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Nanomaterials Versus Ambient Ultrafine Particles: An Opportunity to Exchange Toxicology Knowledge

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Background: A rich body of literature exists that has demonstrated adverse human health effects following exposure to ambient air particulate matter (PM), and there is strong support for an important role of ultrafine (nanosized) particles. At present, relatively few human health or epidemiology data exist for engineered nanomaterials (NMs) despite clear parallels in their physicochemical properties and biological actions in *in vitro* models.

Objectives: NMs are available with a range of physicochemical characteristics, which allows a more systematic toxicological analysis. Therefore, the study of ultrafine particles (UFP, <100 nm in diameter) provides an opportunity to identify plausible health effects for NMs, and the study of NMs provides an opportunity to facilitate the understanding of the mechanism of toxicity of UFP.

Methods: A workshop of experts systematically analyzed the available information and identified 19 key lessons that can facilitate knowledge exchange between these discipline areas.

Discussion: Key lessons range from the availability of specific techniques and standard protocols for physicochemical characterization and toxicology assessment to understanding and defining dose and the molecular mechanisms of toxicity. This review identifies a number of key areas in which additional research prioritization would facilitate both research fields simultaneously.

Conclusion: There is now an opportunity to apply knowledge from NM toxicology and use it to better inform PM health risk research and vice versa. https://doi.org/10.1289/EHP424

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Introduction

The idea of being able to manipulate materials and particles at the molecular level sounds like a film plot; however, over the last 25 y, it has become firmly a part of science fact and a scientific field in its own right: nanotechnology. Although nanotechnology is a rapidly growing area of research with real-world applications in virtually every area of human activity (health care, food and nutrition, water purification, manufacturing, and engineering, to name a few), the introduction of a wide range of novel materials to the environment or to humans either by design or inadvertently raises the possibility of harmful and/or unforeseen adverse effects. In response to this burgeoning field, governments and regulatory bodies have attempted to balance nanotechnology promotion (e.g., the National Nanotechnology Initiative in the United States and the Interagency Working Group on Nanotechnology) with risk assessment and regulation (e.g., the EU NanoSafety Cluster and associated projects such as NANoREG). Nanotoxicology, the study of the toxicity of nanoscale materials, has advanced in line with nanotechnology in terms of the amount of literature being published. Indeed, unlike what has been the case for harmful substances in the past, nanotoxicology is running more in parallel with developments in nanotechnology.

The original concerns about nanotoxicology were born out of research into particulate matter (PM) in air pollution (Figure 1; Beelen et al. 2014; Benbrahim-Talla et al. 2012; Bouwmeester et al. 2011; Brook et al. 2004; Donaldson et al. 2004; Hoffmann et al. 2007; IARC 2014; Künzli et al. 2005; Lelieveld et al. 2015; Li et al. 2002, 2003; Lim et al. 2012; Lucking et al. 2008; Lynch et al. 2007; Lynch and Dawson 2008; Oberdörster 2010; Oberdörster et al. 1990; Pedersen et al. 2013; Peters et al. 2001; Pope et al. 1995; SCENIHR 2007; Stone et al. 2000a, 2000b; Unfried et al. 2007; WHO 2011, 2014). This review examines key findings from air pollution and nanotoxicology health effects research and the comparisons that can be drawn between these disciplines of particle toxicology. In May 2015, the COST MODENA (European Cooperation in Science and Technology–Modelling Nanomaterials Toxicity) project hosted a workshop to exchange and merge knowledge in PM and nanoparticle toxicology. This review outlines the systematic comparison of these overlapping research fields and identifies lessons for advanced understanding as well as priority research gaps that must be addressed.

What Can Be Learned from PM Research That Has Not yet Been Applied Effectively to NM Research?

