 nm. Particle deposition in these regions may be important in the development of airways diseases, such as chronic obstructive pulmonary disease (COPD) or asthma. In addition, once deposited in the respiratory tract, particles may be translocated elsewhere in the body. Experimental data confirm the trend of increasing deposition probability with decreasing particle size, and also demonstrate variations in deposition with breathing pattern and level of exertion [45][46]. Particle deposition probability has been shown to be higher among individuals with lung diseases, such as chronic obstructive pulmonary disease [47].

4.3 Transport of nanoparticles in the body

As described in the previous section, the deposition fraction of inhaled nanoparticles is greater in the alveolar and tracheo-bronchial regions of human lungs, compared to the larger-diameter inhaled particles. Once deposited, nanoparticles may also remain in the lungs longer than larger particles, due to decreased clearance and increased retention of nanoparticles. Studies in rodent lungs and cell cultures have shown decreases in phagocytosis (engulfment) and clearance of nanoparticles by macrophages, compared to the same mass of fine particles [48][49]. Some types of nanoparticles (e.g. titanium dioxide, carbon) have been shown to penetrate the epithelial cell barrier more readily and enter the lung interstitium or the blood circulation in rats or hamsters[39][50]. Once in the blood, nanoparticles may translocate to other organs in the body [40]. Rapid translocation of nanoparticles into the systemic circulation has been reported in humans using radiolabeled carbon particles < 100 nm [39][51]. The fate of inhaled nanoparticles may also depend on chemical composition; while nanometre scale carbon particles have been shown to translocate rapidly to blood and extrapulmonary tissue in rats [40], similar studies with iridium metal nanoparticles have shown only a small fraction of the particles translocating [31].

Nanoparticles may also translocate to the brain, as observed in rats, by a mechanism involving the translocation of nanoparticles along the olfactory nerve following their deposition in the nasopharyngeal region of the respiratory tract [30]. The significance of this path as a route of exposure in humans is unknown at this time.

Current human lung dosimetry models (e.g. ICRP 1994 [1], NCRP 1997 [44]) account for particle size in the predicted deposition fractions for the major regions of the respiratory tract (Figure 1). The particle size-specific effect on clearance is limited in these models, as the rates of clearance from the lungs or translocation to other organs are based on the mass of particles depositing within each of these major lung regions. Thus, due to the data available at the time these models were developed, the mass-based clearance rates do not distinguish between the clearance of nanoparticles and larger particles that deposit in a given lung region. This may be an area for further model development.

4.4 Physical behaviour of nanoaerosols

4.4.1 Formation

The ubiquitous way of nanoparticle formation is from the gas phase [52][53]. This process is characterized by an initial nucleation step, i.e. the formation of very small particle embryos from the molecular phase. These nuclei subsequently grow by coagulation and/or surface growth mechanisms (heterogeneous condensation, surface reaction). The details of the overall process depend very much on the amount of available condensable gaseous materials, their thermodynamic and chemical properties, as well as on the process conditions.

The aerosol is formed from precursor materials which are either vaporized from a liquid or solid reservoir or are existent as intimately mixed reactive gases. The formation of condensable vapour is achieved by cooling and/or by chemical reaction of the precursor gases resulting in supersaturations high enough for homogeneous nucleation to occur. At high nucleation rates where a high-density cloud of embryos is being formed, particle growth will be predominantly controlled by coagulation. This is the case, for example, for silica and welding fumes formed in flame and plasma processes. The temperature history of the process, as well as material parameters of the condensed aerosol matter (such as viscosity, surface and volume diffusion), are key parameters controlling the resulting particle morphology and internal structure. If the aerosol material is in

the liquid state all the time, the particles remain spherical upon coagulation due to rapid coalescence of the colliding droplets. If the intra-particle material flux is quenched throughout the process due, for example, to a sharp temperature drop in the region of particle formation, coalescence is inhibited. Eventually necks between colliding particles will form, resulting in aggregates with strong internal sinter bonds. At the low temperature end of the process, coagulation leads to agglomerates held together by relatively weak Van der Waals or electrostatic forces.

At low nucleation rates leading to small number concentrations of newly formed particles, direct heterogeneous condensation of the aerosol vapour on existing particle surfaces controls the dynamics of the aerosol size distribution. Examples are condensed organic vapours in fugitive emissions, such as asphalt fumes. Condensational growth can result in quite large particles with diameters outside the nanometre size region even if the total mass concentration is small.

Where homogeneous nucleation is concerned, the nucleation rate is a very steep function of the vapour saturation ratio and may change by orders of magnitude when the saturation ratio varies only slightly. From the perspective of uncontrolled particle formation in workplaces, it is often sufficient to consider homogeneous nucleation as an all-or-nothing effect taking place when the supersaturation reaches a critical value. This value is well above one, the saturation ratio necessary for heterogeneous condensation of vapour onto pre-existing surfaces. In many high-temperature processes, such as the flame production of commodities (for example, titanium dioxide by the oxidation of titanium tetra chloride), the reaction of the gaseous precursors leads to new compounds, the critical cluster size of which is extremely small, of the order of the molecular diameter. Here, nucleation is a purely kinetic process and the nucleation rate of the aerosol material is equal to the reaction rate of the gaseous precursors. Further particle growth is primarily by coagulation. The same situation holds for fume formation over hot liquid metals where the precursor is the metal vapour, which nucleates either by cooling or by oxidation in the air. These are very important mechanisms for nanoparticle formation.

When particles are already present in the air, heterogeneous condensation and nucleation compete for the available condensable vapour. Heterogeneous condensation of condensable species will prevail when the surface area available is larger than the one expected to be formed by homogeneous nucleation at the specific thermodynamic conditions. In this case, the formation of new (nanometre-diameter) particles can be inhibited. In contrast, if existing particles are removed from the air (for instance, by filtration), the surface area for heterogeneous condensation is reduced, leading to a possible enhancement of nanoparticle generation through homogeneous condensation of condensable vapours. Such events may occur in the workplace but are extremely difficult to predict in detail due to lack of information on the thermodynamic conditions and the chemical composition of the condensable vapour phase.

The kinetics of heterogeneous condensation of vapour onto pre-existing particles leads to an increase in the amount of condensed vapour material per particle mass with decreasing particle size (enrichment). Since the condensation rate of vapour molecules onto nanoaerosols is proportional to aerosol surface-area concentration, the content of adsorbed toxic material in the nanoparticle fraction is proportional to the specific surface area. This is of toxicological importance in view of, for example, polycyclic aromatic hydrocarbons (PAHs), heavy metals and other condensable toxic substances being found in increased concentrations in the nanoparticle mode, thus serving as an efficient transport vehicle for the respective substances.

4.4.2 Coagulation

In many cases of high-temperature formation of nanoparticles, binary coagulation is the dominant growth process determining the final size distribution. Key parameters controlling coagulation are the relative motion between the particles, their size and number concentration, as well as material properties determining the rearrangement of material inside the particles.

Solid nanoaerosols often appear as agglomerates composed of a large number of primary particles. Examples are diesel soot and welding fumes. The size of the primary particles is controlled by the temperature history of the growth process. In regions of high temperature, colliding particles will coalesce as long as the typical coalescence time between particles is small compared to the collision time and the residence time in the high-temperature zone. As soon as the system cools down, coalescence is quenched and the colliding particles retain their spherical shape and size. Aggregates and agglomerates are formed from these primary particles

upon further particle collision. The structure of the growing agglomerates is fractal-like, leading to aerosols with a high specific surface area.

Close to the respective sources for nanoparticles, for example the evaporation zone of the welding process, the mass concentration of condensed material will typically be much higher than 1 mg/m^3. Particles arriving at the breathing zone of the worker will have experienced many collision events and the coagulating aerosol size distribution will most likely have reached an asymptotic state. This state is characterized by a constant value of the geometric standard deviation and by the fact that the average diameter of the distribution is independent of the initial values of the number concentration and the size of the condensed nuclei. In the asymptotic state, the geometric standard deviation takes a value of approximately 1,35, which is reached earlier when the starting distribution is monodisperse than when the initial distribution is wide.

Under realistic workplace conditions, background dust originating from other sources is generally also present; nanoparticles can deposit onto this dust by diffusion. Since the diffusion coefficient of the nanoparticles is proportional to the inverse of the particle diameter squared, the strength of this loss mechanism decreases drastically with increasing particle diameter of the nanoparticles, resulting in much longer scavenging times. Thus, scavenging by the coarse dust and coagulation between nanoparticles are competing processes. Dust concentrations higher than 5 mg/m^3 are necessary in order for particles of 1 µm in diameter to act as efficient scavengers for nanoparticles.

4.4.3 Transport

For air velocities prevailing in workplaces, nanoparticles can be considered as having no inertia. Particle deposition on surfaces is mainly controlled by diffusion, turbulent diffusion and thermophoresis, although electrophoresis can be important in the presence of high electrostatic fields and charged particles. Deposition is quantified by the so-called deposition velocity, relating the particle flux onto the surface to the particle concentration above the surface. The deposition velocity depends on the particle size and on the structure of the turbulent boundary layer of the flow above the surface. In the small particle size regime and in stagnant air, the concentration boundary layer is formed by the Brownian particle motion only. The concentration gradient in this case is flatter than the average concentration gradient developing under turbulent conditions. Here, turbulent eddies enter the laminar boundary layer randomly, transporting the particles closer to the wall and, thus, reducing the distance for the particles to be surmounted by Brownian diffusion before reaching the wall. Therefore, the particle deposition velocity is larger for turbulent air flow.

In total, however, the nanoparticle (and ultrafine) size range is generally associated with very low deposition velocity to surfaces. Thus, once released in the air, nanoparticles in workplaces will have a long residence time.

4.5 Physiological basis for defining nanoparticles and nanoaerosols

4.5.1 General

Although nanoparticles are nominally defined as particles smaller than 100 nm, there is still insufficient information available on particle behaviour and biological interactions to standardize either the term “nanoparticle” or “nanoaerosol” in the context of human health impact. Three issues need to be resolved before normative definitions can be established.

4.5.2 Biologically-relevant definitions of particle diameter

Diameter is not an absolute measure of a particle’s characteristics, but is dependent on the measurement method. Particle penetration and deposition within the respiratory system are traditionally described in terms of particle aerodynamic diameter, defined as the diameter of a sphere with a density of 1 000 kg/m^3, which shows the same aerodynamic behaviour as the particle in question. However, below approximately 500 nm, particle behaviour is increasingly dominated by diffusion rather than aerodynamics. Thus, in the nanometre size range, it is more appropriate to define particle diameter in terms of diffusional behaviour. Electrical mobility diameter, defined as the diameter of a spherical particle with the same electrical mobility as the particle in question, gives a good representation of the diffusional behaviour and is relatively simple to measure. This would seem to be an appropriate definition for particle diameter in the nanometre size region

where deposition probability and subsequent biological interactions are governed by a parameter equal to or approximated by electrical mobility diameter. However, it is conceivable that the shape, and possibly the density, of some particles will lead to behaviour in the respiratory tract that is not predicted well by electrical mobility diameter. For instance, it is still unclear how electrical mobility diameter is related to the physical characteristics of carbon nanotube particles and how they will behave following inhalation.

4.5.3 Biological significance of particle size

A nominal cut size for nanoparticles is 100 nm, although this is generally unrelated to particle behaviour in the respiratory tract following deposition. It is possible to develop a health-related definition of a nanoparticle based on deposition probability in the lungs [see the two curves (for nasal and oral breathing) for alveolar deposition in Figure 2].

In addition to this, a relevant definition may also need to account for how size and other physicochemical characteristics affect particle translocation following deposition. Below a particle diameter of 200 nm to 300 nm, physical properties do not just scale with size; they change. As particles become smaller, surface curvature, the arrangement (and percentage) of atoms on the particle surface and size-dependent quantum effects, such as quantum confinement, play an increasingly significant role in determining behaviour. These properties may be expressed in particles significantly larger than 100 nm which have a sub-100 nm structure, such as agglomerates of nanoparticles with low fractal dimensions.

4.5.4 Significance of nanoparticle agglomeration/aggregation

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 volume of material. 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. Likewise, 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/aggregates with an open fractal-like structure will conceivably be similar to that from an equivalent dose of discrete particles. On the other hand, if biological interactions following deposition are governed by particle diameter (as would seem to be the case with particle translocation), it is likely that the response to discrete nanoparticles depositing in the respiratory tract will differ from that to an equivalent dose of agglomerated/aggregated particles that do not separate on deposition. A normative health-related standard defining nanoparticles and nanoaerosols needs to be inclusive of discrete nanoparticles and agglomerates/aggregates of nanoparticles where health effects resulting from exposure are similar, but exclusive where resulting health effects are dependent on the size of particles depositing in the respiratory system.

4.5.5 Summary

Until clarification is available on these three issues, it would seem prudent to retain nominal definitions of a nanoparticle as one with a diameter smaller than 100 nm, and of a nanoaerosol as an aerosol comprised of discrete or grouped nanoparticles or nanostructured particles. However, there is a need to be aware that these definitions may not be suited to every occasion and will need to be modified to account for new information and specific particle properties, most notably as research on engineered nanomaterials begins to provide greater insight into the potential hazards they may represent.

5 Sources of occupational nanoaerosols

The greatest prevalence of nanoaerosols within workplaces is associated with particle formation through nucleation and condensation. Hot processes (such as metal refining and processing, thermal metal spraying, welding, gouging and metal grinding) all lead to the generation of metal and/or metal oxide particles with small particle sizes, high specific surface areas and in many cases low solubilities. Combustion also leads to the generation of nanoparticles through vapour reactions and nucleation/condensation. Particle size is dependant on the generating conditions, although primary particles will generally have a modal diameter between 10 nm and 50 nm. These coagulate together rapidly where high particle concentrations are initially generated, forming agglomerates that may lie outside the nanometre size region. However, it is likely that open (fractal-like) agglomerates of these primary particles will demonstrate many similarities to nanoparticles in how they behave following deposition in the respiratory tract. Particles generated from point sources (such as welding) are more likely to undergo rapid coagulation, while disperse sources (such as metal refining) will lead to rapid quenching of the coagulation process in many cases, resulting in a higher fraction of the generated particles lying in the nanometre size range. Simple estimates show that a 50 % reduction in particle concentration is expected within 20 s due to coagulation at a concentration of 10^{14} particles/m^3, while the same reduction will take closer to 55 h at a concentration of 10^{10} particles/m^3 [28].

A further group of hot processes designed to generate aerosols having a high specific surface area include the formation of carbon black, nanoscale TiO_2, fumed alumina and fumed silica. Although the products resulting from these processes are powders with particle agglomerates larger than 100 nm, specific surface areas may be in excess of 300 m^2/g. Other high specific surface-area materials, such as ruthenium black and palladium black (and including some high specific surface-area forms of TiO_2), are produced using wet chemistry but are handled and used as a dry powder.

In addition to established technologies and processes associated with nanoparticles, the emerging field of nanotechnology is leading to the introduction of new processes and materials associated with unique nanoparticles. Nanotechnology relies on the unique physical and chemical properties of materials and devices with nanometre-scale structures and is frequently associated with the generation and use of nano-colloids and nanoparticles. Potential applications of the new technology are widespread, ranging from next generation electronics to medical imaging, drug delivery and cosmetics. Commercial interest in the technology is intense, with a wide range of industries beginning the process of scaling up for commercial production and introducing nanotechnology to a new generation of commercial and consumer products. Many of the raw particulate materials associated with nanotechnology are insoluble particles in the nanometre size region with unusual morphologies and active surfaces.

If materials consisting of or containing nanoparticles are being handled, consideration needs to be given to the likelihood of nanoaerosols being released into the air as the materials are handled and used (their “dustiness”). Powders of nanoparticles will release agglomerates and possibly discrete particles when handled. The rate of nanoaerosol release will depend on the degree of agitation during handling and the nature of the powder. Although very little is known about the release of nanoaerosols from powders, it is likely that the physical and chemical nature of the material will play a significant role in determining how easily a powder is aerosolized.

Nanoparticles embedded in a solid matrix are unlikely to be released during handling, although it is possible that if the matrix is subject to high mechanical and thermal energies, such as when being cut or ground, nanoaerosols may be released. Liquid suspensions of nanoparticles will not lead to inhalation exposure directly. However, fine sprays from the suspension will lead to airborne nanoaerosols. Particles may in principle also be re-suspended from dry deposits, although it is questionable whether disturbances and air movements leading to re-suspension will be sufficient to release large quantities of discrete airborne nanoparticles. Similarly, re-suspension from deposits of nanoparticle powders may lead to nanoaerosol exposure in some cases.

Examples of potential sources of occupational nanoaerosols by aerosol group are given in Table 1.

Table 1 — Examples of potential sources of occupational nanoaerosols

Aerosol group	Source
Hot processes	Metal refining — general
	Aluminium smelting
	Steel smelting
	Iron smelting
	Galvanizing
	Welding
	Gouging
	Metal cutting — thermal torch
	Metal cutting — laser
	Thermal spray coating
	Cooking
	Hot wax application
Combustion	Diesel engines
	Gasoline engines
	Gas-based engines
	Incineration (e.g. power plants, heating, cremation)
	Gas-fired heating
Indoor air quality-related aerosols	Aerosol formation through reaction between gas/vapour emissions from office machinery, cleaning fluids and building materials, and water, ozone and other gases/vapours.
	Infiltration of ambient nanoaerosols
Mechanical processes	High-speed metal grinding and machining
	High-energy drilling
Flame-based powder generation	Carbon black production
	Ultrafine TiO_2 production
	Fumed silica production
	Fumed alumina production
Material handling	Handling unprocessed nanoparticle powders
	Handling dry colloidal deposits
Nanotechnology	Carbon nanotube production
	Gas-phase generation of engineered nanoparticles
	Handling and using engineered nanoparticle powders
	Sprays from engineered nanoparticle suspensions, solutions and slurries

6 Characterizing exposure to occupational nanoaerosols

6.1 Exposure assessment strategies

6.1.1 Introduction

The use of alternative aerosol exposure metrics needs to be considered where there are possible associations between health effects and aerosol parameters other than mass concentration. Some of these exposure quantities are not easy to measure directly and may need to be estimated from parameters that can be measured more easily. The majority of instrument or detection methods are limited to use over a longer period of time, for instance, a full shift. Moreover, most of the measurement and sampling instruments available are not well suited for use as personal devices.

The assessment and characterization of personal occupational exposure to nanoaerosols by direct measurement is restricted by a lack of suitable instruments. However, the combined use of static measuring devices for *in situ* detection and off-line analysis of samples, partly collected by short-term personal sampling as part of an appropriate sampling strategy, could be helpful in providing adequate data on personal exposure to nanoaerosols.

The use of data obtained with static samplers and short-term sampling for assessing personal exposure is confounded by changes in aerosol characteristics with distance from source, leading to spatial and temporal variation of nanoaerosol mass and number concentration. Since hot processes leading to particle nucleation from vapour will often lead to variations in emission rate and concentration over time, a temporal variation of mass and number concentration and particle size distribution is likely to occur. This variation over time will also contribute to spatial variations, as will multi-source emissions in workplaces and air movements due to forced ventilation. Moreover, workers often move from area to area, resulting in complex patterns of residence at selected sampling locations. Therefore, fixed-location data points on the characteristics of nanoaerosol exposure cannot be translated to personal exposure without careful considerations.

To improve the comparability of exposure data, the accepted practice of giving personal exposure as an eight-hour-shift value [54] should also be observed in the case of nanoaerosols. 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.

6.1.2 Considerations for exposure assessment strategies

Since personal exposure estimates will be obtained mostly from data obtained using static samplers, some aspects of a sampling strategy should be considered prior to and during exposure assessment. Table 2 summarizes these aspects and gives examples of useable tools.

Selection of an appropriate sampling location or locations is a key factor for a reliable interpretation of data in view of personal exposure. Firstly, nanoaerosol-emitting sources in the workplace should be identified. For single and multiple sources, the relationship between aerosol emission and work activities should be clear. In addition, it should be checked whether outdoor sources of nanoaerosols (other industrial activities, traffic, etc.) affect indoor aerosol concentration. Background concentrations should be determined. Portable instruments to measure particle number concentration such as condensation particle counters (CPC) can be used for this purpose, assuming there is a good relationship between aerosol emission in the size detection range of the CPC and the airborne nanoparticles.

Secondly, actual air flow patterns should be known, and ideally monitored, as they will determine the spatial as well as the temporal variation of aerosols. Visualization of air flow patterns is preferable. However the most appropriate tool, a smoke generator or tube, cannot be used concurrently with measurements since it is an aerosol generator in its own right. During the measurements, air flow patterns can also be determined by plotting results of air velocity measurements, preferably performed with directional anemometers. However, this is a time- and effort- consuming approach. Results of ventilation measurements using a tracer gas method provide some general information on air movements, for instance air exchange rates, but no specific

information on air flow directions and fluctuations. Air flow pattern characteristics, e.g. direction of air flow relative to source and sampling location, are essential for either selection of a sampling location or interpretation of worker exposure. In general, the sampling location should reflect the location of the worker as well as possible, although size of equipment may hinder the worker and another position (upstream or downstream from the source) may have to be selected.

During exposure assessment, all workplace activities should be observed to provide additional information that can be helpful to interpret results of static samplers. This could include the presence of additional primary aerosol-generating activities, for instance the use of a hand-held tool, fork truck engine exhaust, smoking or secondary sources like re-suspension of deposited aerosols by person, activity- or vehicle- induced air movements. Although re-suspended dusts are likely to be dominated by relatively large particles, they may play a role in scavenging nanoparticles from the air.

Finally, changes in the worker's position relative to source and sample location within the time window of sampling should be monitored. Weighted results of multiple sampling locations may be used for estimating personal exposures.

Table 2 — Considerations prior to and concurrent with sampling

Item	Aim	Tools
Source	Identify and locate single/multiple nanoaerosol-generating source(s) in the workplace, or identify penetration of ambient aerosols in the workplace to select sampling location(s)	Condensation particle counters (CPC); recording of observations on emission-generating activities
Ventilation	Record air flow patterns and transmission of aerosols through the workplace	Smoke tubes; anemometers; tracer gas Observation and registration of opening doors, etc.
Workplace activities	Interpretation of data of direct recording instruments in view of variations in exposure parameters	Observation forms: recording of observations on emission-generating activities; CPC
Worker behaviour	Interpretation of spatial differences in view of time of residence at different locations	Observation forms: recording of observations on workers' position to source/sample location

6.1.3 Sampling

As described in 6.1.1, no specific personal devices are available to determine all relevant exposure characteristics for nanoaerosols. Table 3 summarizes the readily available instruments and techniques for characterizing nanoaerosols.

For mass concentration measurements, there are personal devices that can be used to determine time-weighed mass concentration. However, there is no personal device currently available that allows airborne nanoparticle mass concentration to be monitored exclusively. Therefore static or portable samplers have to be used if just the nanoparticle fraction is to be measured. Characterizing sample location with respect to source, air movement and position of the worker is essential for relating results to personal exposure.

For estimates of surface area concentrations some instruments are discussed in 6.2.3. However, approaches that use the results of a number of measurements are capable of providing initial data on the magnitude of surface area exposure with minimal additional effort.

Table 3 — Readily available instruments and techniques for monitoring nanoaerosol exposure

Metric	Devices	Remarks
Mass	Size-selective personal sampler	No current devices offer a cut point of 100 nm. Off-line gravimetric or chemical detection is necessary. Mass may also be derived from size distribution measurements (see below).
	Size-selective static sampler	The only devices offering a cut point around 100 nm are cascade impactors.
	TEOM®1)	Sensitive real-time monitors such as the Tapered Element Oscillating Microbalance (TEOM®) may be useable to measure nanoaerosol mass concentration on-line with a suitable size-selective inlet.
	SMPS	Real-time size-selective (mobility diameter) detection of number concentration. Data may be interpreted in terms of aerosol mass concentration, only if particle shape and density are known or assumed.
	ELPI	Real-time size-selective (aerodynamic diameter) detection of active surface-area concentration. Data may be interpreted in terms of mass concentration if particle charge and density are assumed or known. Size-selected samples may be further analysed off-line.
Number	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 (no suitable pre-separators are currently available).
	SMPS	Real-time size-selective (mobility diameter) detection of number concentration.
	ELPI	Real-time size-selective (aerodynamic diameter) detection of active surface-area concentration. Data may be interpreted in terms of number concentration. Size-selected samples may be further analysed off-line.
	Optical Particle Counter	These are insensitive to particles smaller than approximately 100 nm to 300 nm in diameter and therefore unsuitable for nanoparticle monitoring.
	Electron Microscopy	Off-line analysis of electron microscope samples can provide information on size-specific aerosol number concentration.
Surface area	SMPS	Real-time size-selective (mobility diameter) detection of number concentration. Data may 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 [3].
	ELPI	Real-time size-selective (aerodynamic diameter) detection of active surface-area concentration. Active surface area does not scale directly with geometric surface area above 100 nm. Size-selected samples may be further analysed off-line.
	SMPS and ELPI used in parallel	Differences in measured aerodynamics and mobility diameters can be used to infer particle fractal dimension, which can be further used to estimate surface area.
	Diffusion Charger	Real-time measurement of aerosol active surface area. Active surface area does not scale directly with geometric surface area above 100 nm. Note that not all commercially available diffusion chargers have a response that scales with the particle active surface area below 100 nm. Diffusion chargers are only specific to nanoparticles if used with an appropriate inlet pre-separator.
	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 may be related to the geometric area for some particle shapes.

To identify the chemical and physical nature of nanoaerosols, off-line analysis is possible. Electron microscopic analysis (SEM and TEM) may be used to characterize particles according to structure, size and morphology. However, such analyses are very sensitive to the level of sample loading, and thus require careful consideration of the sampling time needed (see Annex A). The use of personal samples for off-line analysis is a possible alternative, since this has the advantage that sampling from the breathing zone can easily be adjusted to the maximum level of loading that can be analysed. Electron microscopy is often available with energy-dispersive X-ray or spectrometry detection system for further chemical speciation.

6.1.4 Miscellaneous

Other aspects of sampling strategy, apart from selection of sampling techniques and sampling locations, i.e. selection of persons or work stations to be sampled, timing and duration of sampling, depend very much on the availability and characteristics of instruments, known or foreseen variation of nanoaerosol source strengths and resources for investigation.

6.1.5 Exposure assessment strategies — Summary

Each of the measurement methods that have been discussed previously has its drawbacks, but when used in combination they may give an initial insight into the presence of nanoaerosols in the workplace. Sources of nanoparticle emission can be identified, estimates of size-selective particle number concentrations and surface area can be made and, based on collected (grab) samples, identification of some characteristics of nanoparticles is possible. Field observations are crucial in order to link the results to the various events during the measurements.

Studies have demonstrated that there are spatial variations in both particle number concentration and size distribution in the workplace. Therefore, the use of static samplers at fixed locations restricts the interpretation of the results for personal exposure of ambulatory workers. Even for workers who are positioned at fixed work-stations, interpretation of static samples can be very inaccurate. However, the presence of nanoaerosols in the workplace and the potential for exposure may be demonstrated by an approach as outlined above.

6.2 Particle ensemble characterization methods

6.2.1 General

Ensemble aerosol characterization and monitoring methods respond to many particles simultaneously, either in real-time, or using off-line analysis [55]. They frequently present the most appropriate and cost-effective method for monitoring aerosol exposures using compact and robust instruments. However, by definition the measurements made represent aerosol properties averaged over all particles, and thus the approach may not be suited to every situation.

6.2.2 Mass concentration

Although the available evidence suggests that mass concentration is not an appropriate exposure metric for many nanoaerosols, guidance is required on mass-based techniques where continuity with current sampling conventions and methodologies is necessary. When the aerosol size fraction of interest is known, a pre-separator is needed which allows this fraction to penetrate and remove particles of other sizes from the air stream. The subsequent analysis is often independent of the pre-separator, allowing the most appropriate analytical method to be chosen. For instance, the analytical stage can consist of a *post festum* determination of the particulate mass, surface area or number, of a continuous optical monitor or of a semi-continuous analyser. In some cases, it is possible to select an analytical method which itself effects the pre-separation.

If aerosol surface-area concentration is the quantity of interest, and if the surface area is dominated by the accumulation mode, then the sub-micrometer aerosol mass concentration will also be dominated by the accumulation mode. In this case, it would seem reasonable to sample the aerosol with a 1 µm cut-point pre-separator. However, if there is specific interest in particles of less than 100 nm, one needs a pre-separator and an analytical technique adapted for this requirement.

It is expected that the mass concentrations of nanoparticles will be low, at least in comparison with the corresponding respirable fraction. This would also be true for thermally generated nanoaerosols (for instance, metal fumes), with most of the mass concentration associated with particles smaller than 1 µm. High sampling flow rates will therefore be required to collect sufficient material for subsequent analysis, and it is doubtful if even the best personal sampling pumps presently available (with a maximum flow rate of 10 l/min to 15 l/min) can be used other than in specific sampling situations, for instance, when the analytical method has a very low limit of quantification, perhaps consisting of a direct-reading monitor.

Mass sampling of nanoaerosols will thus require stationary high flow pumps in order to collect samples larger than the limit of quantification of the analytical method. Though such pumps are heavy, bulky and require 115 V to 230 V AC, the sampler (pre-separator and substrate holder) may be relatively compact if it consists of a single stage with a cut-size of 100 nm. This will facilitate positioning the sampler close to the worker's breathing zone in some situations.

At the present time, there are no commercially available occupational aerosol samplers with a 100 nm cut point. It is possible in principle to operate existing devices, such as impactors and cyclones, at sufficiently high sampling rates to provide a cut point at 100 nm, although sampler operation under such conditions needs to be verified. An alternative is to use a low-pressure cascade impactor with a stage cut point at 100 nm.

6.2.3 Surface-area concentration

Although off-line measurements of bulk material surface area have been possible for some time using the BET method [33], instruments capable of measuring aerosol surface area in the field are not widely available at present. BET has been used with some success for measuring aerosol surface area. However, it requires the collection of relatively large amounts of material, and measurements are influenced by particle porosity (which may or may not be important) and collection/support substrate – particularly where the quantity of material analysed is small. The first instrument designed specifically to measure aerosol surface area was the epiphaniometer [56]. This device measures the Fuchs or active surface area of the aerosols by measuring the attachment rate of radioactive ions. As yet, it is unknown how relevant active surface area is to health effects following inhalation exposure. Below approximately 100 nm, active surface is a function of the square of particle diameter, and thus is probably a good indicator of external surface area for nanoparticles. However, above approximately 1 µm, it is a function of particle diameter, and so the relationship with actual particle surface area is lost [57]. Measurements of active surface area are generally insensitive to particle porosity. The epiphaniometer is not well suited to widespread use in the workplace due to the inclusion of a radioactive source.

The same measurement principle may be applied in the aerosol diffusion charger/electrometer. Diffusion charger aerosol surface-area monitors use this combination to measure the attachment rate of positive unipolar ions to particles, and from this the aerosol active surface area is inferred [58]. Following charging, usually using a corona discharge, the aerosol is collected onto a HEPA filter within a sensitive electrometer and the aerosol charge per unit volume of air sampled measured. Other forms of aerosol charging may be used to elucidate information on aerosol surface chemistry. For instance, photoelectric charging using a UV light source enables quantification of attached polyaromatic hydrocarbons [59][60].

Reliance on charging has the potential to lead to sampling errors if the sampled aerosol is already charged, or if the probability of multiple charges per particle is high. Extensive field evaluations of commercial instruments are rare [61]. However, laboratory evaluation of a commercial diffusion charger has shown measurements to correlate well with Transmission Electron Microscopy-derived aerosol surface area for particles below 100 nm mobility diameter [62]. One particular configuration of an aerosol charger has been shown to provide a measurement that correlates well with deposited aerosol surface area in the lungs [63]. Above 100 nm, surface area is increasingly underestimated by the diffusion charger. An alternative method to measuring aerosol surface area is to measure the aerosol size distribution, and to estimate the surface-area-weighted distribution by assuming a specific particle geometry. Details of suitable measurement methods are given in 6.3.

Assuming that the aerosol size distribution is well represented by a unimodal lognormal distribution, it should be possible to characterize the distribution and thus derive the surface-area concentration using just three independent measurements. A suitable approach has been proposed using three simultaneous measurements of aerosol mass concentration, number concentration and charge [64]. With knowledge of the response function of each instrument, minimization techniques may be used to estimate the parameters of a

lognormal distribution leading to the three measurements, and from this aerosol surface area may be estimated. The technique has the advantage that measurements of aerosol number, surface area and mass concentration are made simultaneously. However, assumptions need to be made of the charge distribution on the particles being sampled.

An alternative approach has been proposed whereby independent measurements of aerosol number and mass concentration are made, and the surface area is estimated by assuming the geometric standard deviation of the (assumed) lognormal distribution [65]. This method has the advantage of simplicity and relies on portable instruments that are finding increasing application in the workplace. Theoretical calculations have shown that estimates may be different from the actual aerosol surface area by up to a factor of ten, particularly when the aerosol is bimodal rather than matching the assumed unimodal distribution. Field measurements indicate that much of the time estimates are within a factor of three of the active surface area, particularly at higher concentrations. As occupational aerosol surface-area concentrations can be expected to span up to five orders of magnitude, surface-area estimates within such confidence limits may be suited to initial or preliminary appraisals of exposure levels.

6.2.4 Number concentration

6.2.4.1 General

The number concentration measurement of nanoaerosols requires a detector that is sensitive down to particle diameters of a few nanometres. In all aerosol number concentration measurements, the integration limits over which a particular instrument operates are critical in understanding the reported results. Many instruments become increasingly insensitive to particles smaller than 10 nm to 20 nm. Concentrations measured with instruments with different sensitivities to particles in the size range 1 nm to 10 nm may therefore differ. If the aerosol count median diameter lies below 10 nm, such instruments will significantly underestimate the aerosol concentration. In cases where the aerosol particle number is dominated by nanoparticles, the total number concentration is a relatively good indicator of the nanoparticle concentration. However, if high number concentrations occur in larger size ranges, these particles need to be excluded from the total number concentration if the nanoparticle number concentration is to be estimated. An exception to this may be where nanoparticle agglomeration occurs and there is a need to sample agglomerates, as well as discrete nanoparticles. In all cases, it is advisable to have some understanding of the likely size range of the aerosol being measured and of the response range of the instrument before interpreting aerosol number concentration measurements.

6.2.4.2 Condensation particle counters

The most widely used instrument for detecting nanoparticles is the Condensation Particle Counter (CPC), which exploits vapour condensation on nanometre size (and larger) particles in order to grow the particles to a size range that can be detected optically [66].

CPC techniques can be divided in three main categories:

a) convective cooling laminar flow CPCs;

b) expansion type CPCs;

c) turbulent mixing type CPC.

In all of these techniques, the aerosol is brought into contact with supersaturated vapour which condenses onto the particle surfaces. The particles grow rapidly to large droplets, typically several micrometres in diameter and can be then detected using optical methods.

The convective cooling laminar flow CPC is widely used and also commercially available in several models and from a number of manufacturers. The instrument has a constant sample 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 flow where 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 supersaturation), flows and geometries of the instrument. Devices using butanol are available with detection limits of 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. One of the main concerns is that the instrument has to be operated at the region where homogeneous nucleation of the working fluid does not occur. The instrument can be used in connection with size-classifying instruments, such as a Differential Mobility Analyser (DMA), a Scanning or Stepped Mobility Particle Sizer (SMPS) or a diffusion battery, to determine aerosol size distribution.

One of the earliest aerosol detectors was the expansion type CPC. In this instrument, the aerosol is sampled inside a cylinder, in which the sample air is saturated by wetted surfaces. Rapid expansion of the cylinder causes the supersaturation and consequently activates and grows the particles. This instrument does not operate continuously, but instead in pulses. However, the pulse rate can be several hertz and real-time concentration data can be obtained. This instrument is often used with water as the working fluid. Therefore, it has advantages in that it is cheap and safe to operate.

Mixing type CPCs are based on turbulent mixing of cool aerosol sample flow and warm condensing vapour flow. This technique allows continuous determination of aerosol number concentration and the instrument can be used in connection with size-classifying instruments (such as a DMA, an SMPS or a diffusion battery) to determine size distribution. The principle advantage over laminar flow CPCs is the rapid response time of the instrument.

6.2.4.3 Electrometer

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

6.2.4.4 Other methods

Particle counters (e.g. CPC) need to be used in conjunction with other methods to provide information on the relative contribution of nanoparticles to total aerosol concentration. These methods include the use of a DMA, an SMPS or a diffusion battery in series with the particle counter. Another possibility is to use a pre-separator with a cut-off size at about 100 nm.

One practical solution for separating the nanoparticles from larger particles is to use a CPC in parallel with an Optical Particle Counter (OPC) that is able to detect particles larger than 100 nm to 300 nm. The difference in the readings between these two types of instruments will give information on the concentration of the nanoparticles.

6.3 Size-resolved characterization

6.3.1 Measuring size distribution using particle mobility analysis

The dominant instrument used for measuring aerosol size distributions in the nanometre region is the Scanning or Stepped Mobility Particle Sizer (SMPS) [55]. The SMPS 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. Particles are given a known charge, then passed through a well-defined electrostatic field. Electrostatic forces lead to charged particles moving between the electrodes, and particles with a specific mobility are sampled from the exit of the electrodes and counted. By scanning 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. In an alternative configuration, the voltage between the electrodes may be stepped rather than continuously scanned. The aerosol particles are typically given a known charge by

passing them through a bipolar ion cloud formed from a radioactive source. Charging in a unipolar ion cloud may also be used, although this tends to lead to greater uncertainty in the charge acquired by particles of a given diameter. Particles selected by the electrostatic field are counted by using either a CPC or an electrometer.

The SMPS has the advantage that the mobility diameter is approximately equivalent to the projected-area diameter of particles (defined as the diameter of a sphere with the same projected area as the particles being sized) with compact geometries or fractal dimensions less than 2. In these cases, transformation of the size distribution to a mean aerosol projected surface area does not require further assumptions about particle shape, allowing reasonably accurate determination of aerosol projected surface-area concentration from measured size distributions. When concentric cylindrical electrodes are used, the active surface area of selected particles is directly proportional to the applied voltage between the electrodes, enabling the relatively simple determination of active surface area from measurements [62]. To enhance the comparability of reported SMPS data, further efforts should be made to standardize the operating conditions (e.g. integration limits) of the instruments and to conduct inter-comparisons [67].

Widespread application of the SMPS in the workplace is limited 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. Recently developed mobility diameter-based instruments using a parallel array of electrometers to measure size distribution provide the means of measuring distributions very rapidly without the need for a radioactive source. Sometimes referred to as “Fast Mobility Particle Sizers”, these instruments initially charge the aerosol using a unipolar charger and measure the mobility diameter distribution with a parallel array of electrometer-based sensors. Measurements may be made with a time resolution of one second or less, and operation at ambient pressures reduces evaporation of volatile particles. The instruments are limited to measurements at relatively high aerosol number concentrations, although the lack of a radioactive source may make them a viable alternative to the SMPS in many workplaces. Research has also been conducted on developing a compact aerosol mobility classifier relying on particle migration across an opposing air flow [68].

6.3.2 Measuring particle size distribution using inertial deposition

Inertial impactors with sequentially decreasing cut points may be used in series, or cascaded, to measure the aerosol mass concentration within specific size ranges, or to measure the aerosol size distribution [69]. Cascade impactors are widely available in a number of configurations, allowing either personal or static sampling with a range of particle size cut points. Personal cascade impactors are available with cut points of 250 nm and above, and thus are only able to provide very limited information on size distribution in the nanometre size range. Static cascade impactors are available with lower cut points in the nanometre size region, with some having a lower cut point at 10 nm. Determination of aerosol size distribution in the nanometre size region using cascade impactors is critically dependent on collecting sufficient material on lower stages to allow quantification, while avoiding overloading upper collection stages. Overloading leads to the impactor stage selection characteristics being compromised and the possibility of deposited material being re-suspended and carried over to subsequent collection stages. Particle bounce can also lead to larger particles migrating to lower impactor stages, thus skewing measured size distributions. Common approaches to avoiding overloading are to use multiple-orifice collection stages, rotating collection substrates, and coated and/or porous collection substrates. An alternative approach to avoiding overloading on upper stages while collecting nanoparticle samples is to use a high capacity pre-separator to remove large particles prior to sampling with the cascade impactor.

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 using the 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. A better idea of the size distribution can be obtained by differentiating the cumulative distribution. However, to take full account of the sampling characteristics of each sampling stage, more complex data inversion routines must be applied [70]. These generally require *a priori* assumptions concerning the nature of the size distribution (for instance, the number of modes), and may not provide information that is more representative of the actual aerosol size distribution under all circumstances.

As cascade impactors are usually used to measure the mass-weighted aerosol size distribution, 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.

6.3.3 Electrical low pressure impactor 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 [71]. However, the data from the lowest stage have relatively large uncertainty due to losses and uncertainties of the true size channel width. The upper size limit of the instrument is 10 µm, but in practice, reliable data can be obtained only up to about 2,5 µm due to significant losses at larger particle sizes. Aerosol particles are charged in a unipolar ion charger before being sampled by a cascade impactor. 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 (6.2.3), 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 the effective particle density as a function of size to be known.

As well as allowing on-line measurements of particle concentration and size distribution using a range of metrics, aerosol samples collected by the ELPI are available for off-line analysis. The possibility to examine collected particles post-sampling is useful for validating and calibrating on-line measurements of size distribution and concentration. It also enables a range of off-line analytical methods to be used with samples, including electron microscopy and chemical speciation.

6.3.4 Diffusion batteries

The operation of diffusion batteries is based on the Brownian motion of the aerosol particles [66]. Depositional losses through diffusion are a function of particle diameter; by measuring diffusion-based deposition rates through systems with varying geometries, it is possible to determine particle size distribution. The deposition systems are usually placed together in series to form a diffusion battery. Diffusion batteries operate at the size region where the diffusion of particles is significant and therefore the best information can be obtained for particles smaller than about 100 nm, corresponding rather well with the need to determine nanoparticle size distributions. The primary property measured is the diffusion coefficient of the particles and this has to be converted to particle diameter. Diffusion batteries enable continuous measurements to be made. However, the instrument needs to be operated with a particle counter (typically a continuous flow CPC) in order to determine the number concentration before and after each diffusion stage.

Particle diffusion is a well-known phenomenon and it is straight-forward to design a diffusion battery with the desired properties. The diffusion battery can be designed for determination of particle sizes of less than 100 nm in diameter. However, inversion of the raw data to real size distribution is complex and the solutions of the equations do not give unambiguous results in the case of polydisperse aerosol size distributions.

6.4 On-line chemical analysis

Over the past 10 to 15 years, advances in computing and optics have enabled the development of a class of instruments capable of providing on-line size-resolved chemical speciation of aerosols [72]. While a number of laser-based techniques are used in research, aerosol mass spectrometry is the predominant method to have been commercialized.

Although there are variations in operation and analysis methods, these instruments generally have a number of features in common. Initially, the aerosol is sampled through an inlet where the air is removed and a particle beam formed. This is usually achieved by passing the aerosol through a series of aerodynamic lenses that lead to progressively lower vacuum regions of the instrument. Secondly, the velocity of individual particles is

measured and related to aerodynamic diameter. This is achieved by either measuring the time of flight of individual particles between two laser beams, or selecting particles with a specific velocity using beam choppers. Thirdly, individual particles or ensembles of particles are vaporized and subsequently ionized. Vaporization and ionization are commonly carried out using high-energy laser pulses. This approach is typically used in instruments that analyse single particles. In an alternative approach, ensembles of particles with the same aerodynamic diameter are impacted on a heated block, where volatile components are thermally vaporized, and subsequently ionized by electron impact. Finally, ions are analysed using mass spectrometry to provide chemical information; by correlating the analysis with particle diameter, near-real-time size-specific analysis is possible.

Commercial aerosol mass spectrometry instruments are generally restricted to analysing particles larger than 100 nm in diameter, although methods analysing ensembles of size-differentiated particles are capable of providing information on particles smaller than 50 nm in diameter. With modifications to aerosol beam focusing systems, it is possible to extend these instruments well into the nanometre size region, although there is usually a trade-off between lower particle detection limit and particle losses in the collection system.

These and similar instruments are limited in their usefulness in characterizing nanoaerosols in the workplace, due to their size, cost and complexity. However, they are capable of providing unique compositional information on single particles or ensembles of size-selected particles. In this respect, they are ideally suited to investigations where there are multiple aerosol sources with different chemical signatures, or situations where particle composition is size-dependent.

6.5 Single particle analysis

6.5.1 General

Off-line single particle analysis is a powerful tool for obtaining size, shape, structure and, in some cases, compositional information from aerosol particles [73]. Optical microscopy has been widely used for examining micrometer-diameter particles in the past and is still a key tool for characterizing fibrous particles, such as asbestos. However, particles within the nanometre size region lie beyond the scope of most optical methods. Electron microscopy on the other hand allows the detailed characterization of particles at sub-nanometre spatial resolution, as well as providing a wide range of analytical methods for spatially resolved chemical speciation. Drawbacks of the technique include the need to employ specialist collection techniques and extensive specimen preparation requirements (see Annex A), the need to analyse samples in a vacuum in most cases and the time-consuming nature of the analysis process. However, there are situations where the information available using electron microscopy outweighs the disadvantages. Two generic techniques are generally available: Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM). The latter frequently offers the simplest approach to sample preparation and analysis, and allows topographical imaging of particles. Particles are imaged by scanning a fine electron beam across the specimen and using associated detected signals to modulate the intensity of a display scanning at the same rate as the probe. Conventional SEMs are typically capable of resolving details with a spatial resolution of 5 nm to 10 nm, although this figure becomes significantly poorer under less than ideal conditions. SEMs with bright and coherent Field Emission Electron Guns (FEG-SEM) are capable of a spatial resolution of 1 nm and below. While the conventional SEM requires the specimen to be held under a vacuum during analysis, environmental SEMs (ESEM) enable samples to be studied in a low-pressure gaseous environment. ESEMs allow particles with semi-volatile components to be imaged without loss of volatile material, and enable the repeated hydration/dehydration of specimens to establish the presence and significance of soluble species.

Conventional TEMs use electrons transmitted through the specimen to form images in a manner analogous to a conventional optical microscope, and are inherently more complex than conventional SEMs. They also generally require thin electron-transparent samples and a higher level of sample preparation (see Annex A). They are routinely capable of achieving sub-nanometre spatial resolution, although the achievable resolution is dependent on the sample as well as the microscope being used. TEMs are also available that are capable of operating in a scanning mode (Scanning Transmission Electron Microscopy, or STEM), where a fine electron beam is scanned over the specimen, and signals from transmitted electrons are used to form images. Electron beams smaller than 0,2 nm in diameter may be formed in high-resolution STEMs, allowing sub-nanometre spatial resolution characterization of particles.

6.5.2 Electron microscopy imaging and analysis methods

Electron microscopy analysis provides a wide range of qualitative and quantitative analysis methods that are suited to characterizing individual collected aerosol particles in the nanometre size range. Imaging in the TEM relies on electrons penetrating through the sample, and provides projected two-dimensional morphology and size information on particles [73]. It can also provide information on internal particle structure where this leads to image contrast, including information on the crystalline structure in some cases (although this requires appropriately oriented particles and a high-resolution TEM). Image contrast is governed by elastic and inelastic scattering of electrons as they pass through the specimen and is strongly influenced by crystalline structure and atomic-proton density. When used in scanning mode (STEM) with an appropriate annular detector, images may be formed with an intensity related to the square of the atomic number within the specimen (Z-contrast imaging), allowing intuitive interpretation of images from heterogeneous particles and particle ensembles [74]. The use of transmitted electrons in imaging within the TEM limits the maximum diameter of particles analysed to a few hundred nanometres.

SEM imaging relies predominantly on electrons scattered or emitted from the irradiated surface of the specimen, allowing particles covering a very wide range of sizes to be examined [75]. Standard SEM imaging modes use secondary electrons emitted from the specimen surface following irradiation by the primary electron beam. The resulting image provides topological information, with image contrast predominantly influenced by specimen orientation with respect to the incident electrons and the secondary electron detector. Back-scattered electrons from the primary beam may also be used in imaging. As back-scattering probability is related to the atomic number, this imaging mode provides qualitative information on composition at the specimen surface.

Electron-specimen interactions may be used to quantitatively characterize particles in a wide variety of ways. Characteristic X-rays generated through atomic electron excitation and decay within the specimen are routinely used in TEM and SEM analysis to detect elemental components through Energy Dispersive X-ray analysis (EDX). EDX can provide spatially-resolved information on particle elemental composition and compositional heterogeneity [75][76]. In the SEM, spatial resolution is limited by the electron-scattering volume in the specimen, and is generally not capable of providing much spatial information below a resolution of 1 µm. However, variations in electron-scattering volume with accelerating voltage can be used to characterize elemental composition at the surface of particles with a depth resolution of tens of nanometres [77].

Spatial resolution in the TEM is dominated by the width of the electron beam. In the STEM or combined TEM/STEM, this can be substantially less than 1 nm in diameter, allowing elemental characterization with a high level of spatial resolution. However, X-ray yield falls rapidly with decreasing analysis volume, leading the technique to be relatively insensitive when characterizing very small particles.

In the TEM, quantification of the energy lost by electrons in the electron beam can be used to extensively characterize the sample. The amount of energy lost is characteristic of many sample properties, and can be used to probe elemental composition, atomic structure and electronic properties. In its simplest form, Electron Energy Loss Spectroscopy (EELS) is used to characterize elemental composition by relating features in collected spectra to element-specific orbital electron excitations [78]. In contrast to EDX, EELS is significantly more sensitive to lighter elements. Spatial resolution is dominated by the width of the electron beam used, which may be smaller than 0,2 nm in diameter in the STEM. EELS is generally much more sensitive than EDX for small particles, as many more electron-specimen interactions are detected. This makes it ideal for characterizing the composition of nanoparticles. However, quantification of the spectra is often complex, and interpretation of data not as routine as for EDX.

Both EDX and EELS can be used to map the spatial distribution of elements within a specimen. Images are formed using specific EDX or EELS signals, allowing elemental mapping and the identification of particles with specific compositions. The technique is generally time-consuming as long collection or imaging times are frequently needed to provide sufficient image contrast.

6.5.3 Single particle analysis in the scanning force microscope

The development of scanning probe microscopy (SPM) methods has led to further techniques for imaging nanometre-sized particles. All methods are typified by a fine probe that is scanned in a raster across a surface. Probe position above (or on) the surface is controlled by a range of feedback signals, which are also used to

provide image contrast on the associated display raster. Initial SPM development used the electron tunnelling current between a conducting specimen and probe suspended a few tenths of a nanometre above its surface to map topographic features at sub-nanometre resolution (Scanning Tunnelling Microscopy – STM). Later developments led to the use of Van der Waals forces between the specimen and the probe (Atomic Force Microscopy – AFM), allowing imaging of non-conducting specimens. The use of further feedback mechanisms has led to a number of SPM imaging methods, including magnetic force microscopy, lateral force microscopy, shear force microscopy and near-field scanning optical microscopy. All of these methods can be operated in a range of environments, including atmospheric conditions, liquid immersion and vacuum.

Of all the available SPM methods, AFM is perhaps the most applicable to aerosol analysis, as high-resolution imaging is possible in air, and there are relatively few limitations on the type of sample imaged. However, the clear advantages it has over electron microscopy methods, such as rapid sample analysis, minimal sample preparation and analysis under ambient conditions, are somewhat balanced by a lack of clarity concerning image interpretation and applicability. Although scanning probe microscopy can resolve horizontal and vertical details to fractions of a nanometre, it is unable to deal with large changes in vertical profile occurring over a few nanometres.

Near-field Scanning Optical Microscopy (NSOM) is an SPM technique that has some potential benefits for the analysis of nanoparticles. Conventional optical microscopy is limited to a theoretical spatial resolution of $\lambda/2$. However, if a specimen is illuminated through a sub-wavelength-sized aperture held within a tenth of a nanometre from its surface (the near-field), spatial resolution approaching the diameter of the aperture is possible. By using SPM methods to scan a fine aperture over a sample, optical imaging with a resolution below 100 nm can be achieved. Although resolution doesn't extend far into the sub-100 nm region, the ability to apply optical analysis and detection methods to isolated nanometre-diameter particles presents some interesting possibilities.

7 Summary

An increasing number of studies are indicating that airborne nanoparticles and nanostructured particles may present an inhalation health risk that is not adequately addressed by conventional exposure evaluation methods. However, understanding has yet to advance to a stage where the development of normative standards for evaluating occupational exposures to nanoaerosols is feasible. Before appropriate standards are developed, advances are needed in identifying nanoaerosol attributes which are critical to occupational health, and in the development and implementation of instruments capable of measuring exposure against these attributes.

This Technical Report provides an overview of state-of-the-art nanoaerosol characterization and monitoring, and is intended as a guide to making measurements in the workplace and as a precursor to future monitoring standards. In the absence of health-related terminology standards for nanometre-diameter particles, a nomenclature has been adopted within the report that distinguishes between discrete sub-100 nm diameter particles (nanoparticles) and aerosols of particles with sub-100 nm diameter structural features (nanoaerosols). Further distinction is made between nanoparticles produced as a by-product of an industrial process (ultrafine particles) and those purposely engineered (engineered nanoparticles). In discussing monitoring approaches, the emphasis is predominantly on characterizing airborne nanoparticles. However, the need to consider exposure to larger particles is considered where the presence of nanometre-diameter structures rather than overall particle size may determine health impact.

The monitoring and characterization techniques discussed in this Technical Report enable nanoaerosol exposure to be evaluated in terms of aerosol mass, surface area and number concentration. It is recommended that these methods be used and developed to provide further information on occupational nanoaerosol exposures, and to support the development of exposure characterization standards. However, many of the instruments are not ideally suited to routine use in the workplace. It is therefore also recommended that, as further information on key heath-related aspects of nanoaerosols emerge, these instruments and methods should be developed as appropriate into compact, cost-effective devices for personal nanoaerosol exposure monitoring.

Annex A
(informative)

Electron microscopy sample collection and preparation

A.1 General

Aerosol particle analysis by electron microscopy has stringent sample requirements, leading to the use of specific methods for sample collection and preparation. Because of the different requirements of SEM and TEM, there are significant differences in how samples are collected and prepared for analysis between the two instrument types. However, in each case, there is a need to collect uniform deposits, with minimal particle overlap.

A.2 Scanning electron microscopy

SEM samples are generally mounted on a conducting aluminium or carbon stub or disc. A key requirement of the conventional SEM is the need for a conductive pathway between the point at which the electron beam hits the specimen and ground. Unless conducting particles are sampled directly onto a conducting substrate, the specimen needs to be coated with a thin metal or carbon film to ensure a clear conducting pathway for the electrons. Failure to render the specimen conducting leads to localized charging in the SEM and poor or distorted imaging. Specimen coating is usually carried out in a commercial sputter coater, which allows a layer of atoms a few nanometres thick to be applied under vacuum. Other forms of vapour deposition can also be used.

As aerosol samples invariably have to be coated before imaging in the SEM, there is no inherent disadvantage to collecting particles on a non-conductive substrate. This makes SEM particularly amenable to characterizing samples collected on filters or impactor substrates. In these cases, sample preparation prior to coating consists of attaching a small part of the collection substrate to a specimen support with conductive adhesive. A common mounting method is to use double-sided adhesive conducting carbon pads, augmented with spot-application of colloidal silver or carbon to ensure a clear conducting pathway between the top of the sample and the specimen support. As the specimen will be placed in the SEM under vacuum and possibly undergo localized heating in the electron beam, collection substrates should not contain any oil, grease or other volatile material.

While it is possible to use a wide range of collection substrates for SEM analysis, the most effective substrates are those that provide a featureless support on top of which the particles lie. Filter sampling is one of the simplest methods of collecting aerosols for SEM analysis. Track-etched polycarbonate filters provide a smooth surface punctured by uniform diameter pores, and form an ideal collection surface for subsequent SEM analysis. Although these filters are able to collect particles smaller than the pore size, it is preferable to minimize internal deposition by using filters with a pore size comparable to the smallest particles of interest.

Sampling using an impactor allows the use of smooth collection substrates. Collection with a cascade impactor has the added advantage of providing samples containing particles within a relatively narrow size range. Impactors are prone to overloading, leading to particle overlap on the collection substrate, and care needs to be taken when using them to avoid this.

Sampling particles smaller than a few hundred nanometres in diameter for SEM analysis becomes increasingly difficult using filtration and impaction. Particles down to typically 20 nm in diameter can be sampled directly onto SEM supports using electrostatic precipitation. Charging the aerosol before sampling increases the collection efficiency significantly. 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.

Environmental SEM (ESEM) enables sample analysis under partial vacuum, thus circumventing some of the restrictions placed on specimens prepared for the conventional SEM. With the introduction of a low-pressure gas or vapour into the microscope's specimen chamber, an electron discharge route through the chamber is enabled. Thus, there is no longer the need for samples to be conducting. However, as the imaging and analysis modes in the ESEM provide different information on samples than those generally used in the conventional SEM, care needs to be taken to ascertain whether the ESEM or SEM (if both are available) is the most appropriate analytical tool for a given sample.

A.3 Transmission electron microscopy

TEM samples need to be collected onto a thin electron-transparent substrate that allows transmitted electrons to be used for imaging and analysis. Thus, the constraints on how TEM samples are collected and prepared are significantly more stringent than for SEM analysis. TEMs generally use sample holders designed to accept 3 mm diameter circular grids. These may be made from a variety of metals – copper and nickel are widely used – and are available in a range of designs suited to support different specimens. For aerosol analysis, mesh grids supporting a thin carbon or silicon oxide film are most often used. Specimen grids are widely available with a polyvinyl formal coating, either on its own or supporting a thin carbon film. This type of grid is suitable for analysing larger particles, but can lead to imaging and contamination issues – particularly when examining nanometre-diameter particles in high-resolution TEMs.

Carbon-coated grids are available as either continuous films, films with occasional holes (holey carbon film) or lacy carbon film. The former allow particles to be collected and imaged on large areas of continuous film, and are suited to analysing the size and shape of large numbers of particles. However, it is frequently necessary to have access to specimen areas that don't obstruct the electron beam, either to allow beam alignment or to enable the analysis of particles suspended on the edge of the support without the interference of an underlying substrate. In these cases, the use of holey or lacy carbon film provides a more appropriate collection media.

It is generally preferable to sample directly onto a TEM support grid, thus avoiding a secondary sample preparation stage. Electron conduction of nanometre-sized particles is generally sufficient to avoid the need for coating specimens, as in the case of SEM samples. There are a number of ways in which nanoaerosols can be sampled onto TEM grids, including placing grids on the face of a filter, or pulling air through a lacy carbon film. However, most sampling applications use electrostatic or thermal precipitation.

Thermal precipitation 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. In its crudest form, thermal precipitation involves placing a cold TEM grid briefly into a hot aerosol (such as the plume from a welding operation). Samples taken in this way generally have a uniform particle deposition across the grid. 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. This approach has been used in a number of experimental devices. Electrostatic precipitation provides an efficient alternative method for collecting particles larger than 20 nm for TEM analysis. Particles smaller than 20 nm can be collected using this method, although collection efficiency drops off as diameter decreases. Electrostatic precipitators are generally applicable to both TEM and SEM samples, and are discussed in more depth in A.2.

A.4 Electron microscopy — Sample collection times

When taking electron microscope samples, it is important to achieve sufficient sample loading to allow efficient analysis, while avoiding particle overloading. Where the sampling efficiency is known or can be estimated, it is possible to estimate a minimum sampling time to give sufficient loading. Critical parameters include the sampling efficiency as a function of particle diameter (E_d), flow rate into the sampler (q), particle number concentration as a function of particle diameter (C_d), the minimum acceptable number of particles with diameter d per field of view (N_d) and the minimum acceptable projected area of each particle with diameter d in the field of view, expressed as a fraction of the area being viewed (A_d).

A_d defines the smallest particle that can be comfortably analysed at a given magnification. Assuming spherical particles, the largest field of view A_f (expressed as an area) that should be used to observe particles of diameter d is given by

$$A_f = \frac{\pi d^2}{4A_d} \tag{A.1}$$

where

A_f is the area of the field of view in a microscope for particles of diameter d, in square metres (m^2);

d is the particle diameter, in metres (m);

A_d is the minimum acceptable particle-projected area, expressed as a fraction of A_f.

Thus, the minimum particle density on the substrate n_s is given by

$$n_s = \frac{N_d}{A_f} \tag{A.2}$$

where

n_s is the minimum particle number density on the substrate, in number of particles per square metre (particles/m^2);

N_d is the minimum acceptable number of particles with diameter d per field of view.

For a given sampling flow rate q, collection efficiency E_d and effective substrate area A_s, Equations (A.1) and (A.2) give the sampling time t necessary to achieve the minimum sample loading.

$$t = \frac{n_s A_s}{qC_d E_d} = \frac{4A_d A_s}{\pi d^2} \frac{N_d}{qC_d E_d} \tag{A.3}$$

where

t is the sampling time for the minimum particle loading, in seconds (s);

A_s is the area of the substrate, in square metres (m^2);

q is the sample flow rate, in cubic metres per second (m^3/s);

C_d is the particle number concentration as a function of particle diameter, in number of particles per cubic metre (particles/m^3);

E_d is the sampling efficiency as a function of particle diameter.

For a 3 mm TEM grid, the maximum effective substrate area is defined by the area over which the particles can deposit, which is $7{,}1 \times 10^{-6}$ m^2. When using digital imaging, the smallest particles of interest should ideally cover a minimum of 10 pixels. Thus, in an image with dimensions of 1 024 × 1 024, A_d is $9{,}5 \times 10^{-6}$. A reasonable value for N_d, the number of particles per field of view, is 10. Thus, for example, if 50 nm diameter particles were collected with 80 % efficiency at a flow rate of 5×10^{-6} m^3/s from an aerosol concentration of 10^8 particles/m^3, using Equation (A.3) would give a minimum sampling time of about 0,25 h. However, if 10 nm particles were sampled under the same conditions, the minimum sampling time would be about 6 h.

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