Engineered nanomaterials: current status of occupational exposure assessment

Nanomateriali ingegnerizzati: stato attuale della valutazione dell'esposizione professionale

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An increasing number of studies are indicating that the health risk deriving from exposure to engineered nanomaterials (ENMs) and nanoparticles (ENPs) is not adequately addressed by conventional exposure evaluation methods and strategies. The global aim of this study was to carry out a review of the state-of-the-science of ENMs occupational exposure assessment, with particular concern on the main problems related to ENPs exposure assessment. Original articles and reviews in principal databases of scientific literature were included in this paper; grey literature (released by qualified regulatory agencies and scientific organizations) was also taken into consideration. The paper discusses in particular the main problems found in ENPs exposure assessment, which have been generally identified in: (i) the choice of a proper dosimetric for exposure assessment, (ii) the lack of adequate and reliable measurement techniques, (iii) the need of a harmonized monitoring strategy, (iv) the need of an effective method for the distinction of ENPs from background particles and (v) the difficulties to compare ENPs exposure data with proper Occupational Exposure Limits. On the basis of the considered existing approaches, some key issues related to exposure assessment strategies, derived from direct on-field exposure are then discussed, as well as some identified priorities in the field of ENPs exposure assessment. In conclusion, most of the existing techniques and strategies for occupational exposure assessment to ENPs and ENMs require adjustment, and significant methodological gaps need to be reduced. The rational use of risk management strategies and the application of specifically-developed Occupational Exposure Limits are crucial to mitigate the risk posed for ENPs-exposed workers. The development and harmonization of appropriate exposure assessment strategies and techniques and risk management approaches represent an essential step toward developing health and safety standards for ENPs.

Key words: Engineered nanomaterials; engineered nanoparticles; risk management; exposure assessment

Un crescente numero di studi indica che il rischio per la salute derivante dall'esposizione a nanomateriali (ENM) e nanoparticelle (ENP) ingegnerizzati non è adeguatamente caratterizzato da metodi e strategie convenzionalmente usati per la valutazione dell'esposizione. L'obiettivo di questo articolo è quello di fornire un quadro dello stato dell'arte relativo alla valutazione dell'esposizione occupazionale a ENM, con particolare riferimento ai principali problemi riscontrati nella valutazione dell'esposizione a ENP, mediante una revisione della letteratura tecnico-scientifica disponibile. Vengono discussi in particolare i principali problemi legati alla valutazione dell'esposizione a ENP, che sono riconducibili a: (i) scelta di un'appropriata metrica per l'esposizione; (ii) mancanza di tecniche di misura adeguate e affidabili; (iii) necessità di definire una strategia armonizzata per la valutazione; (iv) necessità di una tecnica adeguata alla distinzione delle ENP dal particelle incidentali e dal fondo ambientale e (v) mancanza di valori di riferimento appropriati. In conclusione, le tecniche e le strategie ad oggi disponibili per la valutazione dell'esposizione a ENM e ENP richiedono un aggiornamento. Lo sviluppo armonizzato di nuovi approcci per la valutazione e gestione del rischio rappresenta un elemento essenziale per mitigare il potenziale rischio per i lavoratori esposti a ENM e ENP.

Introduction

Engineered nanomaterials (ENMs) can be defined as materials with structural features with at least one dimension of less than 100 nm; engineered nanoparticles (ENPs) are nanomaterials produced in form of particles with all three external dimensions in the nanoscale range, between 1 and 100 nm [ISO/TR 12885:2008]. Such materials possess nanostructure-dependent properties, so that potential applications of ENMs are widespread, ranging from electronics to medical applications and cosmetics. Currently, carbon-based ENMs such as carbon nanotubes and fullere-

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nes, metal and metal oxide ENPs, quantum dots (QDs) and silica ENMs are widely used in the manufacturing of several consumer products (i.e. rechargeable and lithium batteries, devices for energy storage, toner formulations, solar cells, biosensors, paints plastics, clothes, shampoo, toothpaste, detergents, deodorants, sunscreens) and are implicated in different industrial processes and applications to obtain a more efficient water purification, stronger and lighter building materials, increased computing power and speed, improved generation and conservation of energy and new tools for the diagnosis and treatment of diseases [Card et al., 2008; Iavicoli et al., 2014]. These intense commercial and industrial interests in nanotechnologies are justified by the unique chemical and physical features of ENMs and ENPs such as dimension, mass, chemical composition, surface area, aggregation and agglomeration status, water solubility, surface chemistry, morphology that are very different from other environmental particulates and that provide a wide industrial use. However, the peculiar physico-chemical properties that are exploited for industrial and biomedical purposes, can also influence the toxicological potential of ENMs and ENPs and could also lead to novel toxicological mechanisms by which humans may become exposed to [Oberdörster et al, 2005a; Abbott, 2010]. Thus, the proliferation and the increasing presence of ENMs and ENPs in the work environment can result in occupationally exposures to ENMs and ENPs [Balbus, 2007] which may possibly cause adverse health effects to exposed worker [Balbus, 2007; Roco, 2011]. Generally, if ENMs (or ENM-containing materials) are handled, consideration needs to be given to the likelihood of ENPs being released into the air as the materials are handled and used, since aggregates, agglomerates and possibly discrete particles may be released when handled. The rate of ENPs release will depend on several factors (e.g. the agitation during handling) and the nature of the material. Although very little is known about the degree of release of ENPs from powders, it is likely that the physico-chemical properties of the material will significantly determine how likely a powder will be aerosolized. In this regard, the control and assessment of the potential exposure of workers to ENMs and ENPs become crucial in occupational health and safety, both to minimize the health risks for workers and for risk management purposes. Over time, the critical importance of exposure assessment and physico-chemical characteristics of ENMs and ENPs was recognized. Nevertheless, to date there is not an unequivocal opinion on (i) the specific correlations between the different physico-chemical ENMs characteristics (i.e. mass or number concentrations, surface area, size, chemical composition, surface functionalization) and the toxic effects exerted by ENMs and (ii) on the best way to characterize the occupational exposure to ENMs/ENPs. Meanwhile specific and toxicology-based occupational health standards are being developed, advances are needed in identifying nanoaerosols properties which are critical to the exposure assessment. Thus, workers may be at risk from occupational exposures, in the absence of a complete characterization of the potential hazards of ENMs and ENPs and of appropriate control of occupational exposures. The implementation of accurate instruments and reliable strategies capable of measuring exposure to ENPs, will permit to increase the accuracy in exposure assessment studies.

Materials and Methods

This paper provides information on the current knowledge about ENMs and ENPs exposure assessment and risk management strategies currently used in occupational environments to evaluate the exposure to these materials. Original articles and reviews in principal databases of scientific literature (PubMed, ISI Web of science, Scopus) were included in this paper; grey literature released by regulatory agencies and scientific organizations was also taken into consideration. The global aim was to realize an overview of the state-of-the-science of ENMs and ENPs occupational exposure assessment, with particular concern on the main problems related to the issue of exposure assessment. Furthermore, based on the considered existing approaches, some key issues related to exposure assessment strategies, derived from direct on-field experiences [Spinazzè et al., 2016a, 2016b] are discussed, as well as some identified priorities in the field of ENMs and ENPs exposure assessment.

Results

What is different when considering exposure assessment to ENMs and ENPs?

Exposure to airborne aerosols has historically been characterized by their size-specific concentrations, corresponding to different deposition regions within the respiratory system. However, ENMs and ENPs show peculiar physico-chemical properties, which may pose a health risk that is not adequately addressed by this conventional exposure assessment method. Three main issue must be considered when approaching an ENMs and ENPs exposure assessment: (i) the very small size and the correspondingly high specific surface area of these materials, which may correspond to a potential for greater reactivity, (ii) their high deposition efficiencies in the respiratory tract and (iii) the peculiar biokinetics of ENMs and ENPs including the possibility of their translocation across cell barriers into cells [Oberdörster et al., 2005b]. These three issues represent the main reason why conventional gravimetric methods for airborne particulate exposure measurement do not provide a reliable indication of the health risks associated with nano-sized particles. In particular, on a mass for mass basis, the toxicity of insoluble materials increases with smaller particle sizes [Donaldson et al., 2000; Li et al., 2003; Johnson et al., 2004; Cassee et al., 2013]. However, the mechanisms by which these materials exhibit higher

level of toxicity at smaller sizes have yet to be elucidated. Many studies also indicate that the biological response depends on the surface-area [Brown et al., 2001; Tran et al., 2000; Donaldson et al., 1996; Hamoir et al., 2003; Oberdörster et al., 1994]. Concerning lung deposition (defined as the mean probability for an inhaled particle with specific diameter to deposit somewhere in respiratory system), the ICRP model outlined that total deposition reaches a minimum within the lungs for airborne particles around 300 nm aerodynamic diameter [ICRP, 1994]. Below this minimum in deposition probability, predicted deposition increases as diffusional forces increase with decreasing particle diameter, so that 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 tracheobronchiolar region, due to their very high diffusional mobility. In any case, a substantial fraction of the inhaled nanoaerosols will deposit within the respiratory tract. Deposition is predominant in the alveolar region for particle larger than 5 nm, while there is a relevant predicted deposition probability in the extra-thoracic and tracheobronchiolar regions, particularly for particles smaller than 5 nm. Experimental data also confirm the trend of increasing deposition with breathing rate and level of exertion [Jacques et al., 2000; Daigle et al., 2003]. Thus, the deposition fraction of inhaled nanoaerosols is greater in the alveolar and tracheo-bronchial regions of human lungs, compared to coarser particles. Once deposited, nanoaerosols may also persist in the lungs longer than coarser particles, due to their decreased clearance. It has been also suggested that, due to their small diameter, nano-sized particles are capable of penetrating lung epithelial cells, enter the bloodstream and reach sites far from their portal of entry [Schlesinger, 1995; Takenaka et al., 2001; Kreyling et al., 2002; Nemmar et al., 2002; Oberdörster et al., 2002; Borm et al., 2004; Semmler et al., 2004; Oberdörster et al., 2005b]. For example, some type of nanoparticles (e.g. titanium dioxide or carbon-based ENMs) have been shown to penetrate the epithelial cell barrier more readily and enter the lung interstitium or the blood circulation; once in the blood, nano-sized particles may translocate to other organs in the body [Larese Filon et al., 2015]. In this regard, the fate of inhaled nanoaerosols may also depend on their chemical composition [Jacques et al., 2000; Daigle et al., 2003]. Therefore, an adequate and comprehensive evaluation of the possible toxic effects induced by ENMs and ENPs exposure should be carried out taking into account both the different points at which these substances enter the body, and also the tissues, organs and systems that may be targeted by ENPs after distribution in the body. In this regard, in the last few years, a number of studies have demonstrated that at cell level ENMs can enhance the formation of Reactive Oxygen Species (ROS), disrupt electron/ion cell membrane transport activity, induce oxidative damage and lipid peroxidation, cause autophagy, lysosome dysfunction and affect the activation of certain signalling pathways [Iavicoli et al., 2011; Feng et al., 2015; Sajid et al., 2015], whereas at body level ENMs may induce adverse effects on several organs and systems such as the respiratory, cardiovascular, nervous, renal and endocrine systems [Iavicoli et al., 2012; Donaldson et al. 2013; Iavicoli et al. 2013a; Lu et al., 2014; Zhang et al., 2014; Iavicoli et al., 2016]. Nevertheless, very few data on the possible health effects of ENMs and ENPs in workers are currently available; furthermore, since these data generally refer to very high accidental exposure or to concomitant exposure to other chemicals, these studies do not allow to confirm a specific risk posed by ENPs for exposed workers. However, it could be supposed, also according to the precautionary principle, that ENMs and ENPs are at least as toxic as the bulk form of the corresponding material [Pietroiusti and Magrini, 2014]. To summarize, even though a number of studies have been conducted in order to evaluate ENPs and ENMs toxicity, further studies are needed in this emerging field since our knowledge of the potentially adverse effects induced by ENMs and ENPs and of the molecular mechanisms of action underlying their toxic effect is still limited and fragmentary [Schulte et al., 2016a, 2016b]. Generally, the literature sources are still not sufficient to reach firm conclusions about the potential hazards of specific nanoaerosols. Undoubtedly, the peculiar physicochemical properties of ENMs and ENPs have a relevant influence on their biological and toxicological activity: in this regard, health effects associated with nano-sized particles exposure are likely to be associated with ENPs particle size, surface-area and number concentration [Oberdoster et al., 2005b]. Thus, further studies are needed to define appropriate methods to quantify and assess the actual occupational and environmental exposure levels.

Problems in exposure assessment

Workers' exposures to ENMs and ENPs might occur during their production, their downstream use for further processing or treatment of ENP-embedded products but also during operations of transport, storage and disposal [ISO/TR 12885:2008]. Different degrees of exposure may occur, as function of the operating conditions (e.g., open or closed production process; physical status of the ENMs themselves). For example, a release of ENMs and ENPs is attended to occur under certain circumstances (e.g., maintenance activity, open gas-phase production process, open handling of nano-products) [Pietroiusti and Magrini, 2014]. Potential routes of occupational exposure to ENP include inhalation, dermal contact and ingestion. The most common route of exposure to airborne particles in the workplace is inhalation [Donaldson et al., 2000]. Despite this paper focuses on inhalation exposure, dermal ENMs exposure may occur (e.g., during production, usage or contact with contaminated surfaces).

It is still not clear if (and, possibly, to what extent) ENPs are able to cause harmful effects following dermal exposure. Most of experiments have been conducted with single types of ENMs such as TiO_2 and ZnO on intact skin. Further evidences indicate that ENPs can penetrate through damaged skin [Larese et al., 2008] or mouse skin [Sonavane et al. 2008]. Besides, a number of study on this topic was recently published [e.g., Tinkle et al., 2003; Crosera et al., 2009; Larese Filon et al., 2015], which also pointed out the need of further research effects on dermal exposure to ENMs. Moreover, another important route of human ENMs exposure is the oral absorption. Indeed, oral uptake of inhaled ENMs, translocated via the mucociliary escalator, may lead to gastrointestinal absorption. In this case, the major absorption site is the small intestine with the major cell types involved in this process being absorptive enterocytes, mucus secretory goblet cells and the immune sampling M cells of Peyer's patches [Ma et al., 2014]. The number of studies focused on ENMs and ENPs occupational exposure assessment, and the current knowledge about the related workers' risk, has increased in the last few years [Pietroiusti and Magrini, 2014; Kaluza et al., 2009; Brouwer et al., 2009; Wooskie et al., 2010; Kuhlbusch et al., 2011]. However, comprehensive characterization of exposure still remain a challenge [Brouwer et al., 2012]. In particular, when considering ENMs and ENPs, the main problems in exposure assessment are amenable to: (i) the choice of a proper dosimetric for exposure assessment, (ii) the lack of adequate and reliable measurement techniques (particularly referring to the assessment of workers' personal exposure), (iii) the need of a harmonized monitoring strategies, (iv) the need of an effective method for the distinction of ENPs from background particles and (v) the difficulties to compare ENPs exposure data with proper Occupational Exposure Limits. These issue will be singularly discussed hereafter.

Which Dosimetric for exposure assessment?

The choice of the proper metric for ENMs and ENPs exposure assessment is still an unresolved issue: with only limited toxicity data and almost negligible occupational exposure data, it is currently unclear how exposure to these materials should be most appropriately monitored and regulated. As previously discussed, aerosol exposure has historically been characterized by the mass (gravimetric) concentration of airborne particles, usually associated with specific size ranges corresponding to different deposition regions within the respiratory system [ISO 7708:1995]. Exceptions to that methodology are represented by particle-number-based metrics for exposure, which can be determined using a large number of commercially-available instruments even though not yet subject of regulations. Although further research is needed on the physical attributes of nanoaerosols which are most closely associated with potential health risk, it is clear that measuring exposures only against particles' mass is not appropriate. Indeed, the mass concentration of ENMs and ENPs is often negligible compared with that of larger particles [Donaldson et al., 2001]. This clearly indicates that, in addition to mass, other metrics must be taken into account to make an adequate and complete evaluation of exposure to nanoaerosols in workplaces. Unfortunately, it is not yet clear which key particulate parameters (mass, surface area, number, and size distribution) would be the most relevant measurements with regard to health in workplaces [Iavicoli et al., 2013b]. However, the adverse health effects induced by ENMs and/or ENPs have been shown to correlate more strongly with particle number or surface area concentration and these metrics may better reflect adverse cardiopulmonary outcomes than respirable mass concentration. For example, in this context, Oberdörster et al. [Oberdörster et al., 1996] demonstrated, in rats exposed to 20-nm polytetrafluoroethylene particles, a correlation between the ENPs number concentration and toxicity, showing a strong dose–response curve between pulmonary inflammation and particle number. Therefore, between the primary physical exposure metrics (mass, surface-area, diameter and number concentration), there is strong evidence to suggest that nanoaerosols should be monitored with respect to particle number or surface-area concentrations, which appear to be more appropriate exposure metrics for low solubility particles, especially when the study is oriented to risk assessment [Brown et al., 2001; Tran et al., 2000, Oberdörster, 2000; Lison et al., 1997]. In particular, surface-area concentrations may be the better descriptor for the biological effects of ENMs and ENPs [Maynard and Aitken, 2007] and have been shown to relate more closely with the toxic effects of inhaled ENPs [Wittmaack, 2007; Monteiller et al., 2007]. However, the measurement of number concentrations is the most widely used method [Pietroiusti and Magrini, 2014] and there are indications that particle number may be an important exposure metric, primarily for particles within specific particle size ranges and when considering specific applications (e.g. measurements for source tracking) [Beck-Speier et al., 2001; Peters et al., 1997]. Finally, some studies have shown that ENMs and ENPs sizes may be critical in influencing the fate and toxicity of respirable particles in the lungs [Li et al., 2003; Hofman et al., 2003]. In any case, while a number of evidences suggest the use of particles surface-area as the most health-relevant exposure metric, it is also necessary to consider characterizing exposure against aerosol mass and/or number concentrations, until further information and reliable instruments for surface-area measurement are available. In this regard, it must be remembered that most of the Occupational Exposure Levels (OELs) are expressed in terms of mass concentration. However, considering the aforementioned considerations, it should be noted that if ENMs and/or ENPs exposures exist regulatory standards based on mass concentration may not protect individuals from either an environmental or an occupational health perspective [Iavicoli et al., 2013b].

Similarly, protective and preventive measures that focus on reducing aerosol mass concentration in the workplaces are not necessarily sufficient to protect the health of workers [Iavicoli et al., 2013b]. Moreover, active sample for the gravimetric determinations (and the subsequent chemical and/or morphological characterization) can act as an efficient measurement only if data on size distribution or specific surface area, particle concentration, agglomeration status are available [ISO/TR 27628/2007].

Which measurement technique?

As previously discussed, at the present time there is not sufficient information to determine which physical exposure metric is the most relevant in terms of possible health effects and, consequently, which is the most appropriate exposure characterization techniques to use. However, currently, no standard sampling methods are available for the assessment of the specific exposure to airborne nanoaerosols. The ENMs and ENPs exposure characterization requires the use of multiple measurement and assessment techniques, as well as leading edge instrumentation [NIOSH, 2009]. As a consequence, exposure measurements can represent a very demanding task in terms of number of required resources. Currently available monitoring and characterization methods allow exposure assessments for ENPs and nanoaerosols in terms of mass and number concentrations and surface area and are the basis for the development of new standards for the exposure characterization [ISO/TR 12885:2008; ISO/TR 27628/2007].
In fact, nanoaerosols in workplaces can be monitored using a variety of tools in order to obtain useful information about different particle metrics, for example, number concentration and particle size distribution (condensation particle counter, optical particle counter, scanning mobility particle sizer, electrical low pressure impactor (ELPI), micro-orifice uniform deposit impactor) or the surface area (diffusion chargers). Moreover, to achieve a comprehensive characterization of ENMs and ENPs sampled in a specific workplace there is the need to adopt a combination of real-time and time-integrated sampling techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and inductively coupled plasma mass spectrometry (ICP-MS) because these laboratory analyses are useful for determining the elemental composition and morphology of the particles and aid interpretation of real-time instrument data [Iavicoli et al., 2013b].
Environmental monitoring techniques for the assessment of occupational exposure to airborne ENMs and ENP can generally be classified as a function of the metric used, the performance in terms of temporal and particle size resolution and the performance in discriminating ENPs from the background ultrafine particles (UFPs) (i.e. background discrimination performances) [Kuhlbusch et al., 2011].
As told, the gravimetric analysis is very little sensitive to ENMs and ENPs; further, size-selective sampling would be necessary to ensure only particle within the relevant size range (namely with diameters <100 nm) are sampled [ISO 7708:1995]. In this regard, the use of conventional impact techniques for determining ENMs and ENPs concentrations is currently limited, as the lower cut-off 50% is restricted to aerodynamic diameters in the 200 - 300 nm interval, anyway. With low pressure impactors, it is possible to measure particle concentrations (size-resolved, time-integrated) of up to 10 nm as static samplers [Marjamäki et al., 2000], though their dimensions and complexity do not allow the use for personal sampling (in the breathing zone of individuals). This can lead to biases in the determination of the actual exposure [Cattaneo et al., 2010]. Nevertheless, personal cascade impactor with a cut point of 250 nm also allow an approximation of nanoaerosol mass concentration in the worker's breathing zone [ISO/TR 12885:2008]. The advantage of all this techniques is that they consist of the sampling of size-fractioned ENPs collected onto sampling membranes, thus they enable off-line investigations on ENPs through chemical analysis and electron microscopies. The most widespread method is the determination of ENMs and ENPs particle number concentration (PNC). Two of the most widely used instruments are the Scanning Mobility Particle Sizers (SMPS) and the Fast Mobility Particle Sizer (FMPS): these two determine airborne nanoaerosol size-resolved and time-resolved PNC, based on electrical mobility analysis of the particles measured. SMPS and FMPS measure particles size-distribution (dimensional range: 3 - 800 nm) [Flagan et al., 2011]. These techniques are not able to distinguish single ENPs from agglomerates of smaller particles; further, the SMPS method showed some limitations (i.e., the technique is too bulky, complicated to use and too expensive for routine operation) [Asbach et al., 2009]. Smaller (often handheld or portable) instruments can be used to monitor the efficiency of engineering controls in the control of workers' exposure to ENMs and ENPs. Portable instruments are available to measure PNC of nano-sized particles. For example, handheld Condensation Particle Counters (CPCs) are able to measure particle number concentrations, in an adeguate dimensional range (typically 10 - 1000 nm). The use CPCs is relatively easy and can be extended without great difficulty for particles until 3 nm. These instruments are widely used to measure airborne concentratoions of nano-sized aerosols (size-integrated, time-resolved) both in workplaces [Liu et al., 2014] and in the urban atmosphere [Hind et al., 1999; Kim et al., 2002; Aalto et al., 2002; Marconi et al., 2007]. Optical Particle Counters (OPCs) provide PNC by size in multiple channels. As the lower detection limit of many portable OPCs is approximatively 300 nm (with upper limits ranging from 10 to 20 μm), these instruments can be used to obtain a rough approximation of exposures to ENMs in particular situations, for example when ENMs and ENP are dispersed in liquid aerosols (spray applications). As CPCs are not size selective (except for an initial cut-off selection), it is difficult to distinguish the different sources of ENMs and ENPs generated

by industrial processes from those present in the background (ultrafine particles of composite origin). Nevertheless, these kind of measurement can be used for the raw identification of nanoaerosols emitted by identified or supposed sources at the workplace [Brouwer et al., 2004]. Such devices can be typically used in a static way only. In the absence of reliable field-portable instrument, capable of sizing and counting particles <100 nm, strategies recently suggested (Nanoparticle Emission Assessment Technique developed by the National Institute for Occupational Safety and Health) are based on a combined CPC and OPC method (count-difference method: number concentrations of particle <300 nm are calculated by subtracting OPC-measured number concentrations from CPC-measured concentrations) [NIOSH, 2009; Methner et al., 2009]. It must be considered that PNC measured with this method are not true estimates of ENMs and ENPs concentrations but actually "very fine particles" number concentration, which are certainly higher [Schmoll et al., 2010]. Furthermore, particle counters are not sensitive to particle sources or chemical composition, making it difficult to differentiate between background nano-sized particles (i.e. ultrafine particles), incidental nanoparticles (e.g. those generated in some work activities and by some production processes but not typically engineered) and process-related ENPs using number concentration alone. The instrument traditionally used for the measurements of aerosols' surface-area is the epiphaniometer. Nevertheless, more recent devices such as diffusion chargers (operating by generation and adhesion of positive unipolar ions to the aerosol particles surface) might find a wider use [Keller et al., 2001]. Recently, different types of instrument provided data well correlated to the surface area of particles deposited in the human respiratory tract [Wilson et al., 2004; Fierz et al., 2008, Fierz et al., 2011; Fierz et al., 2014]. However, as reported in Levin et al., 2015 [Levin et al., 2015] there is still insufficient reliability and comparability between methods in the measurement of surface-area concentrations, and technical developments are required to enable use of surface-area for reliable dose metric and regulatory enforcement of exposure. Measurement techniques have to be optimally combined to allow sensitive and cost effective determination of airborne ENMs/ENPs at workplaces, with the aim of deriving detailed and health-relevant information. At present, no instrument capable to detect single ENP with high specificity/selectivity exist, then every attempt to assess the occupational exposure to ENMs and ENPs requires the use of multiple sampling and monitoring techniques. However, some of them are not yet fully evaluated in terms of accuracy and reliability (in particular those measuring number concentration and surface-area) and are not portable (i.e. multistage impactors for the determination of mass concentrations) so that exposure characterization is deeply limited by the lack of availability of reliable instrumentation for collecting high-quality data, especially for personal monitoring.

As a consequence, nano-specific monitors and samplers are subject to studies on their comparability, accuracy and field performance [Kaluza et al., 2009; Asbach et al., 2012a; Kaminski et al., 2013; Zimmerman et al., 2014; Price et al., 2014; Liu et al., 2014 Levin et al., 2015]. These studies are expected to improve the performances of most of instrumentations currently available on the market, as well as their compactness, portability and costs for routinary applications. To date, the combined use of devices for in-situ measurement (direct reading measurement) and offline sample analysis (chemical and morphological analysis of ENP-loaded filters) represents, the best way for the assessment of ENMs and ENPs exposure in workplaces. However, it should be noted that, even if the environmental monitoring of ENMs and ENPs is carried out placing the aforementioned instruments as close as possible to the breathing zone of selected workers, the measurements obtained reflect particle characteristics and concentrations at the area close to a job activity and, consequently, results from this type of sampling should not be interpreted as representative of personal worker exposure [Methner et al., 2010]. In recent years, a few novel sampling, monitoring and analysis techniques have been introduced that allow for an assessment of the personal exposure to engineered ENMs, based on number or lung deposited surface area concentration [Asbach et al., 2012]. Instruments intended for individual exposure assessment of workers to ENMs in workplaces, measuring particle number or lung deposited surface area concentration concentrations, are currently being tested and validated, since, due to their rather recent introduction to the market, the number of studies that investigated their comparability and accuracy are still rather scarce [Asbach et al., 2012].

Which monitoring strategy?

When defining the exposure measurement strategies, it is necessary to ensure the need of a sensitive and accurate determination of airborne ENMs and ENPs concentrations. Measurement strategies can be based on tiered approaches, such as NEAT [Methner et al., 2009; Eastlake et al., 2016a]; NanoGEM [Asbach et al., 2012b] or approaches proposed by other institutions [Cornelissen et al., 2012; BAuA et al., 2011]. Measurement techniques and measurement strategies have to be optimally combined also to allow a cost-effective assessment; in this regard, tiered approach are cost- and time-effective, since whether an initial screening reveals that ENMs and ENPs concentrations are below a defined threshold value, no further investigations are necessary. Conversely, if ENMs and ENPs release is suspected (concentrations above the threshold value), a more detailed investigation is required, possibly including personal exposure or process-related measurements [Kuhlbusch et al., 2011]. The preferable measurement strategy should include the use of devices for the personal exposure assessment. As an alternative, a micro-environmental modeling approach, based on static measu-

rements combined with the recording of personal activity patterns, could be used to allow the estimation of occupational exposure. Process-related strategies are based on real measurements, derived from the simultaneous use of several sensitive devices in locations close to the work activity of interest. Finally, other strategies are toxicological-oriented, and typically use a measurement approach aimed to obtain an exposure characterization with health relevant metrics (i.e. surface area and oxidative potential). Considering also these different approaches, several occupational safety and health institutes agreed on a convention for the measurement of ENMs and ENPs, to allow for comparison of measurement results [Riediger and Möhlmann, 2001]. The core points of this convention includes that (i) the particle number concentrations (PNC) should be measured in the range form approximately 10 to 600 nm; (ii) the entire particle size distribution should be measured and (iii) a concentration range of up to 1 x 108 particle/cm^3 should be covered. Reflecting the current state of the art, it is possible to conclude that effective strategies for measuring exposure to a wide range of ENMs and ENPs and discriminating them from the background nanoaerosols are needed for exposure and risk assessment purposes, in particular those capable to determine health-relevant ENMs and ENPs properties. Further, the exposure assessment study design and strategy should follow the main available technical recommendations on the harmonization of the strategies for measuring exposure to ENMs and for data analysis [Methner et al., 2009; Cornelissen et al., 2012; Brouwer at al., 2012, EN 689:1995; EN 481:1993]. In this regard, no strictly-binding decisions were taken, but preliminary recommendations were agreed, based on discussions among experts and from current general practices. These include indication on (i) the type of the measurement (real-time task-based and peak measurements or real-time measurements averaged over a shift, for which repeated sampling is to be encouraged); (ii) the need for a multi-metric approach to exposure assessment (i.e. representative data on number concentration and particle size distribution, or particle number concentration with information about surface area or particle number concentration for at least particles $<$100 nm and particles $>$100 nm; a qualitative morphologic and elemental characterization of the EMNs, mass concentration of respirable fraction); (iii) the approach for the determination of the contribute of background aerosol due to the presence of other sources of ENPs (a representative size distribution analysis of background and process concentrations is also encouraged). In summary, at the current state of knowledge and technical development, a time-resolved, multi-metric approach including the assessment of mass concentration, surface area concentration and number concentration, followed by physico-chemical characterization represents the ideal approach.

Distinction from background

During sporadic events (e.g. leakage in the production line) very high PNC may be encountered (i.e. peak concentrations in the order of 10^5 - 10^6 particle/cm^3). As aforementioned discussed, if the initial screening reveals that the workplace is suspected to be contaminated by ENMs and ENPs due to increased PNC levels, the successive level of investigations of the tiered approach need to be more detailed [Methner et al., 2009]. In this regard, very important information can be obtained from the distinction of ENMs and ENPs from background nanoaerosol (i.e. ubiquitous ambient ultrafine particles deriving from various sources). Besides ENMs and ENPs exposure is often put into perspective by comparing it with the ambient background concentration of ultrafine particles [Berges et al., 2013]. Following direct-reading measurement strategies (that is characterized by high temporal resolution measurements), the identification of the ENMs sources (peak concentrations) is in general fairly easy. Nevertheless, the specific quantification of the ENPs concentration requires further analyses and remains a challenge. The following different strategies for the distinction of the single ENPs from the background aerosol have been proposed elsewhere [Kuhlbusch et al., 2011]: (i) time series approach: (any increase from background concentrations during the work activity can be attributed to the process ENPs); (ii) spatial approach (any difference between the defined background concentrations in a specific location and workplace concentrations can be linked to the work activity); (iii) comparative studies of the process with and without presence of the ENMs; (iv) size resolved chemical and/or morphological analysis. Most of the recent studies are likely to use a combined approach of time-series and spatial analysis, [Kuhlbusch et al., 2011]. However, it must be noted that there are several possible errors associated with use of the count-difference methods for background distinction. Firstly, incorrect estimates of PNC by size can results when the shape and refractive index of measured particles as having a smaller than actual diameter [Heim et al., 2008]. Secondly, during production and handling of nanoparticles the workplace ENPs number concentration is likely to be close to background concentrations [Brouwer et al., 2009]. Thus, it is plausible that sources of incidental nanoparticles (i.e. solid or liquid particulates emitted by the production process within the same size range but not corresponding to the production ENMs) and the large PNC fluctuations that may result from the presence of incidental nanoparticles, may make it impossible to identify actual releases of ENMs or ENPs [Methner et al., 2009]. In this regard, the selectivity (namely the ability to identify and discriminate ENPs in a mixture of generic ultrafine particles and nanoaerosols) is a critical issue when characterizing exposure using particle number concentrations because nano-scale particles often originate from multiple sources (e.g., combustion, vehicle emissions, and infiltration of outside air).

Consequently, background distinction approaches are considered only as a proxy for assessing ENMs and ENPs exposures. Thus, when chemical or morphological analysis is not feasible, and while appropriate standard methods are developed, the distinction from background nanoaerosols should be conducted with a precautionary and conservative approach, attributing the whole differential particle concentrations to ENPs (without any differentiation between incidental and production ENMs). Newly developed methods and instruments with good specificity, accuracy and sensitivity (i.e., based on morphological, chemical or surface activity analysis) are needed to overcome the problem of background distinction, anyway.

Comparison with OELs

To date, no legal-binding framework concerning ENMs or ENP-specific occupational limit values (OELs) exists, but several ENM-specific OELs were proposed by national organizations. In a recent review a summary of the currently proposed OELs for the various ENMs by national institutions is reported [Pietroiusti and Magrini, 2014]. In the absence of regulatory OELs, alternative strategies are required for risk assessment as, for example, the determination of technical exposure thresholds for the protection of workers' health. In this regard, nano reference values (NRVs) were developed to provide provisional threshold values in situations where recognised OELs and DNELs are not available. NRVs represent a warning (i.e. they act as an 'action level'): when these value are exceeded, exposure control measures should be taken [Cornelissen et al., 2012]. Besides, different limits have been proposed in different countries, since the assumptions upon which OELs have been derived may differ, as for example the size range of the particles to which these OELs refer (e.g., IFA, Germany: only ENPs with size of 1 - 100 nm; SWA, Australia: ENPs including aggregates and agglomerates $>$100 nm) or the safety factors used to derive OELs for ENPs from the bulk form of the material. Finally, other reference values (DNEL) for selected ENMs (e.g. fullerene, CNTs, silver, TiO_2) were proposed by experts [Stone et al., 2010]. It must be noted that NRVs, as the majority of the proposed OELs for ENPs, used number concentration as reference metric (although some of the proposed OELs are also expressed as mass concentration). Until reliable, easy-to-use and inexpensive devices are developed, this metric should be considered the reference to allow for data comparability. Other metrics, such as surface-area concentration, still has limited use and no proposed OELs have been developed in this regard. Besides, OELs and NRVs are typically 8-hour time-weighted average standards, but it should be considered that exposure in the workplaces can be often represented by transient peak concentrations, occurring during some specific work procedures. Taking this consideration into account, it was established a short-term exposure criteria to be considered to evaluate the need of further assessment: (i) short-term exposures should not exceed 3 times the particle control value for more than a total of 30 min per 8-h working day or (ii) a single short-term value should not exceed the particle control value by 5 times [ACGIH, 2010]. Furthermore, the scientific community agreed on the need of a more precautionary exposure threshold for ENMs and ENPs, that is twice the 8-h OEL over a 15-min TWA (time weighted average) [Van Broekhuizen et al., 2012]. Moreover, to complicate the issue of the definition and subsequent use of adequate OELs, it still has to be stressed that ENMs are not a uniform group of substances [Borm et al. 2006]. For example, there are currently about 50000 different types of carbon nanotubes due to different raw materials, production processes and catalysts and the same diversity applies to many other types of manufactured ENMs [Schulte et al. 2009]. In summary, although different limits have been proposed in different countries and harmonization would be desirable, the existing OELs and NRVs provide a reference to check the reliability of existing engineering and personal protective measures for exposed workers. Thus, as widely discussed in Pietroiusti and Magrini, 2014 [Pietroiusti and Magrini, 2014], the rational use of provisional OELs for ENPs may be advisable in occupational settings where exposure to ENMs is possible.

Risk management

As known, it's mandatory to ensure the health and safety of workers in every aspect related to their work, by conducting a regular and standardized risk assessment process, as specified in the 'Framework' Directive 89/391/EEC [EEC, 1991], including also possible risks from ENMs exposures. In addition, Directive 98/24/EC on chemical agents at work [EEC, 1998] imposes more restrictive indications on the management of risks from substances at work, according to the hierarchy of prevention strategies that strengthens elimination or substitution as priority measures. This also applies to ENMs, which fall within the general definition of 'substances'. Moreover, when an ENM or the corresponding macro-scale equivalent is carcinogenic or mutagenic, Directive 2004/37/EC on carcinogens and mutagens at work must be fulfilled [EEC, 2004]. In any case, national legislation may have stricter provisions and should be consulted. Moreover, being ENMs considered "substances", the REACH regulation [European Parliament, 2006] and the CLP regulation [European Parliament, 2008] are relevant anyway. The complexity and detail needed is dependent on the hazardous substance involved and the activity being undertaken. Standards and Guidelines developed on nano-safety in the past decade by government agencies, academia, and occupational health organizations are summarized in Table 1 and Table 2. The European Commission issued some communication documents [CCE, 2008] illustrating a plan of action to implement a safe, integrated and responsible approach to nanotechnologies, in order to properly develop, modify and implement legislation

through the improvement of scientific knowledge about risk assessment and management. These communications are also relevant for the protection of the general population (consumers) and the environment. The European Agency for Safety and Health at Work also developed more specific recommendations on how to manage risks of nanomaterials in occupational settings [EU-OSHA, 2013]. Because of the above-mentioned gap of knowledge on the toxicity and eco-toxicity of ENMs, traditional approaches for environmental, health and safety risk assessment of dangerous substances cannot always be applied.

Table 1: Standards and guidelines relevant for nano-safety

Type	*Field*	*Year*	*Institution/N°*	*Title*
IS	O	2008	ISO/TS 12885	Nanotechnologies - Health and safety practices in occupational settings relevant to nanotechnologies
IS	O	2011	ISO/TR 13121	Nanotechnologies - Nanomaterial risk evaluation
IS	O	2012	ISO/TS 12901-1	Nanotechnologies - Occupational risk management applied to engineered nanomaterials - Part 1: Principles and approaches
IS	O	2014	ISO/TS 12901-2	Nanotechnologies - Occupational risk management applied to engineered nanomaterials - Part 2: Use of the control banding approach
IS	C	2013	CEN ISO/TS 13830	Nanotechnologies - Guidance on voluntary labelling for consumer products containing manufactured nano-objects
IS	-	2009	CEN ISO/TS 27687	Nanotechnologies - Terminology and definitions for nano-objects - Nanoparticle, nanofibre and nanoplatelets
EG	O	2013	EU-OSHA	E-fact 72: Tools for the management of nanomaterials in the workplace and prevention measures
EG	C	2009	SCENIHR	Risk Assessment of Products of nanotechnologies
IG	C	2009	EFSA	Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain
NG France	O	2010	Agencie Nationale de Sécurité Sanitare	Development of a specific control banding tool for nanomaterials
NG Nederl.	O	2012	IVAM/version 4.2	Working safely with engineered nanomaterials and nanoproducts - A guide for employers and employees
NG U.K.	O	2008	BSI/PD 6699-2	Nanotechnologies - Part 2: guide to safe handling and disposal of manufactured nanomaterials
NG U.S.A.	E	2007	U.S. EPA	Nanotechnology white paper (EPA/100/ B-07/001) Concept Paper for the Nanoscale Materials Stewardship Program under TSCA
NG U.K.	E	2006	U.K. Defra	UK Voluntary Reporting Scheme for engineered nanoscale materials Consultation on a Proposed Voluntary Reporting Scheme for Engineered Nanoscale Materials
NG Canada	E	2007	Environment Canada Health Canada	Proposed Regulatory Framework For Nanomaterials Under The Canadian Environmental Protection Act, 1999 Requirements for nanomaterials under the New Substances Notification Regulations

IS = International Standard; EG = European Guideline; IG = International Guideline; NG = National Guideline; W = Workplace; C = Consumers; E = Environment

Table 2: Scientific publications and reports on risk management of ENM

Author	*Year*	*Citation*	*Title*
Schulte et al.	2016a	Nanotoxicology, DOI: 10.3109/17435390.2015.1132347	Assessing the protection of the nanomaterial workforce
Schulte et al.	2016b	J. Nanopart. Res., 2016, **18**, 159.	Taking stock of the occupational safety and health challenges of nanotechnology: 2000 - 2015
GAO	2014	GAO-14-181SP	Nanomanufacturing. Emergence and Implications for U.S. Competitiveness, the Environment, and Human Health
Read et al.	2014	In book: Handbook of Nanosafety - Measurement, Exposure & Toxicology, Chapter 2; 17 - 58	Nanotechnology and Exposure Scenarios
Berges et al.	2014	In book: Handbook of Nanosafety - Measurement, Exposure & Toxicology, Chapter 8; 279 - 326	Risk Assessment and Risk Management
FIOH	2013	http://www.ttl.fi/en/publications/electronic_publications/pages/default.aspx	Nanosafety in Europe 2015-2025: Towards Safe and Sustainable Nanomaterials and Nanotechnology Innovations
Roco et al.	2011	ISBN: 978-94-007-1167-9 (Print), 978-94-007-1168-6 (Online)	Nanotechnology research directions for societal needs in 2020
OECD	2009	ENV/JM/MONO(2009)18	Report of an OECD Workshop on Exposure Assessment and Exposure Mitigation: Manufactured Nanomaterials
Aitken et al.	2009	IOM Report TM/09/01	EMERGNANO: A review of completed and near completed environment, health and safety research on nanomaterials and nanotechnology
Stone et al.	2010	Project Final Report	Engineered nanoparticles: Review of health and environmental safety (ENRHES)
Nel et al.	2010	WTEC panel report (http://www.wtec.org/nano2)	Nanotechnology environmental, health, and safety issues
Maynard et al.	2005	J. Nanopart. Res., **7**, 587 - 614.	Airborne nanostructured particles and occupational health

Thus, according to the precautionary principle [COM, 2000], is necessary to minimize exposures at workplace where high exposure levels can occur, but also control industrial emissions of ENMs in air, water and soils for the protection of the general population. Available data indicate that relevant occupational exposures may occur whenever the implementation of protective measures is inappropriate or neglected. Besides, in the absence of regulatory OELs for most ENMs, a strategy is needed to correctly assess the hazard and determine the appropriate levels of exposure control to protect workers' health. In this regard, a number of guidelines and tools have been developed in order to facilitate workplace risk assessment and management. However, the validation of the effectiveness of the exposure controls and measurement methods for ENMs remains a key research need. The adoption of a risk management strategy is generally recommended: a tiered approach for preventing and mitigating exposure should be implemented to eliminate or control the exposure associated with the occupational handling of ENMs and ENPs. It is globally recognized that a general hierarchical approach in risk management must be implemented to eliminate the hazard when possible (i.e., substitute with a less hazardous material) or, if not feasible, control the hazard as close to the source as possible [Mirer et al., 2008]. Risk management programs should include detailed guidelines on engineering controls, education and training of workers (e.g., good work practices), selection and use of personal protective equipment (e.g., clothing, gloves, respirators). The hierarchy of controls typically includes engineering controls (chemical fume hoods, enclosed production processes, gloveboxes and high-efficiency particulate air-filtered vacuums), warnings, training and procedures (reducing the exposure durations and the number of people exposed) specific work practices, and use of personal protective equipment (PPE), with the aim to reduce the envi-

ronmental concentrations of ENMs, the exposure duration and the number of people exposed. A number of control banding strategies have been also proposed for workplaces where exposure to ENMs can occur; to date, however, it is unclear if the use of such techniques can effectively reduce worker exposure to nanomaterials [Eastlake et al., 2016b; Liguori et al., 2016].

Discussion

Occupational exposure to ENMs and ENPs may pose a health risk that is not fully addressed by conventional and existing exposure assessment methods. At the current state of knowledge and technical development, a multi-metric approach including the assessment of mass concentration, surface area concentration and number concentration with high selectivity for ENPs, represent the ideal study design. However, challenging measurement strategies can't always be transferred to real occupational scenarios, and in practice, a measurement approach based on number concentration seems the most practical one. In spite of these limitations, provisional (non legal-binding) OELs, both based on number and mass concentrions, have been developed by some national organizations. Although different limits have been proposed in different countries, they may provide affordable reference values to check the reliability of existing engineering and personal protective measures for exposed workers.

Priorities and needs

Despite the progress made in recent years on measurement techniques and strategies for the exposure assessment of ENMs and ENPs, several gaps need to be filled and the following priorities have been identified:

(i) better definition of the health risk for workers occupationally exposed to ENPs. Further research is still needed in the emerging field of nano-toxicology, starting from the assumption that the peculiar physicochemical properties of ENPs and ENMs (e.g., chemical structure, thickness, lateral size, surface charge, surface area) may have significant influence on their biological/toxicological activity. In this regard, future *in vivo* studies should investigate all the different possible adverse effects induced by ENM exposure on different organs and systems, whereas *in vitro* studies should identify the several molecular mechanisms of action underlying the toxic effects. Consequently, systematic studies addressing the role of ENPs and ENMs properties in determining adverse health impacts are urgently required, also in order to orientate the definition of appropriate methods to characterize the actual occupational and environmental exposure levels;

(ii) harmonization of strategies for measuring exposure to ENM or ENPs and for the analysis of data is needed. Despite preliminary recommendations were agreed upon some aspect of the exposure assessment procedures (i.e., measurement type, multi-metric approach, distinction from background aerosol), univocal, standard monitoring methods are not currently available for the assessment of exposure to airborne nanoaerosols. Other needs that have been identified include: criteria on statistical approaches to analyze time-series data, guidance on ENMs analysis and its agreed reporting in nano-specific exposure terminology [Brouwer, et al., 2012];

(iii) development of reliable and easy-to-use instruments for high-selectivity and high-accuracy ENMs and ENPs measurements. The development of easy to use devices by occupational hygienists for exposure measurements is urgently needed even though no agreement on the best metric for exposure exists. To date, every attempt to assess the occupational exposure to ENMs and ENPs requires the use of multiple sampling and monitoring techniques; some of them are not yet fully evaluated in terms of accuracy and reliability. Nano-specific monitors and samplers are expected to improve their performance (inter-comparability, accuracy, selectivity to ENPs), as well as the compactness, portability and costs for routinary applications. In this regard, the definition of clear performance criteria to achieve comparability is deemed. Particular emphasis should be reserved to the development, testing and validation of devices to evaluate the workers' personal exposure to ENMs and ENPs, possibly delivering reliable results that can be used in exposure assessment, health impact assessment and risk management studies;

(vi) the development of realistic exposure scenarios for the comparative assessment of different tasks and industrial processes, as well as the harmonization of OELs, is desirable. It is worth noting that the definition of reference regulatory standards needs to take into account some fundamental aspects; in particular, the OELs need to take into consideration both discrete ENPs and agglomerates/aggregates, if analogies in effects of human health from exposure are identified (with respect to a potential independence of the health impact assessment from particle size); otherwise, differentiated exposure limits must be established.

Proposal for the development of exposure assessment protocols

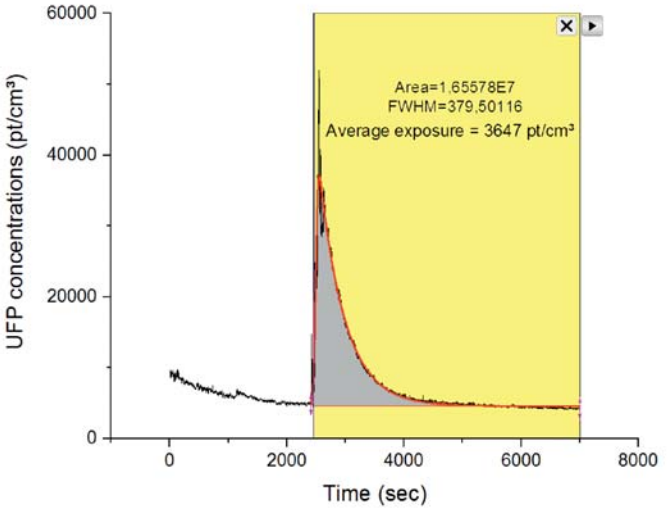
On the basis of the existing approaches, an exposure assessment study should be performed following a multilevel protocol, which may result to be cost- and time-effective. A comprehensive proposal for an exposure assessment study should involve firstly a preliminary assessment (analysis of the work practices, literature review, observational survey of the production area) for the identification the potential source(s) of engineered nanomaterial emissions, to identify the processes/tasks that require an in-depth monitoring, the frequency and duration of each operation and the type of equipment used for handling and containment of ENMs. Secondly, environmental (and,

when feasible, personal) measurements, based on the information collected in the preliminary analysis, should then performed in accordance with a tiered approach, such as NEAT [Methner et al., 2009; Eastlake et al., 2016a] and NanoGEM [Asbach et al., 2012b] or strategies proposed by other institutions [Cornelissen et al. 2012; BAuA et al., 2011]. Some key issues derived from direct on-field exposure and regarding the exposure assessment approaches, are then described hereafter. Note that since number concentration is the most widely used method in ENPs exposure assessment, the following recommendations are mainly referred to measurement based on this exposure metric:

(i) determination of the "background" particle number concentration (natural and anthropogenic nanoparticles in the ambient and environmental air): direct-reading instruments should be used to measure, while ENPs are not handled, applied or produced, the total particle number concentrations (PNC), both for particle >100 nm and <100nm, and the mean size-dependent characteristics of particles (mean diameter, surface-area concentration). Average airborne PNC can be measured before and after the production or handling of ENMs in each workplace location, to obtain location-specific average background number concentration, which can be then subtracted from the measurements made during processing, manufacturing, or the handling of ENMs, assuming that the emissions during process are stable during the measurements [Mether et al., 2009; Brouwer et al., 2009; Koivisto et al., 2012a; 2012b; 2014; 2015; Koponen et al., 2015; Jensen et al., 2015];

(ii) determination of the total particle number concentration during the production process (in presence of ENPs) by means of micro-environmental and personal measurement: direct-reading instruments should be used to evaluate the total particle number concentrations (PNC), including that of the background and the mean size-dependent characteristics of particles (mean diameter, surface-area concentration), while filter-based particle sampling should be used for the determination of size-resolved particle mass concentrations. Once the background particle number concentrations have been determined, specific measurements can be made with all of the available instruments simultaneously at different locations: the results from this type of measurement should be interpreted as an indicator of workplace's micro-environment exposure concentration, which can be preliminarily compared with proper threshold limits (i.e., in order to evaluate the need of a more in-depth study). The monitoring time should match the length of time necessary to complete an entire work shift (or at least half of the work shift). Direct reading instruments should be placed at both the process/task location where operations involve ENPs emissions or application, and at the process/task location where ENPs were not directly involved (i.e. offices), as a "control area". A time-activity diary should be used to separate the continuous concentration data as a function of the different monitored environments and working tasks. Filter-based, size-selective air sampling should be performed at least in one selected location (i.e. following a worst-case exposure scenario approach, where highest expected exposure concentrations). Personal monitoring should be performed, when feasible, in the breathing zone of workers who can be exposed. All of the identified tasks/locations that required in-depth monitoring should be monitored preferably simultaneously for the entire length of the work shift;

(iii) distinction of ENPs from background aerosols, to obtain occupational ENP exposure concentrations (OEECs) for exposed workers. Exposure to ENPs should be estimated by subtracting the workplace ENPs concentrations with the location-specific background concentration. As discussed previously, different approaches can be applied for background distinction [Berges, 2013; Kuhlbusch et al., 2011]. Note that all these approaches are considered as a proxy for assessing ENPs exposures, since there being several possible errors associated with use of the count-difference methods for background distinction. One of the better solution, is to perform the distinction from background aerosols with a precautionary and conservative approach, attributing the whole differential particle concentrations as ENPs (without any differentiation between incidental and process-related engineered nanomaterials). The issue of background distinction in of particular interest and is deepen in a dedicated chapter (Distinction from background). Note that further indication could be obtained from the distinction from background: a high particle number concentration for particles <100 nm, combined with a high particle count in the sub-micrometer size range (0.3 - 0.5 μm), indicates the possible presence of nanoscale particles; contrariwise, a high count in the size range above 1.0 μm in presence of a low count for particles <100 nm, would indicate the presence of larger particles and/or agglomerates [Methner et al., 2009]. Moreover, a more accurate and quantitative distinction of occupational exposures to ENPs from the background contamination by ultrafine particles can be also achieved by peak fitting methods using suitable profile functions (convolutions of log-normal, gaussian, exponential functions with exponential decay functions) after background least squares interpolation (i.e. using polynomial functions) and subtraction. Multiple peak fitting routines are included in some data processing programs. This approach, commonly used in analytical chemistry, can be useful when an accurate determination of time-integrated exposure of workers to multiple process/activity-related spikes is needed (Figure 1);



Model	GaussMod
Equation	double z = (x-xc)/w - w/t0; y = y0 + A/t0 * exp(0.5*(w/t0)^2 -(x-xc)/t0)*(erf(z/sqrt(2))+1)/2;
Plot	UFP concentrations
y0	4591,23728 ± 21,62798
A	1,66712E7 ± 63144,45095
xc	2491,98917 ± 0,36577
w	31,32607 ± 0,47548
t0	434,05854 ± 2,30884
Reduced Chi-Sqr	1,28142E6
R-Square(COD)	0,97561
Adj. R-Square	0,97559

Figure 1: Example of determination of time-weighted occupational exposures and distinction from the background contamination by ultrafine particles, for a worker involved in an activity characterized by transient peak of ENPs, by means of peak fitting method (Model: GaussMod; Software: Origin Pro 2016)

(iv) estimation of 8-hour Time Weighted Average (8-hr TWA) exposures for ENPs. The occupational exposure concentrations (obtained by background concentration subtraction) should be used to estimate the 8-hr TWA exposure to ENPs for workers involved in different working tasks. If feasible, a microenvironmental model can be used: following this approach, time-weighted average exposure can be defined as the sum of partial microenvironmental exposures, which are determined by the product of the concentration of ENPs and the time spent in each microenvironment or work task. For this purpose, time-activity patterns should be defined for each worker, or derived from time-activity diaries and a questionnaire to be submitted to workers;

(v) comparison of these exposure values with occupational exposure thresholds or nano reference values. When available, OELs and NRVs consist typically in 8-hour TWA standards and are typically expressed as number or mass concentrations. In order to evaluate short term exposures, the criteria proposed by ACGIH [ACGIH, 2010] should be used (short-term exposures should never exceed the 8-hr TWA threshold value by 5 times, and should not exceed the defined threshold by 3 times for more than a total of 30 min per working day). Further, it should also be considered that a short-term (15-min) threshold can be set as twice the 8-h OEL [Van Broekhuizen et al., 2012].

Conclusions

Exposure to ENMs and ENPs pose specific challenges and possible risks to the occupational, health and safety, due to the great diversity of ENMs manufactured and used in industrial setting and in general life environments. Despite some recent research efforts, nanotechnology progresses are growing faster than the scientific knowledge about the health and safety aspects of ENMs. There are still knowledge gaps regarding the impacts of nanomaterials on workers' health and safety and regarding exposure assessment methods. Thus, it is important that priorities in risk assessment (with particular emphasis for exposure assessment) and risk management processes are given not only to ENMs with known effects on health and safety, but also to the large part of ENMs for which there is missing, incomplete or uncertain information regarding their hazards and exposures, in which case a precautionary approach to prevent exposures to ENMs in the workplace should be applied. Reflecting the current state of the art, it is possible to conclude that effective approaches for measuring the occupational exposure to ENMs and ENPs and discriminating them from the background nanoaerosols are needed for exposure and risk assessment purposes, in particular those capable to determine peculiar ENPs properties, such as surface-area concentrations. Further, due to the encountered rather low concentrations of ENPs in comparison to those of UFP at workplace, additional methods and novel instruments with good specificity, accuracy and sensitivity (i.e. based on morphological, chemical or surface activity analysis) are necessary in order to overcome the problem of background distinction. To our knowledge, limited workshift average surface-area concentrations exposure levels are currently available in the scientific literature, but the need to measure the surface area of ENPs is justified by a stricter correlation with the potential biological effects on humans. In conclusion, the comprehensive characterization of exposure will probably remain a challenge in the near future: some technical and scientific improvements and the harmonization of strategies for measuring exposure to ENPs for the analysis of data are needed. In this regard, preliminary recommendations were agreed upon

some priority needs, including: (i) a multi-metric approach to exposure assessment and (ii) a number of contextual information to be collected. The combined use of devices for in-situ assessments and offline sampling analysis represents, today, the best tool for the estimation of ENPs exposure.

Conflict of interests

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