The Ultrafine Hypothesis and Nanomaterials

At the end of the previous century, several epidemiological studies identified health effects induced by airborne PM at levels that, at that time, were considered safe (e.g., Brunekreef and Holgate 2002; Dockery et al. 1993). Particles <10 μm in aerodynamic diameter (PM_{10}) can be inhaled by humans and deposit in the respiratory tract (ICRP 1994) (Appendix I), with smaller particles having higher fractional deposition in the alveoli. Consequently, ambient PM is frequently regulated as PM_{10} and $PM_{2.5}$ (<2.5 μm in aerodynamic diameter), the latter of which reflects the fine fraction of PM_{10}. The composition of PM is complex and variable (Appendix I). Although they do not contribute substantially to the (regulated) mass, ultrafine particles (UFPs) have also been identified as one of the components that are responsible for the adverse health effects observed at typical outdoor levels. Evidence also exists for the involvement of other components in the toxicity of PM, such as metals (Frampton et al. 1999; Jiménez et al. 2000; Pope 1991) and biological components (Schins et al. 2004). The relative importance of each component is likely to differ with composition, reflecting differences in location and time.

In the 1990s, the UFP fraction was hypothesized to be responsible for driving the acute respiratory and cardiovascular effects of PM (Oberdörster et al. 1995; Seaton et al. 1995). The "UFP hypothesis" was derived from toxicological evidence from rodent models that smaller titanium dioxide (TiO_2) particles (20 nm) were more likely than larger TiO_2 particles (250 nm) to cross the lung barrier and induce inflammation (Ferin et al. 1992; Oberdörster et al. 1994). Soon after, this hypothesis was supported by epidemiological evidence (Peters et al. 1997). Owing to the lack of readily available PM samples, health effect studies in the following decade used surrogate particles (e.g., carbon black, diesel engine soot, TiO_2, and polystyrene beads) to investigate the mechanisms of toxicity of UFPs, the results of which were then extrapolated to PM (e.g. Li et al. 1996; Stone et al. 1998).

In contrast to ambient PM, which is derived from natural and combustion processes, nanomaterials (NMs) are prepared deliberately at the nanoscale because they exhibit properties that provide technological advantages compared with the bulk form of the same material (The Royal Society and The Royal Academy of Engineering 2004) (Appendix I). For example, elemental (graphitic) carbon has semiconductor properties at the nanoscale (e.g., carbon nanotubes). These advantages expand the number of possible products and applications, offering great opportunities and economic gains. Although UFPs and NMs are often derived from very different sources and processes, their physicochemical characteristics can overlap (Appendix I), suggesting that their properties, their behaviors, and importantly, their toxicities might also overlap. In the early 2000s, a number of high-profile national and international reports highlighted the importance of nanotechnology, but they also recognized the potential risks (e.g., SCENIHR 2005; The Royal Society and The Royal Academy of Engineering 2004). These reports led to an increased interest in UFP toxicology accompanied by a change in terminology from the mid-2000s.

Ambient PM and UFP Health Effects

Cardiopulmonary: Epidemiologic Evidence

Epidemiology studies clearly demonstrate links between PM_{10} and $PM_{2.5}$ with both short-term and long-term health effects, particularly on the respiratory and cardiovascular systems (Dockery et al. 1993). However, PM includes a range of particle sizes, and very few studies have included UFP *per se* as a variable. Using particle number concentration as a surrogate for UFP, exposure has been associated with hospital admissions for acute asthma and increased systolic blood pressure in children (Andersen et al. 2008; Pieters et al. 2015) as well as with rehospitalization in patients with prior myocardial infarction (von Klot et al. 2005). For hospitalization with ischemic stroke, a stronger association was reported with particle number than with PM_{10} mass concentration (Andersen et al. 2010). Conversely, greater associations for particle number than mass metrics have been less convincing for acute myocardial infarction (Lanki et al. 2006).

Particle number concentrations have also been associated with surrogate markers of cardiovascular health. For example, elevated levels of fibrinogen, prothrombin factors 1 and 2, and von Willebrand factor are associated with exposure to UFP (Hildebrandt et al. 2009). Independent associations have been observed for UFP and $PM_{2.5}$ with heart rate and heart rate variability in patients with diabetes mellitus and glucose intolerance (Peters et al. 2015; Sun et al. 2015). In patients undergoing cardiac rehabilitation, modulation of the parasympathetic innervation of the heart, increased blood pressure, and markers of systemic inflammation were all associated with exposure to UFP (Rich et al. 2012). Epidemiological studies involving biomarkers related to oxidative stress and inflammation revealed that primary combustion markers from quasi-UFP ($PM_{<0.25}$) were positively associated with systemic changes in interleukin 6 (IL-6) and tumor necrosis factor alpha (TNFα), platelet activation, and erythrocyte antioxidant enzyme activity in an elderly population (Delfino et al. 2009). Similarly, elevated plasma fibrinogen and white blood cells have been associated with UFP exposure (Gong et al. 2014).

Cardiopulmonary: Preclinical and Clinical Evidence

Several preclinical and clinical studies have addressed the short-term inhalation and respiratory effects of UFPs. For example, field studies have observed associations for UFPs and carbon with reductions in lung function among asthmatics (McCreanor et al. 2007), and healthy adolescents and adolescents with asthma in New York exhibited an increase in indicators of inflammation (Patel et al. 2013). The majority of preclinical and clinical studies on UFPs have been conducted with diesel exhaust and diesel exhaust particles (DEPs), an especially rich source of UFPs

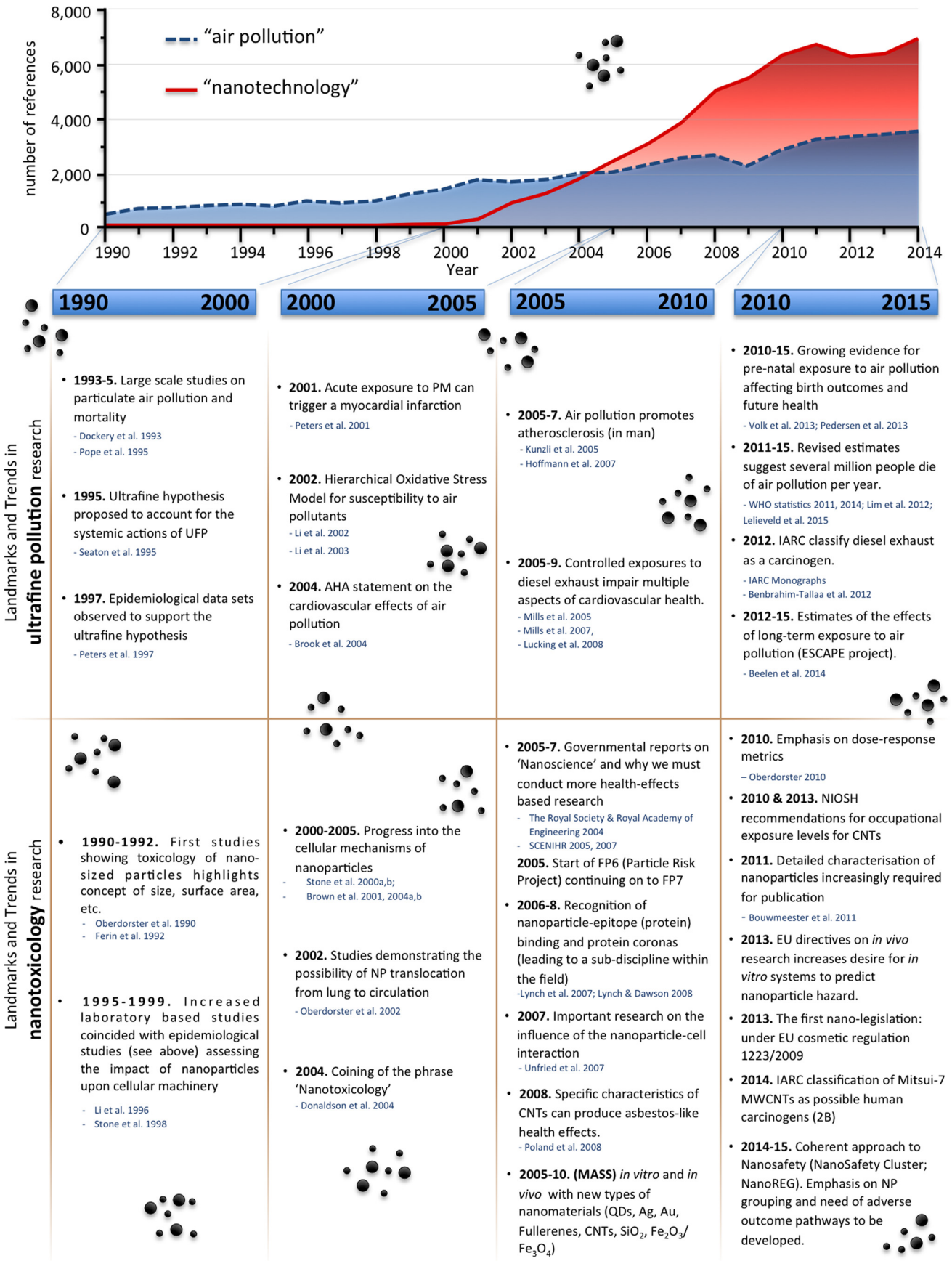

Figure 1. Time line showing the increased interest in particulate matter (PM) and nanomaterials (NMs) over the last three decades, highlighting key studies and research trends in both areas. Number of references per year (noncumulative) based on Pubmed (https://www.ncbi.nlm.nih.gov/pubmed/) search without further limits applied.

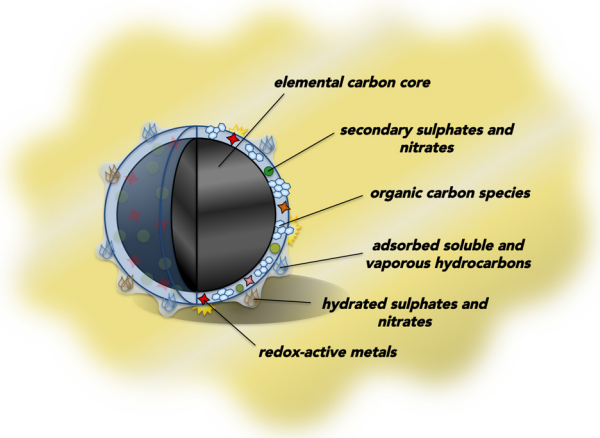

Figure 2. Schematic providing an example of the complex composition of ultrafine particles (UFPs) [e.g., urban particulate matter (PM) or particles in vehicle exhaust], which in urban air often have a carbon core coated with a diverse range of chemical species including reactive transition metals and organic hydrocarbons. Detail is not to scale.

(Figure 2). These studies have shown airway inflammation in healthy individuals, including elevated levels of inflammatory cells and mediators (Ghio et al. 2012; Xu et al. 2013; Yamamoto et al. 2013).

Inhaled UFPs modify numerous aspects of cardiac function, reducing heart rate variability (Cassee et al. 2011; Pieters et al. 2012), a predictor of cardiovascular risk, and increasing the incidence, duration, and severity of arrhythmia (Delfino et al. 2005; Robertson et al. 2014). Furthermore, UFPs in urban air (Weichenthal 2012) or diesel engine emissions (Mills et al. 2007) exacerbate myocardial ischemia (Cascio et al. 2007; Robertson et al. 2014). Blood vessels finely regulate blood flow through changes in the tone of vascular smooth muscle, and UFPs generally alter the balance in favor of constriction (Møller et al. 2011). The resulting increased blood pressure (Bartoli et al. 2009) and the reduced ability of the arteries to relax are usually detrimental. Vascular dysfunction can be caused by a loss of mediators such as nitric oxide released by the vascular endothelium (Courtois et al. 2008; Miller et al. 2009; Møller et al. 2011), by increased sensitivity to vasoconstrictor factors (Langrish et al. 2009), and by alterations in baroreceptor/neuroregulatory feedback (Rhoden et al. 2005; Robertson et al. 2012). Blood components are also dysregulated, with UFPs tending to increase blood coagulability (Kilinç et al. 2011; Nemmar et al. 2004), encourage platelet activation (Cascio et al. 2007; Lucking et al. 2011), and reduce blood clot clearance (Mills et al. 2005). The cellular and biochemical mechanisms underlying these effects are wide-ranging, with oxidative stress and inflammation being key drivers (Miller et al. 2012) (Figure 3). In combination, these actions promote cardiovascular disease. Indeed, long-term exposure to UFPs in animal models (Araujo et al. 2008; Miller et al. 2013) has been shown to worsen atherosclerotic vessel disease.

Other Target Organs

Although research has predominantly focused upon the inhalation of UFPs and their impact upon cardiovascular function, a number of additional, secondary target organs have been investigated (see Figure 3). Such research has been based upon the hypothesis of alveolar translocation of UFPs to the bloodstream allowing for nonspecific interaction with other essential organs such as the brain and kidneys. UFP exposure and mucociliary clearance from

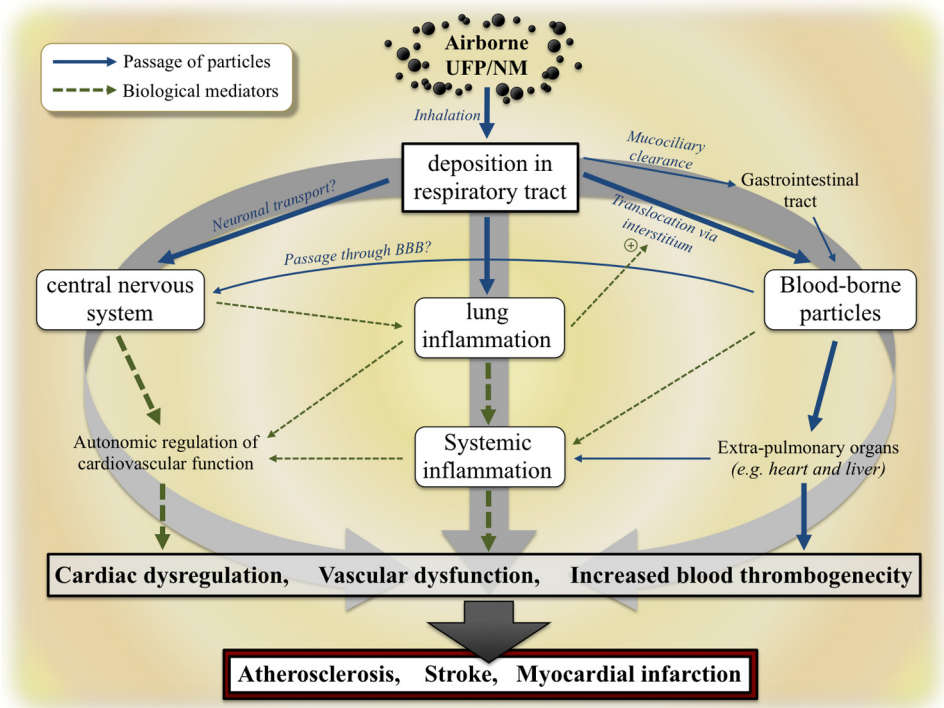

Figure 3. Schematic illustrating some of the key mechanisms through which inhaled ultrafine particles (UFPs) may influence secondary organs and systemic tissues, with emphasis on the means through which inhaled particles may cause cardiovascular events. Note that there are three main pathways linking the pulmonary and cardiovascular systems (grey arrows, left to right): autonomic regulation, passage of inflammatory mediators, and particle translocation. The arrows between these three pathways highlight the degree of interaction between mechanistic pathways and the challenges involved in broad categorization of the wide-ranging biological actions of inhaled UFPs. Added to these pathways is the potential for desorbed components to exert effects.

the lungs into the gut might also be linked with adverse effects on lipid metabolism and intestinal villus shortening (Li et al. 2015), conveying evidence of effects with potential clinical relevance for gut or liver diseases.

Starting approximately 15 y ago, the effects of PM in the central nervous system (CNS) gained recognition with reports that exposure to polluted Mexico City air resulted in oxidative stress, inflammation, neuropathology, and cognitive and behavioral changes in humans and in animals (Calderón-Garcidueñas et al. 1999, 2011). Other studies using a myriad of PM collection techniques upheld the early findings related to PM-induced brain-centric inflammatory processes, including those in regions related to learning and memory (Campbell et al. 2005; Fonken et al. 2011). Such health outcomes could be explained by findings that inhaled particles can travel to the brain via the blood following alveolar deposition, via nose–brain transport following olfactory mucosa deposition (Balasubramanian et al. 2013; Elder et al. 2006), or via the spillover of systemic inflammation to the CNS; a combination of these processes is also possible. Although acute CNS inflammatory processes cannot be directly measured in living humans, it is interesting to note that neurodegenerative diseases are on the rise and that there is a well-established—albeit mechanistically murky—link between inflammation and neurodegeneration (Akiyama et al. 2000; Amor et al. 2010). Recent research has focused on one area where animal and human outcomes have good concordance, namely, behavior and cognition. For example, Fonken et al. (2011) showed that mice exposed to $PM_{2.5}$ (which includes UFPs) had deficits in spatial learning and memory. Using mice exposed to concentrated UFPs as neonates, Allen et al. (2014a, 2014b) showed that males had behavioral outcomes that were associated with persistent enlargement of the ventricles and innate immune cell activation. In population-based studies, several investigators have now reported associations between traffic aerosol exposures and reduced cognitive function in the elderly (Ranft et al. 2009) and in children (Freire et al. 2010; Suglia et al. 2008). Two U.S.-based case–control studies have also reported increased odds ratios for autism in association with early-life exposure to traffic-related pollution, specifically $PM_{2.5}$ (Becerra et al. 2013; Volk et al. 2013). With the exception that NM research has demonstrated plausibility for PM translocation to the brain, very little has been investigated in terms of the nervous system impacts of NM.

Priority research gap. PM research provides a basis for developing a strategy to identify potential neurological effects of NMs in which physicochemical characteristics could be responsible.

Epidemiological studies have also related $PM_{2.5}$ and PM_{10} air pollution to reproductive toxicity and to adverse effects on the progeny. A recent systematic review (Stieb et al. 2012) reported an association between exposure to $PM_{2.5}$ and PM_{10} and low birth weight, preterm birth, and small-for-gestational-age birth. Additionally, van Rossem et al. (2015) found that maternal exposure to $PM_{2.5}$ and black carbon were associated with increased blood pressure in newborn children. The effect seems to be mediated by altered placental vascular structure induced by $PM_{2.5}$ (Veras et al. 2008). Preclinical studies indicated that adverse health effects of UFP exposures cannot be excluded, although the potential for hazard has not been well characterized (Hougaard et al. 2015).

The evidence outlined above demonstrates the impact of ambient PM on a range of targets, but in particular the adverse effects on respiratory, cardiovascular, neurological, and reproductive systems. For cardiovascular studies, this evidence extends to UFPs, but direct evidence for the role of UFPs in the induction of the other disease targets is, in general, still lacking.

Investigation of NM Impacts on Human Health

A few studies are now emerging that demonstrate the effects of nanomaterials on human health, particularly in an occupational setting. Lee et al. (2015) investigated workers manufacturing multiwalled carbon nanotubes and found that although there was no impact on hematology and blood biochemistry, they did see an increase in a range of markers of lipid peroxidation in exhaled breath condensates of the workers, including malondialdehyde, 4-hydroxy-2-hexenal and *n*-hexanal. Multiwalled carbon nanotubes have also been reported to have impacts on a range of end points in workers exposed for ≥ 6 mo. These end points include the targeting of genes associated with cell cycle regulation, progression, and control, as well as genes involved in apoptosis and proliferation (Shvedova et al. 2016). The same study also identified targeting of pathways involved in pulmonary and cardiovascular effects, as well as pathways involved in carcinogenic outcomes in humans.

Another study followed workers in 14 nanomaterial manufacturing or application (or both) factories in Taiwan for 6 mo (Liao et al. 2014). The nanomaterials that were produced or handled included silver, iron oxide, gold, titanium dioxide, carbon nanotubes, and silicon dioxide. The group working with nanomaterials exhibited higher levels of antioxidant enzymes and cardiovascular markers than the workers who handled other materials. In addition, the study also reported that markers of small airway damage (Clara cell protein 16) and lung function were significantly associated with handling nanomaterials.

Liou et al. (2015) reviewed 15 studies that investigated the effects of engineered nanomaterials on workers. Of these 15 studies, 11 were cross-sectional, four were longitudinal, and one was a descriptive pilot study. All of the 11 cross-sectional studies showed a positive relationship between various biomarkers and exposure to engineered nanomaterials. Three of the four longitudinal studies demonstrated a negative relationship, with the fourth showing a positive relationship after 1 y follow-up. In general, the exposure levels identified were not very high compared with those used in human inhalation chamber studies; however, there were some exceptions with higher exposures. The studies were generally found to be limited by small numbers of participants, a lack of consistent exposure information, the detection of generally low exposures, and short intervals between exposure and effect.

Taken together, these initial human health studies suggest that occupational exposure to nanomaterials may have detrimental impacts on human health. Further work is required over the long term to ascertain the nature and extent of these effects as well as their relevance to different types of materials.

Lesson 1. A rich body of literature exists that has demonstrated adverse human health effects following exposure to PM, with a proportion of that literature providing support for UFP involvement. In contrast, although initial studies have suggested an association between exposure to nanomaterials and human health, relatively few clinical or epidemiology data are available at the present time.

Figure 4 outlines a range of health effects and biological indicators of disease reported in the literature. This information can be used to better inform and justify NM study end points.

Mechanisms of UFP-Induced Health Effects

Cardiovascular Effects

Three hypothetical pathways to explain the cardiovascular effects of PM predominate: "inflammation," "autonomic regulation," and "particle translocation" (Figure 3). The classical hypothesis

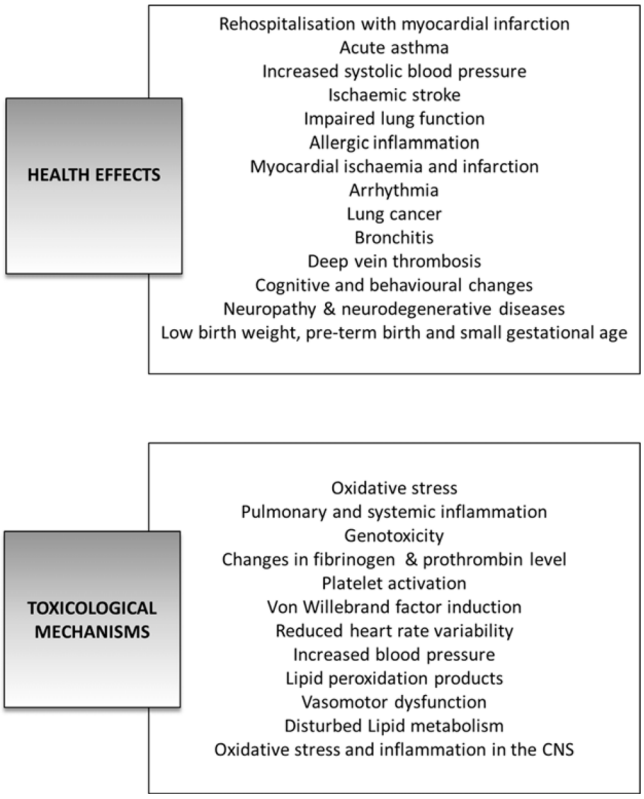

Figure 4. A range of health effects and biological indicators of disease that can be used to identify relevant end points for study design.

is that particles inhaled into the lung are taken up by alveolar macrophages, triggering an inflammatory response within the lung. A sufficient particle dose, reactivity, or lack of clearance leads to amplification of the response with a resultant "spill-over" of inflammatory mediators into the blood causing systemic inflammation (Seaton et al. 1995), which is strongly associated with cardiovascular disease. Alternatively, inhaled particles (or the inflammatory response resulting from inhalation of the particles) stimulate alveolar sensory receptors (Ghelfi et al. 2010; Hazari et al. 2011; Robertson et al. 2014), providing a signal to the CNS. This response manifests through alterations in autonomic nervous system activity, which directly regulates cardiac function and, indirectly, other aspects of the cardiovascular system (Pope et al. 1999; Rhoden et al. 2005). The identification of the UFP fraction of PM paved the way for a third hypothesis: The minute size of UFPs allows them to translocate across the thin alveolar-capillary wall (by an as-yet undetermined mechanism) and enter the circulation themselves to directly affect cardiovascular function (Nemmar et al. 2001; Oberdörster et al. 2002).

There is a wealth of evidence for and against each of these hypotheses, but in truth, all three are likely to occur; the contribution of each is dependent on the physicochemical properties of the UFPs, the cardiovascular end point under investigation, and the susceptibility of the person/model being explored (Miller 2014). Furthermore, it is highly likely that many of the subtleties of these pathways have yet to be identified. The intricacies of these processes may encompass nonclassical inflammatory/oxidative biological mediators such as acute-phase proteins (Saber et al. 2014) or oxidized phospholipids (Kampfrath et al. 2011), the release and accumulation of chemical particle surfaces and constituents within biological compartments (Murphy et al. 2008; Totlandsdal et al. 2015), particle/plasma–protein interactions (Deng et al. 2011; Monopoli et al. 2012), and the role of proteins/inflammatory cells in carrying/accumulating particles to susceptible areas of the body (Schäffler et al. 2014). Reports are rapidly emerging from preclinical models that demonstrate cardiovascular effects for NMs similar to those shown for UFPs, such as altered autonomic function (Harder et al. 2005), impaired vasodilatation (Leblanc et al. 2010; Møller et al. 2011), blood hypercoagulability (Kim et al. 2012; Radomski et al. 2005), and aggravated atherosclerosis (Li et al. 2007; Mikkelsen et al. 2011; Niwa et al. 2007). Identification of the biological mechanisms for these parallel observations will have important consequences for both fields of research.

Lesson 2. The information obtained from PM epidemiology and mechanistic research has provided an evidence base on which to develop hypotheses to stimulate research into the potential modes of action for NMs.

Genotoxicity/Carcinogenicity

Markers of genotoxicity, such as elevated levels of oxidized DNA nucleobases, bulky DNA adducts, and clastogenic end points in leukocytes have been documented in biomonitoring studies of humans. Positive associations between UFP and oxidatively damaged DNA in mononuclear blood cells have been observed (Bräuner et al. 2007; Vinzents et al. 2005). In contrast, there is a paucity of studies on UFP-generated oxidative DNA oxidation products in cultured mammalian cells and experimental animal models (Møller et al. 2014), as well as a lack of studies on neoplastic lesions in the respiratory tract (a priority research gap). Studies including exposure to traffic-related outdoor air pollution or to DEPs (Stinn et al. 2005; Valberg and Crouch 1999) have identified increased lung adenomas in rodent models.

The carcinogenic mechanism is believed to involve genotoxicity by both oxidative reactions and formation of bulky DNA adducts from polycyclic aromatic hydrocarbons (PAHs), which may give rise to mutation and structural chromosome damage. Early genotoxic events such as DNA adducts and small nucleobase oxidative lesions can be generated by primary (direct) mechanisms in relevant target cells, whereas oxidative-mediated DNA damage may also occur as a consequence of secondary inflammation-driven events (Schins and Knaapen 2007). Importantly, the last mechanism has been discussed as a major contributor to the mutagenic and carcinogenic properties of DEPs, as well as to those of poorly soluble nanoparticles such as carbon black and titanium dioxide in long-term high-dose inhalation studies in rats (Knaapen et al. 2004). However, the relevance of this mechanism towards the carcinogenicity of PM and the specific contribution of the UFP component herein remains to be elucidated (a priority research gap). Recent reviews comparing the genotoxicity of DEPs and NMs have indicated similar mechanistic causes of DNA damage (Magdolenova et al. 2014), although the dose metric of "mass concentration" causes difficulties in comparison across studies (Møller et al. 2015). Interestingly, it has recently been reported that single- and multiwalled carbon nanotubes could interfere with the mitotic spindle apparatus (Sargent et al. 2012; Siegrist et al. 2014).

Lesson 3. NM research provides an opportunity to better understand the (mechanistic) role of UFPs in the genotoxicity, mutagenicity, and carcinogenicity of PM.

Lessons Learned from NM Toxicology That Could Be Applied to PM

Regulatory standards for ambient PM are promulgated from a rich body of literature that has demonstrated adverse human health effects following exposure. