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OVERVIEW

Nanomaterial exposure, toxicity, and impact on human health

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The use of engineered nanomaterials (ENM) has grown after the turn of the 21st century. Also, the production of ENM has globally grown, and exposure of workers especially via the lungs to ENM has increased. This review tackles with effects of ENM on workers' health because occupational environment is the main source of exposure to ENM. Assessment of exposure to ENM is demanding, and today there are no occupational exposure level (OEL) for ENM. This is partly due to challenges of such measurements, and in part to the unknown causality between ENM metrics and effects. There are also marked gaps in systematic knowledge on ENM hazards. Human health surveys of exposed workers, or human field studies have not identified specific effects of ENM linking them with a specific exposure. There is, however, a consensus that material characteristics such as size, and chemistry influence effects of ENM. Available data suggest that multiwalled carbon nanotubes (MWCNT) affect the immunological system and cause inflammation of the lungs, or signs of asthma whereas carbon nanofibers (CNF) may cause interstitial fibrosis. Metallic and metal oxide nanoparticles together with MWCNT induce genotoxicity, and a given type of MWCNT has been identified as a possible human carcinogen. Currently, lack of understanding of mechanisms of effects of ENM renders assessment of hazards and risks of ENM material-by-material a necessity. The so called "omics" approaches utilizing ENM-induced alterations in gene and protein expression may be useful in the development of a new paradigm for ENM hazard and risk assessment.

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KEYWORDS
assessment of hazards and risks, engineered nanomaterials, exposure, health effects

1 | INTRODUCTION

The use of engineered nanomaterials (ENM) and their applications have spread widely since the turn of the 21st century due to multiple technological benefits of material at a nanoscale. Technologies utilizing ENM are called nanotechnologies even though the only common issue in different nanotechnologies is the use of often very different materials at a nanoscale for very different purposes. For the above reasons, nanotechnologies have been recognized as highly cross-cutting ones, whose products, based on the use of ENM, utilize physical and chemical properties of nanoparticles, different from their chemically identical bulk counterparts (Schmid, 2010).

However, during the production and use of ENM there is the chance of exposure for workers, consumers and the environment (Savolainen et al., 2010; Valsami-Jones & Lynch, 2015). The effects of such exposure cannot be predicted based on our current understanding of chemicals, given the fact that material at nanoscale has both particulate identity and molecular identity which are responsible for the biological effects. To this end, novel approaches for the prediction of material at nanoscale need to

be developed (Kinaret et al., 2017). There are currently a number of ongoing attempts to develop such hazard prediction tools and frameworks (www.nanosolutionsfp7.com/; www.guidenano.eu/; www.nanomile:eu-vri.eu/; www.sun-fp7.eu/).

Although it has been argued that size as such does not cause harmful effects (European Commission, 2011), a number of studies have convincingly shown that nanomaterials cause toxic effects not induced by their chemically similar but larger particles (Catalán et al., 2016; Palomäki et al., 2011; Rossi et al., 2010). Hence, engineered nanoparticles may in many cases enable harmful effects in biological organisms, not possible for the chemically identical, but larger particles. These effects are likely due to exposure to the unique features of engineered nanoparticles, either to their intrinsic properties or to their small entity that allows them reaching targets not reachable by their larger, chemically identical counterparts (Kreyling et al., 2009). An important issue in this context is the biocorona formed to surround every single nanoparticle, and a larger particle, once it reaches biological environment. It is though likely that the formation of biocorona has a larger impact on the features of nanoparticles, among others because of the proportionally larger change in particle dimensions as compared with larger particles. Hence, the layer of proteins and lipids attached to the surface of a given nanoparticle greatly affects dimensions thereby markedly influencing molecular and cellular targets the particles can reach (Monopoli, Aberg, Salvati, & Dawson, 2012).

Another major challenge in the assessment of hazards of ENM to experimental animals, humans, and environmental species is, that being in a particulate form, the behavior of ENM differ dramatically from that of traditional soluble chemicals having an impact, not only on the kinetics of ENM in biological environments, but also on their potentially harmful effects (Savolainen & Alenius, 2013).

The properties of nanomaterials cause marked challenges to the assessment of hazard of ENM via the lungs, but also via other exposure routes. However, in the lungs, when the nanoparticles most readily reach the body, they also immediately become covered by biomolecules rendering the kinetic behavior and effects more difficult to assess. The special features of the airways hence add to the complexity to the hazard assessment of ENM via the inhalational route. In general terms, assessing effects of ENM is demanding because the associations of harmful effects of ENM features (physicochemical and biological) are not well understood.

Exposure to ENM via oral route may not yet be important for human health, but the importance of this issue is likely to increase when the use of ENM especially in food items becomes more widespread. Thus, reliable exposure and hazard assessment methods should be developed quickly in response to future demands.

The more than 1,600 "nanoenabled" products in commerce, all required workers for that to happen (http://www.nanotechproject.org/cpi/), and, according to recent estimates, 6 million workers will be potentially exposed to ENPs in 2020 (Roco, 2011).

Some of the known effects of engineered nanoparticles include those of titanium dioxide (National Institute of Occupational Safety and Health [NIOSH], 2011) and of metal oxide and metal (Saber et al., 2013), and carbon containing material induced pulmonary inflammation (Kinaret et al., 2017; Mercer et al., 2013; Palomäki et al., 2011; Ryman-Rasmussen et al., 2009). These effects include among others inflammation, granuloma formation, and fibrosis of the lungs (Rossi et al., 2010; Ryman-Rasmussen et al., 2009; Saber et al., 2013). It has also been shown that both tangled and rigid rod-like carbon nanotubes (Mitsui-7) can reach the lung and subsequently the sub-pleural space and cause collagen deposition. Subpleural space is also the site of pulmonary mesothelioma initiation (Mercer et al., 2013; Ryman-Rasmussen, Cesta, et al., 2009). However, only rigid, rod-like carbon nanotubes (CNT) have been shown to induce mesothelioma in rodents (Sargent et al., 2014; Takagi, Hirose, Futakuchi, Tsuda, & Kanno, 2012). In addition, several fibrous and crystalline ENM have been shown to induce genotoxic effects in vivo and in vitro (Catalán et al., 2016; Kinaret et al., 2017).

However, even though there is a plethora of detailed information on specific toxicity of several ENM, a systematic toxicity database does not exist rendering ENM risk and safety assessment challenging, especially because information on exposure to ENM in occupational setting or other environments is lacking for most of the materials (Savolainen & Alenius, 2013; Valsami-Jones & Lynch, 2015). This situation is reflected by the fact that there are no occupational exposure limits (OEL) implemented for any of the ENM anywhere (Van Broekhuizen & Reijnders, 2011; Van Broekhuizen, van Veelen, Streekstra, Schulte, & Reijnders, 2012). Hence, it is not surprising that there are concerns regarding the safety of ENM in the occupational environment, in consumer products, and in causing burden to the environment.

In this context, issues relevant to occupational environment will be mainly dealt with in this review because the most significant exposure to ENM takes place in workplaces. Also, much less is known of the consumer and environmental exposure.

2 | EXPOSURE

2.1 | Nanomaterials in the workplace

ENM are present at an increasing amount in the workplace, as indirectly shown by the number of nano-enabled products on the market, as reported in the Introduction. In 2004, 20,000 to 114,000 jobs in 450 nanotechnology enterprises were reported in Germany (Kaluza et al., 2009).

TABLE 1 ENM involved in different activities in industry and research laboratories (Elaborated from Ding et al. (2017))

Activity	Metal/metal oxide	Carbonaceous material	other
Collection, sorting & processing (including sieving)	TiO_2, SiO_2, Al_2O_3, CeO_2, iron oxides, Mn, Ag, Co, Si	carbon black, CNT, SWCNT, MWCNT, carbon nanofibers (CNFs), carbon nanopearls, nano diamond, carbon nanodiscs/carbon nanocones, graphene	
Physical & chemical synthesis	TiO_2, Al_2O_3, Ag_2O, MgO/Y_2O_3/CaO/FeO, $BiPO_4$/Bi_2O_3/NaCl/$CaSO_4$/ZnO/ZrO_2/WO_3/Ta_2O_5/Pt/Ba, lithium titanate, Ag, Si, other metal-based nanoparticles	CNT, MWCNT, SWCNT, CNFs, carbon black, graphene	
Weighing, transferring & mixing	TiO_2, Al_2O_3, CuO, SiO_2, ZnO, MgO/Y_2O_3/CaO/FeO, CeO_2, Ag, Si, Indium tin oxide	MWCNT, CNTs, carbon black, carbon nanofibers, fullerenes, carbon nanodiscs/carbon nanocones	Ceramic powders
Machining & abrasion	Alumina fiber	MWCNT, carbon fibers	Nylon 6 nanofiber
Cleaning & maintenance	TiO_2, Mn, Ag, Co, Al, Al_2O_3/SiO_2/CeO_2 [49], Metal oxides (Ag,Cu,Co,Ni,Fe,Mn)	SWCNT, fullerenes, carbon nanofibers, Graphene	
Finishing	CeO_2, TiO_2, silica-iron nanomaterial, indium tin oxide, ZnO, SiO_2	Carbon black, MWCNT	
Packing and bagging	Si, TiO_2, SiO_2	Carbon black, Fullerenes, CNFs, MWCNT, carbon nanodiscs/nanocones	CaCO3
Sonication	Ag_2O, CeO_2	Fullerenes, MWCNT, carbon black	
Testing	Cadmium-zinc/selenide quantum dots		Nylon 6 nanofiber
Ball milling		MWCNT	
Feeding	Silver		
Recycling	SiO2/Al2O3/CeO2	CNTs	

However, a precise identification of companies producing and/or processing ENM is not simple, because ENM may be used in many activities by companies that are not labeled as nanotechnology companies and which do not identify themselves as such (Schulte et al., 2016). Therefore, in order to assess the presence and extent of ENM in the workplace it is better to refer to the activities rather than to the type of industry. Using this approach, Ding et al. (2017) recently reviewed the ENM more frequently present in various activities in industry and research laboratories, as shown in Table 1.

Of course, much higher amounts of ENM are produced and handled in industrial activities in comparison to laboratory activities. The order of magnitude in industrial activities ranges from kg per batches to thousands of tons per year, whereas in research laboratories the range is grams to kg. The ENMs produced and handled in industry in the largest amounts are $CaCO_3$ and carbon black, which were in the market since a long time before the "nanotechnology era" (Tsai et al., 2011), whereas among the nanotechnology products, TiO_2 is the ENM produced in the largest quantity (Yang et al., 2012).

2.2 | Routes of exposure

There is no doubt that the respiratory system is the most prominent exposure route for ENM in the occupational environment (Borm et al., 2006; Savolainen et al., 2010; Shvedova et al., 2005). There are of course other routes through which exposure may take place. They include the skin, gastrointestinal tract and the eyes. In the respiratory system, the size of the particles has a major impact on their systemic distribution in the body, or accumulation in the lungs (Kreyling et al., 2009). The reason why the lungs are important, is that ENM most easily enter the body in an aerosol form, whereby they can, if of correct size, reach the alveolar region in the lungs (ICRP, 1994). Overall, the size distribution of the particles markedly influences the possible target areas of the nanoparticles in the airways. Key-issues in particle deposition in the lungs include the dynamic behavior of particles in an aerosol, influencing the aerodynamic particle size through agglomeration or aggregation of primary particles. At the end, the particle size is crucial in determining how deep in the respiratory tract the particles can enter (Seipenbusch et al., 2014). Furthermore, especially hygroscopic particles will grow in the saturated air of the airways, and the deposition in the respiratory tract will depend on the 'wet size' instead of the commonly measured 'dry size' (Brouwer, Liden, Aschbach, Berges, & van Tongeren, 2014).

Particles will also upon contact with the biological milieu in the lungs become surrounded by biomolecules such as albumin and proteins in the surfactant (Monopoli et al., 2012). This increases the diameter of the particles and has an impact on

the particle kinetics in the airways, that is, whether it is caught in the respiratory tract wall or macrophages due to impaction, or whether it reaches the alveolar region, where it can be phagocytized by alveolar macrophages or pass through the alveolar wall and reach the systemic circulation. Larger particles, at a micrometer scale, are caught in the nose or upper respiratory tract and removed by macrophages or by the mucociliary escalator from the airways, and then swallowed (ICRP, 1994).

The primary region of the deposition of small ENM—in the range of few tens nanometres—is the alveolar region of the rodent and human lung. Of those ENM that reach the alveoli, the smallest ones, in the range of few nm can pass the alveolar wall and reach the systemic circulation (Kreyling et al., 2009). Many ENMs are characterized by a high surface reactivity and may therefore induce inflammation and generation of reactive oxygen species (ROS) at the site of deposition, causing local injury (Donaldson, Poland, & Schins, 2010; Mercer, Scabilloni, Hubbs, Battelli, et al., 2013; Ryman-Rasmussen, Tewksbury, et al., 2009). A small fraction of ENM that reach the alveolar region can translocate to internal organs and organ systems via lymphatics and blood, and may cause damage through direct and indirect mechanisms (Kreyling et al., 2009). In addition, even in the absence of ENM translocation, systemic damage may arise from the release of inflammatory mediators into the systemic circulation (Erdely et al., 2011; Saber et al., 2013). Donaldson et al. (2013) emphasize the use of the Biologically Active Dose (BED) paradigm in exposure and hazard assessment. In inhalation toxicology. BED is defined as the dose that actually reaches the target organ and hence drives a critical patho-physiologically relevant form of toxicity such as oxidative stress, genotoxicity, or inflammation. BED is relevant for different routes of exposure to ENM, but is most relevant for inhalational exposure to particles. In routine assessment of exposure to ENM the BED approach is not used because it adds to the complexity of ENM exposure and hazard assessment. However, in rare cases, lung burden estimates have been used in the context of risk assessment of nano titanium dioxide (National Institute of Occupational Safety and Health, 2011) and CNT and nanofibers (National Institute of Occupational Safety and Health, 2013).

The gastro-intestinal route is potentially important for consumers (Pietroiusti, 2012). However it is considered less relevant for workers, at least in comparison to the pulmonary route. It is noteworthy that a substantial percentage of inhaled nanoparticles are cleared by the muco-ciliary escalator cells into the oral cavity and thereafter by swallowing into the gastrointestinal tract (Geiser & Kreyling, 2010). One should also note that ENMs deposited in the skin may reach the gut lumen through hand-mouth contact. Once having reached the gut, ENMs may exert local toxic effects (Bergin & Witzmann, 2013; Nguyen, Lin, & Mustapha, 2015). An interesting new perspective is represented by the possible effects mediated by the interaction of ENMs with the gut microbiota (the community of organisms living within the gastrointestinal tract). There is in fact recent evidence that the gut microbiota may have a relevant role in modulating both local and systemic biological effects of ENMs (Pietroiusti, Magrini, & Campagnolo, 2016). The importance of the gastrointestinal tract as a route of exposure to ENM may markedly increase in the future when the use of a variety of different kinds of ENM will be utilized in different food items. This may become an important area of research, because, when in food items, ENM are largely bound to various biomolecules, and very little is known of the kinetics of ENM when such mixture enters the gastrointestinal tract of humans or other mammals. Hence, ENM behavior in the intestine and its microbiota may represent an important area of research in coming years.

Skin is the largest organ in the human body, and hence the potential contribution of dermal exposure to ENMs should not be ignored. Estimates of possible dermal exposure to manufactured ENMs in the workplace have been reported (Van Duuren-Stuurman et al., 2010). However, no clear evidence exists on the penetration of ENM through intact or even damaged or inflamed skin into the systemic circulation. It is plausible though that dermal exposure may lead to penetration of nanoparticles into the superficial layers, the dermis, of the skin, causing there a local inflammatory reaction (Gulson et al., 2010).

2.3 | Methods and strategies of ENM evaluation in the occupational setting

European Commission (EC) has defined nanoparticles as being "natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm" (European Commission, 2011). This definition implies that the number concentration, that is, the number of ENM usually in a cubic centimeter of air, of ENMs should be known in the process of risk assessment at all times, also suggesting the need for the evaluation of this parameter. To this end, the EC definition has mainly legislative purposes, which means that adverse health effects may occur even at a lower level of exposure (i.e., an exposure to material having less than 50% of the particles in the nanoscale range). Furthermore, in reality, the measurement of nano-sized particles in aerosols containing ENM at any given moment is not possible because such aerosols are always in a continuous, dynamic change (Seipenbusch, Binder, & Kasper, 2008). Hence, the recommendation has regulatory significance, but in research context it is unlikely to be applied.

The above EC definition of nanoparticles has usually not been utilized in assessing exposure to ENM. One also needs to take into account that there are very few studies in which health effects of ENM have been explored using particle number

concentrations, that is, number of particles per unit space (e.g., cm^3) as a measure of exposure (Van Broekhuizen et al., 2012; van Broekhuizen & Reijnders, 2011). This has regulatory impacts, because experimental rodent studies are traditionally conducted using ENM mass as a measure of exposure, and comparison of ENM mass versus the number of ENM/cm^3 is far from simple. In addition, assessment of exposure of workers to ENM is often carried out by measuring on-line ENM number concentrations, which are not easy to routinely transform into mass units.

The use of different measures of exposure to ENM renders the comparison of results of studies with different exposure assessment approaches a challenge.

There are currently several sampling strategies to assess the exposure to engineered nanoparticles for different purposes, such as whether exposure events to ENM take place, for modeling purposes, for surveillance of workplace air, or risk assessment, which have been summarized by Brouwer et al. (2009). A rather similar approach has been developed simultaneously by a number research organizations in the world with the aim of separating the exposure to engineered nanoparticles from exposure to the ubiquitous background nano-sized or ultrafine particles whether from traffic exhausts or energy production. A good description of one typical approach of an exposure assessment strategy for risk assessment has been provided by NIOSH (National Institute of Occupational Safety and Health, 2009). The NIOSH 8 h recommended exposure limit value (REL) for CNT and carbon nanofibers requires the measurement of the respirable fraction followed by off-line determination of the amount of elemental carbon (EC) in the sample (Brouwer et al., 2014; National Institute of Occupational Safety and Health, 2013).

In workplaces, exposure to ENM is often carried out by using a direct-reading hand held device such as condensation particle counter (CPC) or optical particle counter (OPC) which provide information on the particle numbers and size distribution but not the source or chemical composition of the particles (Maynard et al., 2004). In addition to CPC or OPC, also fast mobility particle sizer (FMPS), scanning mobility particle sizer (SMPS), and electric low pressure impactor (ELPI) can be used to measure the nanoparticle number concentrations (Methner, Hodson, Dames, & Geraci, 2010). Background particle number concentrations in the surroundings need to be measured and compared with the particle concentrations near the source to assess whether the occupational levels exceed the background levels suggesting occupational exposure. Similar measurements can be made in the workers' breathing zone, and these measurements should be repeated before and at the end of the work shifts where ENM are being used. If there is a clear indication that the nanoparticle number concentration exceeds that of the number concentration of background nanoparticles, one can assume that the workers are exposed to process-derived ENM (Methner, Hodson, & Geraci, 2010). A good example of this approach is the study by Peters et al. (2009) in which they measured nanoparticle concentrations before and after the workday and throughout the day, and overlaid the particle number concentration on daily work activities, trying to find associations between those activities and alterations in nanoparticle number concentrations in the workplace air. The association between the working periods and increased exposure was clear and has been confirmed by other studies (Hämeri, Lähde, Hussein, Koivisto, & Savolainen, 2009).

Particles can also be collected on filters: background sampling, source specific sampling, and personal sampling are performed. The collected material is thereafter transferred for scanning electron microscopy (SEM), or transmission electron microscopy (TEM), which may be coupled with energy dispersive X-ray analyzer (EDS) for a complete structural and chemical analysis. Elemental analysis may also be performed by means of inductively coupled plasma-mass spectrometry (ICP-MS), but this is laborious, time consuming and expensive, and not suitable for routine workplace exposure assessment. In order to validate the procedure, the background particle number concentration shall be measured again (Brouwer et al., 2014).

The appeal of the described procedures is that they are relatively simple and may be performed on the field with portable instruments of relatively low cost. This process cannot be considered a definitive response to the issue of workplace evaluation of nanoparticles. At some time, in the not too distant future, it is likely that workers' exposure standards will be promulgated, and therefore a quick, simple repeatable and inexpensive exposure assessment should be developed, in order to compare the exposure levels with a permissible (PEL) or OEL. In the meantime, however, assessments in occupational settings should be performed, adopting uniform, standardized strategies.

A challenge with the procedure based on the measurement of nanoparticle number concentrations is that our knowledge on the physical–chemical parameters of ENM does not yet enable us to conclude which measurements are justified to assess potential health hazards to the exposed workers. Rather than number concentrations, mass or particle surface area may also be relevant in many cases, and the suitable metric for different engineered nanoparticles may vary (Juric, Meldrum, & Liberda, 2015; Savolainen et al., 2010; Savolainen & Alenius, 2013).

In this context it is important to note that using particle counters in combination with sample collection for chemical analysis allows a good evaluation of worker exposure to ENM, but if this assessment strategy will continue to rely only on static or area sampling, some uncertainty will always exist in estimating real worker exposures. A specific problem is posed by the

high aspect ratio nanoparticles (HARN) (e.g., CNT). Owing to their very small diameter, high magnifications are necessary for their detection, decreasing the likelihood of finding countable fibers (i.e., with both ends counted). The highly agglomerated nature of some types of HARN (e.g., single wall carbon nanotubes-SWCNT) adds further problems to the identification of single fibers.

2.4 | Occupational exposure limits

OELs are important means through the use of which workers can be protected against harmful exposures. OELs are generally described as airborne concentrations of chemicals or other agents, which should not be exceeded, as health hazards may then occur. OELs are set, for example, by national authorities for specific chemicals to which workers may be exposed. The OEL-values are often health-based, and derived from no-observed adverse effect level (NOAEL) findings in human or animal studies (Nielsen & Øvrebø, 2008). In addition to health hazard data, OELs may also take into consideration technical feasibility to reach exposure levels below the limit values. When assessing the impact of ENM exposure levels in occupational environments, and comparing exposure concentrations with the OELs, the distinction between background ultrafine or nano-sized particles and ENM becomes especially important (see description before).

Currently, no regulatory binding, health-based OELs for ENM have been given by authorities. One reason for this is that, for most ENM, there is not yet conclusive information available on the dose-responses related to their hazardous effects. Furthermore, approaches for grouping and read-across, which could be used if substance-specific data are lacking, are still under development. Unofficial OELs and benchmark values for different ENMs have, however, been released by different institutions and in several research projects (Mihalache, Verbeek, Graczyk, Murashov, & Van Broekhuizen, 2017; Pietroiusti & Magrini, 2014). Different approaches have been utilized for the derivation of the values, and there is a lack of uniformity both in metrics used to express the OELs and in the OELs themselves. As an example, the National Institute of Occupational Health and Safety (2013a) proposed a mass-based OEL of 1 μg/m^3 for all types of CNTs and carbon nanofibers (as elemental carbon). This value is based on the inflammatory effects of various types of CNTs. The Japanese New Energy and Industrial Technology Development Organization (AIST), in turn, proposed an OEL of 30 μg/m^3 (AIST, 2011). Other organizations, such as the British Standard Institute (BSI), Safe Work Australia (SWA), the German Social Accident Insurance (IFA), and the Dutch Social and Economic Council (SER) express their limit values as number concentration (BSI, 2007; IFA, 2009; Morawska et al., 2012; van Broekhuizen et al., 2012). Furthermore, IFA and SER make a distinction between rigid, biopersistent CNTs, and other CNTs, to which higher OELs can be applied. In case of soluble ENMs, the OELs of the chemically similar materials in bulk form are generally applicable, if such ones are available. (Table 2). The current state of recommended OELs for ENMs has been extensively reviewed by Mihalache et al. (2017) and by Pietroiusti and Magrini (2014).

TABLE 2 Examples of occupational exposure limit and benchmark value approaches proposed by different organizations

ENMs	BSI (UK; 2007)	IFA (Germany; 2009) and SER (Netherlands; 2012)	NIOSH (USA; 2013a, 2011)	SWA (Australia; 2012)
Fiber-like ENM				
Rigid, biopersistent CNTs	0.01 fibers/cm^3	0.01 fibers/cm^3		0.1 fibers/cm^3
CNTs and CNFs			0.007 mg/m^3	
Fiber-like metal oxides	0.01 fibers/cm^3			
CNTs for which asbestos-like effects can be excluded		40 000 particles/cm^3		
Biopersistent granular ENM	0.066*OEL of coarse material or 20 000 particles/cm^3			0.03*inhalable OEL or 0.1*respirable OEL of coarse material
ENM with a density < 6000 kg/m^3		40 000 particles/cm^3		
ENM with a density > 6000 kg/m^3		20 000 particles/cm^3		
Titanium dioxide			0.3 mg/m^3	
CMAR*-classified substances	0.1*OEL of coarse material			0.1*OEL of coarse material
CMAR*-classified substances for which OEL of coarse material is not available				0,003 mg/m^3
Soluble ENM	0.5*OEL of coarse material	Same OEL as for coarse material		0.5*OEL of coarse material
No OEL of coarse material available				1,5 mg/m^3

*Carcinogen, mutagen, allergen, reprotoxic. An example of Health surveillance for workers exposed to carbon nanotubes proposed by the National Institute of Occupational Safety and Health (NIOSH). Adapted from NIOSH CIB 65, April 2013.

2.5 | Biomarkers of exposure

A biomarker of exposure is defined as a "chemical, its metabolite , or the product of an interaction between a chemical and some target molecule or cell that is measured in the human body" (Committee on Human Biomonitoring for Environmental Toxicants, National Research Council, 2006). This kind of biomarker is relevant in occupational medicine, since it reflects cumulative (recent and past) exposure by any route and gives insights on the different toxicokinetics for a given substance among individuals. Exposure biomarkers are generally measured in blood, urine or saliva. Several studies have shown the potential relevance of pulmonary cytokines as potentially relevant biomarkers of lung exposure to ENM, as reviewed by Bergamaschi, Guseva-Canu, Prina-Mello, and Magrini (2017). Given the relevance of the pulmonary exposure in workers, the evaluation of pulmonary cytokines might be a useful parameter of recent exposure to ENM. Their assessment, however, is generally performed by means of invasive procedures, such as the collection of brocho-alveolar lavage fluid (BALF), which cannot be easily performed as a screening procedure in humans. However, it has been recently reported that the presence of cytokines (along with oxidative stress markers) may be evaluated by means of non-invasive assessment of exhaled breath condensate (Bergamaschi et al., 2017). Furthermore, a characteristic pattern of serum cytokines has been reported after pulmonary exposure to MWCNT, thus suggesting that their presence in serum might represent a useful indirect biomarker of pulmonary exposure (Erdely et al., 2009). It is not clear, however, if, in the case of exposure to multiple agents potentially able to cause pulmonary inflammation (a not uncommon event in several occupational settings), the local and systemic alterations in cytokine expression detected after pulmonary exposure may allow the identification of the specific role of ENM.

In general, the identification of reliable biomarkers of exposure is more difficult for ENM than for traditional substances, because of the current incomplete knowledge of the ENM toxicokinetics, and also due, among others, to inherent technical problems in studying their absorption, biodistribution and excretion. For example, the lack of detailed knowledge on the half-life of several ENM makes difficult to select the most appropriate timing for measuring their concentration in blood.

It should be also taken into account that ENM are particulates with a particle surface and core molecular identity, both contributing to their novel/enhanced biological activity. Therefore, measuring the concentration of, for example the elemental component of metallic ENM in blood, is not a specific marker of exposure, given the fact that this is the same marker used for the compound in its bulk form. This fact may have relevant implications for the interpretation of the data: for example, the titanium dioxide is suggested to be more biologically active in its nano-form than in the bulk form (National Institute of Occupational Safety and Health, 2011). Using elemental titanium, which is representative of both forms, as a biomarker of exposure is therefore useless for a correct interpretation of the risk of the exposed workers. Last but not least, in experimental biodistribution studies, doses largely exceeding those expected in the occupational setting are generally used (Pietroiusti, 2012) rendering the translation of these data to humans questionable.

With these limitations in mind, a good relationship has been reported in experimental animal studies between the amount of inhaled metallic ENM such as silver, gold and iron and the concentration of the elemental metal content in blood, as recently reviewed by Iavicoli, Leso, Manno, and Schulte (2014). Some data, regarding exposure to indium tin oxide and silver ENM, available for a very limited number of workers, seem to confirm the experimental findings (Lee, Mun, Park, & Yu, 2012; Liu, Chen, Chen, Lee, & Chen,, & H.L., 2012). Information on exposure biomarkers for non-metallic ENM is limited to one report on polystyrene ENM (Sarlo et al., 2009), whereas no data are available for CNT, which are the ENM posing the potentially highest hazard to humans.

As far as the cutaneous exposure is concerned, the available literature has shown minimal penetration of ENM through the skin (Monteiro-Riviere & Larese-Filon, 2017), with the possible exception of UV damaged skin to which ENM based sunscreen are applied (Gulson et al., 2010). However, this is a typical consumer context, and therefore exposure biomarkers valid for workers cannot be developed on this basis.

Exposure through the gastro-intestinal route is generally considered of minor relevance in the occupational setting in comparison to inhalation. However it should be considered that the gastro-intestinal tract of workers may be exposed (a) to inhaled ENM cleared from the lung through the muco-ciliary escalator (which is a major clearance pathway for ENM from the lung as compared with translocation through the alveolo-capillary barrier, Geiser & Kreyling, 2010, (b) to ENM directly ingested while breathing air (the so called "aerophagia"). Hemmink, Weusten, Bredenoord, Timmer, and Smout (2009); and (c) through the hand-mouth contact in poor hygiene contexts. Therefore, the data reported above for the exposure biomarkers of the inhalation route probably incorporate the gastro-intestinal component.

There are also attempts to relate the urinary and fecal concentrations of ENM to exposure in experimental animals (Iavicoli et al., 2014). However, the lack of knowledge on the efficiency of the kidney filtration barrier for different ENM made the extrapolation of these data to humans very problematic. In workers exposed to titanium dioxide, Pelclova et al. (2015) detected substantial amounts of elemental titanium in urine in most cases (18/20) of tested people. The same workers underwent the evaluation of titanium content in the exhaled breath condensate. No difference between pre- and post-shift values was detected, suggesting that, at best, this local exposure biomarkers might be useful for assessing long-

term exposure only. Of note, in this study workers were exposed both to nano- and micrometer sized titanium dioxide. It is evident that using fecal samples to develop useful biomarkers of ENM exposure is problematic due to practical reasons and resources required.

In conclusion, the search for reliable markers of exposure to ENM is still at its very early stages for metallic ENM, and practically absent for ENM such as CNT. Improved knowledge on the kinetics and degradation of ENM in the human body will be of great help in the development of specific nano-related markers, suitable for evaluation in the context of health surveillance of workers.

2.6 | Short-term exposure

Most experimental studies on ENM toxicity, especially in the past, have been performed in rodents by using a single high dose of ENM followed by the evaluation of the effects in the short- and, less frequently, long-term course; therefore, these experiments mainly refer to the acute effects of short-term exposure. Some degree of toxicity has been reported for virtually all tested ENM, however, whether this information may be translated to the accidental exposure of workers and consumers is still a controversial issue. In fact, problems for the correct interpretation of the available data arise from the dose and the methods of exposure.

In spite of the recurrent claim about the "unrealistic doses" used in short-term toxicity studies regarding ENM (Krug, 2014), there are no systematic guidelines or authoritative suggestions on what "realistic doses" (or dose-ranges) actually are. Therefore, with the potential exception of very extreme experimental settings, sometimes showing no substantial toxicity (Wang et al., 2007), we simply do not know at what extent currently available information can predict the risk posed by accidental acute exposure in humans. A guiding principle might be represented by the different amounts of ENM produced in industrial activities: testing acute toxicity at relatively high doses might be reasonable for ENM produced in large amounts (e.g., TiO_2), whereas lower doses should be tested for less widely produced and used ENM, such as gold ENM.

The matter is further complicated by the methods used for exposure. As an example, for mimicking inhalation exposure, ENM are delivered in several studies dispersed in a liquid forcefully introduced within the respiratory tree, which is a quite different situation in comparison to the expected exposure in humans. However, recent observations with MWCNT suggest that oropharyngeal aspiration, provided that appropriate doses are used, can serve as a highly useful and reliable surrogate for inhalation exposure (Ilves & Alenius, 2016). In theory, inhalation, or in some cases oropharyngeal aspiration, exposure may in most cases represent the best method for performing reliable acute toxicity studies via the pulmonary route. However, in practice, there are no resources for full-scale inhalation studies of a wide range of ENM. Hence, the attempts to replace inhalation studies with studies utilizing oropharyngeal aspiration may provide a useful substitute (Kinaret et al., 2017).

The chance of substantial acute toxic effects as a consequence of skin exposure to ENM seems to be remote (Monteiro-Riviere & Larese-Filon, 2017), whereas relatively few studies testing acute toxicity are currently available for gastrointestinal and ocular exposure.

2.7 | Repeated dose exposure

Data on repeated dose toxicity are mainly available for the oral and dermal route, which are typical settings of consumers. For inhalation, which is the typical exposure route for workers, one assumes either an accidental exposure to high concentration of ENM over a short-time period (see above the acute toxicity chapter) or a long-term exposure to low ENM concentrations for several years. Indeed, the proposed unofficial OELs for ENM have mainly been developed to predict clinical effects of a constant exposure of workers for long time periods. However, the findings of onsite studies do not completely fit with this vision: they show frequently short-term peaks of ENM exposure, followed by longer periods of virtually no exposure (Pietroiusti & Magrini, 2014). Whether the biological effects of this type of exposure overlap the effect of chronic low grade continuous exposure, is uncertain. Recent data suggest that cells can adapt to low level chronic ENM exposure, whereas repeated short-term exposure may induce more marked persistent damage (Falagan-Lotsch, Grzincica, & Murphy, 2016).

Some studies based on intermittent intra-tracheal instillation over a relatively long time are available. They generally show a variable degree of pulmonary damage (Christophersen et al., 2016; Christophersen et al., 2016; Présumé et al., 2016), even at occupationally relevant doses (Présumé et al., 2016). It should be considered, however, that intra-tracheal administration has several limitations for translating experimental data to humans. As reported above, there have though been attempts to develop exposure using oropharyngeal aspiration as a surrogate for inhalation (Kinaret et al., 2017).

The effects of intermittent exposure of healthy skin to ENM range from no effects (Ryu et al., 2014) to mild inflammation (Adachi, Yamada, Yoshida, & Yamamoto, 2013) or acceleration of skin aging (Wu et al., 2009).

Current literature is conflicting with respect to health risks of repeated ENM ingestion, in part due to inadequate knowledge of changes in ENM features such as agglomeration or binding of biomolecules and reactivity following exposure to

biological fluids (Warheit, 2008). For example, it is not known whether enterocytes encounter ENMs in the initial, smaller size range (<100 nm) or whether they predominantly encounter larger particles or agglomerates. Furthermore, the vast majority of available data does not take into account the role of the interaction of ENM with the intestinal microbiota (the collection of micro-organisms living in symbiotic relationship with the host in the gut lumen), which may cause relevant local and systemic biological effects (Pietroiusti et al., 2016). Further studies, taking into account the above mentioned factors, are needed for a reliable assessment of repeated dose toxicity of ENM on the gastro-intestinal system.

2.8 | Health surveillance

Health surveillance and medical screening are used for assessing the health of exposed workers. Based on the reported findings, a connection between adverse health outcomes and exposure may be identified. Typically, health surveillance and medical screening are used when the health effects of a substance are reasonably well known. In the case of ENM, the scientific knowledge on the health effects is still limited. No ENM-specific biomarkers, which could predict occupational diseases have been identified, nor have medical tests been established to investigate specific effects of nanomaterials. At the moment no comprehensive data on health surveillance of workers exposed to ENM have been published.

So far, some general recommendations related to health surveillance have been published. National Institute of Occupational Safety and Health (2013b) has given recommendations for the medical surveillance and screening of workers exposed to CNT or carbon nanofibres (CNF). The recommendations for the medical screening of these workers focus on the respiratory system and include spirometry tests and a baseline chest X-ray. In addition, other examinations or medical tests may be included if considered relevant by the responsible health-care professional (Figure 1). National Institute of Occupational Safety and Health does not recommend specific medical screenings for workers potentially exposed to other types of ENM, due to the insufficient scientific and medical evidence. If the ENM is composed of a bulk chemical for which medical screening recommendations exist, those recommendations are also applicable to workers exposed to ENM (National Institute of Occupational Safety and Health, 2009).

The Health Council of the Netherlands (2012) has taken the view that there is no need for separate medical surveillance of workers exposed to ENM. In the Netherlands there is already a concept with national health registries designed for the continuous input of disease data, covering a great range of health effects. The Health Council recommends setting up exposure registries for workers exposed to insoluble ENM and solid materials in which ENM is incorporated. The health data from the existing registries can then be linked to the new data in exposure registries. Ideally it can then be determined whether there is an association between exposure to nanomaterials and certain health effects or not.

The Health and Safety Executive (HSE, 2013) has concluded that there is no legal requirement to perform health surveillance among workers exposed to ENM, as there are no links between exposure and occupational diseases. However, employers are encouraged to establish a health monitoring program. As a minimum, HSE recommends to keep a record of all employees working with ENM. If health surveillance is required for the non-nanoform of a material on the basis of risk assessment, then it is likely to be appropriate also for the nanoform.

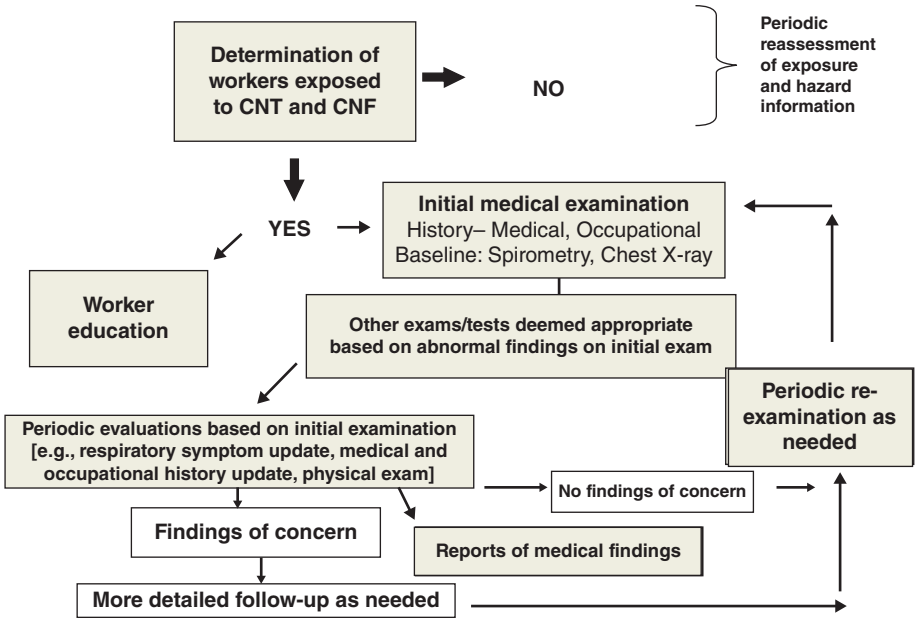

FIGURE 1 Medical Surveillance Recommendations-CNT and CNF. Understanding of mechanisms of health hazards of nanomaterials has markedly increased during recent years. Additional steps are, however, required to enable the prediction of nanomaterial-induced hazards and risks, especially by increasing the use of omics technologies and bioinformaticsAn example of Health surveillance for workers exposed to carbon nanotubes proposed by the National Institute of Occupational Safety and Health (NIOSH). (Reprinted from NIOSH CB 65, April 2013.)

In the EU FP7 SCAFFOLD project (http://scaffold.eu-vri.eu/), recommendations were given on health surveillance of workers exposed to ENM in the construction sector (Hyytinen, Väänänen, Uuksulainen, Stockmann-Juvala, & Oksa, 2014). It was concluded that health surveillance practices may vary between countries, but in the case of construction workers, the examinations are generally carried out at intervals of 1–5 years, and cover several parameters, including spirometry and chest X-ray. In the project it was therefore recommended to continue applying established medical surveillance approaches. In case the risk level is evaluated as being high (e.g., CNT or fibrous ENM are handled and exposure occurs), it is recommended to include regular follow-up examinations, focusing on respiratory (and cardiovascular) systems.

2.9 | Risk prediction and management

All employers are obliged to perform risk assessments at the workplace, taking into consideration the hazards of all chemicals (in this case ENMs) used, and evaluating the potential exposure of the workers. If the hazard and exposure assessment indicate that there may be a risk in relation to the activities involving ENMs, it is the obligation of the employer to take actions in order to reduce the risks. As for traditional chemicals, the first option should be to substitute the chemical (in this case the ENM) with a less harmful one. In nanotechnological applications, a particular ENM has usually been selected because of its unique material properties, and substitution is thus perhaps not an option. Instead, it might for example be possible to select the ENM supplied as a dispersion instead of a powder, and in that way the dustiness is decreased and the likelihood of inhalation exposure minimized. In addition, it is important to check the working routines and occupational hygiene in order to minimize the exposure. If exposure still may occur, the next step is to consider whether procedures can be isolated or enclosed, or whether the situation can be solved by using local exhaust ventilation. The final option, according to the traditionally used hierarchy of controls, is the use of personal protective equipment (e.g., respirators, dermal protection). The effectiveness of personal protective equipment has been tested in several projects, and for example normal respiratory protective equipment, used for other kinds of dust, are seem to be effective and work well also in the case of exposure to ENMs by inhalation. Training and information are however always important in relation to the use of personal protective equipment (European Commission, 2014; National Institute of Occupational Safety and Health, 2013).

2.10 | Consumer exposure

ENM are currently used in thousands of consumer products. Consumer exposure to ENM has, however, generally been considered less critical than occupational exposure due to lower concentrations and shorter exposure duration. It has been estimated that in the majority of situations in which consumers are in contact with ENM-containing products, the potential route of exposure is the dermal route. Examples of dermal exposure include use of cosmetic products applied on the skin and touching solid materials with ENM incorporated in the matrix. Exposure via inhalation may occur, for example, when using spray products containing ENM. Exposure by ingestion is likely to become more common as the use of ENM in food and food package materials is increasing (Dekkers et al., 2016; Vance et al., 2015).

The consumer exposure potential is higher in situations where the ENM is in free form, and not embedded in a solid matrix. The data on release of ENM from consumer products is so far very limited. Some data has been collected in laboratory studies (Saber et al., 2013). Different modeling tools have also been used to estimate the potential exposure and identify relevant exposure scenarios (Mackevica, 2016; The Danish Environment Protection Agency, 2015). As indicated before, consumer or environmental exposure will not be dealt with in detail, and this paper focuses on occupational exposure because the marked exposures currently take place in occupational environments.

3 | SPECIAL FEATURES OF NANOMATERIAL TOXICITY

3.1 | Physicochemical properties influencing biological effects

ENM display remarkable and peculiar physicochemical properties—size, shape, surface area and charge, aggregation- which primarily influence, individually or cooperatively, their interaction with target cells, and may cause toxic effects such as inflammation and cell death (Fu, Xia, Hwang, Ray, & Yu, 2014; Palomäki et al., 2011), probably at least partly mediated by oxidative stress in the majority of cases (Khanna, Ong, Bay, & Baeg, 2015), and especially associated with the presence of different kinds of CNT (Nel et al., 2009; Palomäki et al., 2011; Rossi et al., 2010; Shvedova et al., 2007).

Indeed, the size makes ENM not only chemically more reactive, but also able to have an easier entry into the cells and to exert their potential damaging action in sites precluded from larger particles (Pietroiusti, Campagnolo, & Fadeel, 2013). The inverse relationship between size and toxicity seems to apply also in the nano-metric range: in comparison to larger

nanoparticles, 10 nm Ag nanoparticles showed in vivo significantly higher diffusion and dispersion properties (Asgharian & Price, 2007), and in vitro a greater ability to induce apoptosis (Kim et al., 2012).

As far as surface charge is concerned, ENM with cationic surface charge interact with cell membranes and genetic materials more easily than anionic or neutral ENM, inducing higher toxicity (Navya & Daima, 2016). Surface charge seems also to be responsible for possible alterations of blood–brain barrier integrity and for changes of particle shape and size, through production of aggregates or agglomerates of particles (Gatoo et al., 2014). Assessing the reactivity of nanomaterials is further complicated by the quick formation of the biocorona around the nanoparticles as soon as they get into contact with biological milieu. Formation of biocorona influences at least the surface properties of the particles and their size. This influence of biocorona needs to be assessed further (Nel, Xia, Mädler, & Li, 2006 and 2009; Monopoli et al., 2012).

Another crucial property of ENM is represented by shape which, among others, influences the membrane wrapping processes during endocytosis or phagocytosis (Verma & Stellacci, 2010). Spherical nanoparticles seem to be less toxic than rod shaped or fiber like ones. They are more easily and fastly uptaken and ingested by endocytosis, when compared to nanoparticles with different shapes (Lee, Lim, & Kim, 2007). On the other hand, nonspherical nanomaterials are more likely to spread through capillaries causing adverse effects at distant sites (Kim et al., 2012).

When translating these findings to the "real world," it should be kept in mind that the above reported structure/activity relationships have been derived from experimental studies, generally performed under well controlled conditions. Workers, involved in activities including the processing and/or handling of ENM, are likely to be exposed to materials different from those tested in laboratory experiments. For example, most "in field" studies reveal the formation of large agglomerates after the release of ENM (Pietroiusti & Magrini, 2014). The extent at which the toxic effects elicited by the exposure to these agglomerates are similar to those reported in experimental studies is still an unsolved issue.

3.2 | Immunotoxicity and sensitization

The interaction between nanoparticles and immune system may involve both the innate and adaptative immune responses. As far as the innate immunity is concerned, several in vitro and in vivo studies show that exposure to ENM results in inflammation, characterized by inflammasome activation and induction of proinflammatory cytokines (Castranova, Schulte, & Zumwalde, 2013; Dobrovolskaia, Shurin, & Shvedova, 2016; Palomäki et al., 2011). Probably, however, ENM are not proinflammatory by themselves, but may induce inflammation if they aggregate/agglomerate and form larger particles easily recognized by phagocytes (Geiser, 2010), or if they adsorb on their surface bacterial products such as LPS, which are strong inducers of inflammation (Li & Boraschi, 2016).

As far as the adaptive immunity is concerned, it is well known that once ENM gain access into the body of living organisms (including humans) a bio-corona of proteins and other biomolecules is formed on their surface (Monopoli et al., 2012; Nel et al., 2009). It has been suggested that in some cases the autologous proteins adsorbed on the surface of ENM may display altered characteristics, so that they are recognized as non-self by dendritic cells (DC), which can then process and present them thereby initiating an autoimmune response (Tavanti, Pedone, & Menziani, 2015). There is evidence that ENM can also act as adjuvants, that is, as substances that are added to the antigen in order to stimulate immune response (Li, Aldayel, & Cui, 2014; Niikura et al., 2013). The putative mechanism of the adjuvant action is represented by the promotion of the antigen uptake and the stimulation of DC (Dobrovolskaia & McNeil, 2007). Once again, it should be emphasized that the described immunological alterations may probably arise in a very limited number of cases, characterized by still unidentified physicochemical characteristics of ENM, and enhanced susceptibility of the host.

The potential role of ENM in immunotoxicity is not limited to the activation of the immune response, but may extend to immunosuppression. Indeed, nanoparticle-loaded immune cells may be less fit for exerting their defensive and scavenging functions, making the host more vulnerable to bacterial infections (Shvedova et al., 2008). Furthermore, it seems that nanoparticle-loaded immune cells may undergo apoptosis sooner than normal cells, or be subjected to anomalous autophagic mechanisms that affect immunological fitness (Stern, Adiseshaiah, & Crist, 2012). Interestingly, the defective clearance of apoptotic cells by scavenger phagocytes has been suggested as a plausible mechanism of ENM linked development of autoimmunity (Witasp, Shvedova, Kagan, & Fadeel, 2009).

ENM given by inhalation can induce asthma and allergic-like reactions (pseudoallergy) in rodents (Horie, Stowe, Tabei, & Kuroda, 2015), and may exacerbate Th2 inflammation in mice (Girtsman, Beamer, Wu, Buford, & Holian, 2014). For example, mice exposed to MWCNT, without preceding sensitization by OVA, showed typical signs of allergic asthma (Kinaret et al., 2017). Taken together, the current data suggest that exposure to given ENM, especially carboneous fibrous materials, might trigger allergic reactions in healthy susceptible individuals, and might induce relapse in people with known allergy. This conclusion is in agreement with a recent report of severe nickel allergy in a man occupationally exposed to nickel nanoparticles (Journeay & Goldman, 2014). The induction of damage-associated molecular patterns, the secretion of the IL-1 family members, and the functional alterations of antigen presenting cells such as DC may be the underlying

mechanisms (Smith, Brown, Zamboni, & Walker, 2014). However, the exact pathogenesis of ENM-related allergic responses is not completely known.

3.3 | Genetic in vivo and in vitro toxicity

Assessment of genotoxicity is important for the protection of workers from the potential harmful effects of ENM. The number of studies exploring genotoxicity of ENM in vivo or in vitro has grown exponentially during last 10 years, even though it is still limited considering the large number of ENM on the market. There have been doubts whether one can make conclusions on ENM genotoxicity (Cunningham, 2007; Gonzalez, Lison, & Kirsch-Volders, 2008; Landsiedel, Kapp, Schulz, Wiench, & Oesch, 2009) because many of the commonly used genotoxicity tests have been tailored for soluble chemicals rather than for particulate matter. For example, the widely used test utilizing the *Salmonella* bacteria for genotoxicity assessment is not suitable for testing particulate matter, bulk or nano-sized, because it does not penetrate the bacterial cell wall (Landsiedel et al., 2009; Lindberg et al., 2013; Warheit et al., 2007). However, a number of studies performed in vitro and in vivo have provided useful information of genotoxicity of ENM relevant for work environments (Lindberg et al., 2013). Recently, alterations in global mRNA and ncRNA expression profiles in the blood of workers exposed to MWCNT have been reported (Shvedova et al., 2016).

Lindberg et al. (2009) have shown that exposure of pulmonary epithelial cells to low doses of CNT or graphite nanofibres (CNF) induces genotoxic effects. Catalán, Järventaus, Vippola, Savolainen, and Norppa (2012) confirmed that single-walled (SWCNT) and multiwalled (MWCNT) CNT induce chromosomal aberrations during exposure of isolated human lymphocytes at low to moderate doses (6.25–300 ug/ml) for 24, 48, or 72 h. The findings of Catalán et al. (2012) were largely in agreement with those of Lindberg et al. (2013), who observed an increase of oxidative stress markers such as malon dialdehyde in human mesothelial cells and BEAS 2B cells at corresponding doses and exposure times, and to those of Sargent et al. (2012) who showed that SWCNTs were incorporated into the centrosome structure of human airway epithelial and induced DNA damage. Notably, these findings were observed at doses of SWCNT compatible with those expected in an occupational setting. Nymark et al. (2014) found that exposure of BEAS 2B cells to rigid rod-like MWCNT induced cytotoxicity and production of hydroxyl radical more than Printex 90 carbon black, asbestos, or glass wool. However, cellulose nanocrystals were not cytotoxic or genotoxic whereas larger crystals induced a marked cytotoxicity (Catalán et al., 2015). Finally, Muller et al. (2008), reported that MWCNT induced clastogenic as well as aneugenic events in a human epithelial cell line (MCF-7). On the other hand, MWCNT decreased dose-dependently DNA damage in BAL and lung cells in mice after a single oropharyngeal aspiration; however, in vitro exposure of BEAS 2B cells to rigid rod-like MWCNT induced DNA strand breaks at low doses, but such effects were not observed with tangled MWCNT, except at high doses. Even though the results are not always consistent, there is remarkable amount of data relevant to the occupational environment demonstrating that genotoxicity is a highly relevant measure of the safety of ENM, and this is particularly true for carbon-based ENM.

For the time being there seems to be a need to assess the genotoxicity of different ENM material by material to reveal especially relevant hazards to workers in occupational environments. These findings also emphasize the importance of development of an accurate and predictive approach for the testing of genotoxicity of ENM.

3.4 | Carcinogenicity

There have been doubts from the beginning of the use of ENM that they might cause long-term health effects like other airborne particulate matter (Pope & Kanner, 1993), among them increased risk of cancer as analogy of the carcinogenicity of asbestos (Savolainen et al., 2010; The Royal Society, 2004). There were concerns especially regarding high aspect ratio nanomaterials (HARN), notably SWCNT, DWCNT, MWCNT, and CGF. Poland et al. (2008) showed that 7 days after a single intraperitoneal injection of long (> 4 um) rigid, rod-like MWCNT at low dose (Mitsui-7 CNT) alterations in mouse intraperitoneal cavity mesothelium similar to those induced by asbestos, and increased the amount of exudate and inflammatory cells were present. Potential carcinogenicity of MWCNT has been also suggested by Sargent et al. (2014), reporting the ability of these particles to act as tumor promoters in mice, and by Kasai et al. (2016), who demonstrated that MWCNT act as initiators of lung tumors (but not of mesotheliomas) after a 2-year inhalation in rats. Another study (Takagi et al., 2008) showed that a single high (1,000 μg/mouse) i.p. injection of the same material caused a dose-dependent increase of mesotheliomas in the peritoneal cavity of p53+/− mouse during an 18 month follow up. These findings were criticized, for example, by Poland et al. (2008), but Kane and Hurt (2008) considered that both findings were in essence in support of each other. In a later study, Takagi et al. (2012) demonstrated that also low doses of Mitsui-7 MWCNT (3, 30, and 300 ug/mouse) induced a dose-dependent increase of mesotheliomas in the mesothelial lining of the p53+/− mouse after a 18-month follow up.

Ryman-Rasmussen, Cesta, et al. (2009) and later Mercer, Scabilloni, Hubbs, Wang, et al. (2013) showed that upon inhalation, this same material could reach the primary target location from carcinogenicity point of view, notably the subpleural space. Other investigators have shown that inhalational exposure to the same material causes an increased incidence of lung cancer in rats (Sakamoto et al., 2009). Another study (Muller et al., 2009) suggested that exposure of rats to high doses of MWCNT does not induce mesotheliomas. It should be noted that in this study the fibers were short, less than 4 um, which might explain the findings. Schinwald et al. (2012) have shown that 4 μm is the threshold length of fibers to produce acute pleural inflammation in rats. Based on these studies, the International Agency for Research of Cancer (IARC) classified Mitsui-7 MWCNT into category 2B, possibly carcinogenic to humans (Grosse et al., 2014). Other types of CNT were not classified because of lack of data. Current evidence points to the direction that long and rigid, rather than tangled, CNT may be associated with increased risk of cancer.

Titanium dioxide induced lung tumors in rats upon exposure at high doses using both nano-sized titanium dioxide (Heinrich et al., 1995) and larger particles (Lee, Trochimowicz, & Reinhardt, 1985). A possible carcinogenicity potential of titanium dioxide has also been suggested by the NIOSH document on the consequences of titanium dioxide exposure (NIOSH, 2011). The relevance of these observations, and the role of possible of particle overload of the lungs, have been questioned. However, for example the Risk Assessment Committee of the European Chemicals Agency recently recommended that titanium dioxide should be classified as a substance suspected of causing cancer through the inhalation route (European Chemicals Agency, 2017). This applies for titanium dioxide powder of all sizes. Also IARC has concluded that titanium dioxide is possibly carcinogenic to humans. The particle size was not specified (IARC, 2010).

3.5 | Toxicity to reproduction

There are data available pointing to possible effects of ENM on reproduction. Toxicity to reproduction may occur in two ways: by interfering with the ability to reproduce (fertility) and/or with the outcome of pregnancy. In the first case, both sexes may be affected, whereas in the second one the mother and the concepts may be injured. As far as the first types of effects are concerned, most available evidence is indirect, consisting of organic and functional (i.e., hormone secretion) alterations of the gonads in rodents, reported after exposure to various ENM such as nickel (Kong et al., 2014), gold (Li et al., 2013), carbon black (Yoshida et al., 2009), silver (Castellini et al., 2014), titanium dioxide ENM (Tassinari et al., 2014), and zinc oxide ENM (Moridian, Khorsandi, & Talebi, 2015). For silica ENM and MWCNT, it has been shown that the alterations may be transient, with a return to normal after about 2 months for silica ENM (Xu et al., 2014) and 3 months for MWCNT (Bai et al., 2010). Of note, direct studies (i.e., including fertility as an outcome) reported only mild effects such as a delayed delivery of the first litter after the administration of MWCNTs to female mice 24 hr before their co-habitation with male mice (Hougaard et al., 2013), and a reduced incidence of pregnancy after chronic pulmonary exposure to cadmium oxide ENM (Blum, Edwards, Prozialeck, Xiong, & Zelikoff, 2015), whereas no effect on fertility was observed in male mice intravenously exposed to MWCNT (Bai et al., 2010) or gold (Li et al., 2013). Almost all the above reported studies on gonadal effects used non-physiological routes of administration (e.g., intravenous administration (Li et al., 2013) or very high doses (e.g., Yoshida et al., 2009), making the interpretation of the findings uncertain. It should also be noted that there are suggestions from experimental studies in rodents that certain ENM such as fullerene (Bal et al., 2011), zinc oxide ENM (Afifi, Almaghrabi, & Kadasa, 2015), fullerenol (Srdjenovi et al., 2010) and cerium oxide ENM (Kobyliak et al., 2015) may be successfully used to treat male infertility, a finding once again supporting the concept that even small modifications in the physicochemical properties of ENMs, or changes in the way they come in contact with living organisms, may lead to quite divergent outcomes.

Furthermore, there are several studies indicating that virtually all ENM can adversely influence the outcome of pregnancy (Campagnolo & Hougaard, 2017). It has also been suggested that in-utero exposure to ENM may cause the development of post-natal disorders, sometimes clinically evident only in adult age. As an example,

Stapleton et al. (2013) showed that inhalation of titanium dioxide by pregnant mice may cause microvascular dysfunction in the fetus (Stapleton et al., 2013) which may be detectable post-natally, sometimes in the adulthood (Stapleton, 2016; Stapleton et al., 2015). However, in addition to the caveats regarding in general the interpretation of the toxic effects of ENM observed in experimental studies (high doses and non-physiological routes). it should be considered that for this specific context the chance of pregnant women being exposed to ENM at work is rather limited, at least in western countries, due to laws protecting pregnant workers from exposure to harmful chemicals at work. Since many experimental studies refer to the late stages of pregnancy, their findings may at best be related to the therapeutic use of ENM in pregnancy or to exposure in occupational context where there is insufficient tutelage of pregnancy, more likely to occur in developing countries. Therefore, the findings of studies in which pregnant animals are exposed during the very early stages of pregnancy may be more generalizable. Unfortunately, only one study focusing on this specific gestational age is available. In that report, Pietroiusti et al. (2011) showed that single walled carbon nanotubes (SWCNT) given at low doses soon after implantation caused severe

malformations in embryos. Further studies are clearly needed, but precautionary avoidance of working with ENM for women in their reproductive age seems reasonable on the basis of the current evidence.

4 | IMPACT ON HUMAN HEALTH

4.1 | Studies in humans

Epidemiological studies suggest that that the ultrafine (nano-sized) fraction of particulate air pollution has a remarkable effect on the exacerbation of cardio-respiratory disease and increased morbidity. Experimental studies in rodents suggest, in turn, that the increased concentration of nanoparticles and higher reactive surface area per unit mass, alongside unique chemistry and functionality, is important in the acute and chronic inflammation upon such exposure. Some animal models have shown that nanoparticles which are deposited in one organ, for example, lung and gut, may access the vasculature and target other organs such as brain and liver (Tetley, 2007). Air pollution epidemiological studies, especially in occupational environments, have though emphasized the importance for worker protection against these nano-sized particles, including exposure quantification and confounder characterization (Peters, Rückerl, & Cyrys, 2011). The difference between engineered and other nano-sized particles is in the fact that engineered ones have been tailored for a given purpose whereas the other ones are unintentionally produced, usually unwanted particles of processes such as combustion. Therefore, a marked need to carry out epidemiological studies focusing on effects of ENM on human health has been identified. The fact that that ENM have been in a wide use only for a relatively short time, with rather limited groups of persons exposed to them, has though slowed down these attempts because the number of individuals exposed to ENM has been small and the duration of exposure fairly short. In 2017, there were nine published epidemiological studies on engineered nanomaterials. These included a pilot case study (Lee et al., 2012), seven cross-sectional studies (Fatkhutdinova et al., 2016; Lee et al., 2015; Liao, Chung, Lai, Lin, & Liou, 2014; Liou, Tsai, Pelclova, Schubauer-Berigan, & Schulte, 2015; Pelclova et al., 2015, 2017; Wu et al., 2014), and a longitudinal study (Liao et al. 2014b). The workers in these studies were exposed to nanosilver (Lee et al., 2012), MWCNT (Fatkhutdinova et al., 2016; Lee et al., 2015), titanium dioxide (Pelclova et al., 2015, 2017) and concomitantly to various ENM (CNT, silica dioxide, titanium dioxide, nanosilver, and nanoresin) (Liao et al., 2014; Liao, Chung, Lai, et al., 2014; Liou et al., 2015; Wu et al., 2014). In detail, four of the studies involved a single group of workers in Taiwan (Liao et al. 2014a, 2014; Liou et al., 2015; Wu et al., 2014), two involved workers in Korea (Lee et al., 2012, 2015) and Czech Republic (Pelclova et al., 2015, 2017), and one was performed in Russia (Fatkhutdinova et al., 2016). Possible differences in safety control at work between Asain and Eastern and Western European countries do not allow therefore a generalization of the findings.

The detected effects ranged from changes in markers of oxidative stress (Liao et al., 2014b; Liu et al., 2012; Pelclova et al., 2015, 2017), increased levels of several biological markers of inflammation (Liu et al., 2012), increased cardiovascular markers (Liao et al., 2014b; Liou et al., 2015) and local and systemic markers of pulmonary damage (Fatkhutdinova et al., 2016; Lee et al., 2015; Liao et al., 2014b; Wu et al., 2014). Increased symptoms of sneezing and allergic dermatitis and alterations of lung functional parameters were reported by Liao et al. (2014a and 2014b). Conversely, no biochemical or clinical adverse effects were found by Lee et al in a pilot study performed in 2012 (Lee et al., 2012).

In most of these studies, the exposure levels were low, but there were some exceptions. Furthermore, the sample size was small and ranged between 2 and 258 workers. Based on the available information on exposure of workers to ENM, it is premature to conclude whether exposure to engineered nanoparticles is associated with health effects in humans. The currently available studies create an inconsistent group with usually small number of cases, inconsistent, and in some cases inadequate assessment of exposure, and often short intervals between exposure and effect. It though appears that several categories of biomarkers may be influenced by exposure of workers to ENM, and this observation should guide future studies on this topic. The current knowledge, hence, serves as the basis for further epidemiological studies.

4.2 | Lessons from ultrafine epidemiological data

Ultrafine particles (UFP) and ENM share similar physicochemical properties, however, differently from ENM, robust epidemiological data are available for UFP. These data show mainly an association between UFP exposure and cardio-pulmonary effects (Robertson et al., 2014; Xu et al., 2013) andtherefore, similar adverse health effects might be expected in humans exposed to ENM. This suggestion is reinforced by data coming from in vitro studies, showing that both UFP and ENM can generate ROS and induce oxidative stress, which is a major factor in the pathogenesis of cardio-pulmonary disorders (Miller, Shaw, & Langrish, 2012). As far as ENM are concerned, the amount of ROS production is probably related to their physicochemical properties, with some material like copper ENM showing an intrinsic ability to generate ROS (Rushton et al.,

2010), whereas other ENM show no inherent capacity to generate ROS (Napierska et al., 2012), or may do so only following interaction with cellular targets (Hussain et al., 2009). Since epidemiological data on UFP do not generally include the evaluation of their physical–chemical properties, it is not possible to predict at which extent the different ability of various ENM to generate ROS shown in experimental studies may translate to human diseases of different severity. Of note, no threshold limit value is currently available for UFP.

4.3 | Implications for human health from experimental studies

Generally, the toxicity of engineered nanomaterials seems to be higher than the toxicity of their chemically identical bulk size counterparts (Fu et al., 2014; Khanna et al., 2015; Rossi et al., 2010). Indeed, the nanometric size makes ENM not only chemically more reactive, partly due to their large surface to mass ratio, but also due to their ability to enter the cells and to exert their potential damaging action in sites precluded from larger particles (Pietroiusti et al., 2013). In fact, it has been demonstrated convincingly that, for example, nanosized TiO_2 more readily induces pulmonary inflammation in mouse lung than chemically identical but larger TiO_2 particles. This is likely due to the much large surface to mass ratio of nanosized particles (Rossi et al., 2010). In addition to these general considerations, there is convincing evidence on the toxicity of several ENM. Especially certain types of metal and metal oxide nanoparticles frequently induce inflammation and even genotoxicity, but their potential to induce harmful morphological permanent changes in their target organ is often quite limited (Fu et al., 2014). However, fibrous materials such as different types of CNT, especially rigid rod-like CNT, readily induce genotoxicity and have proven to be carcinogenic to rodents (Catalán et al., 2016; Ilves & Alenius, 2016; Kinaret et al., 2017; Palomäki et al., 2011). Evidence of carcinogenicity of Mitsui-7 MWCNT is compelling from experimental animal studies which has lead IARC to classify Mitsui-7 as a possible human carcinogen (Grosse et al., 2014). So far, the data on other types of CNT is too limited to warrant any conclusions, but they have been suggested to be strong immunotoxic and inflammatory materials (National Institute of Occupational Safety and Health, 2013).

One can conclude that there are a number of metal and metal oxide nanomaterials which may cause harmful effects judged on experimental in vitro and in vivo studies (Khanna et al., 2015). For example, the reactivity of nano-gold increases as a function of size towards nanoscale (Gulumian & Savolainen, 2012), and several nanometals and nano-sized metal oxides induce oxidative stress, inflammation and genotoxicity (Fu et al., 2014). However, as a group, HARN materials seem to stand out as a peculiar group of materials that may harm human health. Currently, the ability of various methods to predict toxicity of various materials is very limited and hence these results should be interpreted with a caution. However, new approaches, utilizing transcriptomics and proteomic methods and bioinformatics are emerging and may be able to provide new possibilities to predict the toxicity of different types of ENM (Ilves & Alenius, 2016; Kinaret et al., 2017). Before such approaches are fully available, one has to execute ENM risk assessment based on the current material by material approach. In addition, in the current situation, results from the existing epidemiological studies (Liou et al., 2015) and studies on exposure (Guseva Canu et al., 2016) are valuable in putting the results of experimental studies into perspective. It is clear, however, that due to increasing exposure of workers, consumers and the environment to different ENM, effects of ENM on humans and the environment will increase, and hence a caution is warranted and possible harmful health effects of ENM shall be followed carefully. At the same time, actions towards setting of various exposure limits, especially OEL, is a highly topical issue. The demand of technologies and products utilizing ENM has grown constantly, and safety of ENM is a global issue, as has been recently emphasized (Nano on reflection, 2016; Pietroiusti & Magrini, 2014).

5 | CONCLUSIONS

Production of engineered nanomaterials for commercial use has increased exponentially since 2000 and, therefore, safety and risks of ENM have become global issues for nanotechnology innovations and for the commercialization of these innovations. Due to increased production of ENM and exposure of different groups in the society, and due to the burden to the environment, ENM have received attention and raised concerns regarding their safety. Workers have been identified as the main exposed group because concentrations of airborne ENM tend to be much higher in the industrial setting than in other exposure situations (Schulte et al., 2009). Special features of ENM have provided challenges to the research on their safety and risks, evaluation of hazards, exposure assessment, and the regulation of risks of ENM. ENM are particulate material, and many of them are non-soluble. For this reason, many principles and routines applied for traditional soluble chemicals in their hazard and risk assessment do not apply to ENM. This has contributed to the still very limited knowledge base of exposure and toxicity of ENM, and their mechanisms of action, all vital prerequisites for risk assessment and subsequent regulatory actions, when deemed necessary (Fu et al., 2014; Ou et al., 2016; Savolainen et al., 2010; Savolainen & Alenius, 2013).

Important knowledge gaps of ENM include data on: (a) toxicokinetics of ENM in organisms and cells; (b) mechanisms of toxicity; (c) identification of nano-specific early bioindicators of ENM toxicity or ENM-induced diseases; (d) development of approaches enabling prediction of ENM toxicity, for example, based on in vitro models utilizing omics technologies and bioinformatics; and (e) quantitative risk assessment enabling regulatory actions, including setting of OELs or limitations of use for workplaces, when necessary. A principle that obtained much support recently includes the 'safe by design' concept, meaning that safety issues are taken into consideration when the structure, features and production of novel nanomaterials are being designed (Stone, Johnston, Balharry, Gernand, & Gulumian, 2016). There have been recent attempts to utilize in vitro and in silico methods for ENM hazard assessment and prediction to be used in ENM risk assessment and management (http://www.nanosolutionsfp7.com; http://nanomilefp7.eu/; http://nanomile.eu-vri.eu/). These attempts have been completed in research endeavors developing tools for ENM hazard and risk assessment and risk management during their whole life cycle (http://www.guidenano.eu/; http://www.sun-eu-vri.eu/ http://www.sun-fp7.eu/). These novel approaches recently developed are based on the use of smart use of transcriptomics and proteomics and bioinformatics to analyze the data, in addition to the existing methods based on phenotypic toxicity endpoints. Implementation of the novel approaches will be highly important to provide affordable and reliable means for ENM safety assessment. For this, they still have to be validated for regulatory purposes.

These are urgent issues for the successful promotion on nanosafety globally to protect workers, consumers and the environment. Even though data on ENM toxicity exist, it is not systematic, it is scattered, and contains large knowledge gaps (Savolainen & Alenius, 2013). For this reason increased efforts to understand key elements between the physicochemical and biological features of ENM have to be continued systematically and used for the protection of humans and the environment. The above mentioned approaches will alleviate these concerns. However, in the rapidly changing world safety of ENM and of ENM-enabled products will increase in importance. It is clear that safety continues to be an increasingly important selling point of all nanomaterial and nanotechnology-driven innovations and novel products on the market.

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CONFLICTS OF INTEREST

The authors have declared no conflicts of interest for this article.

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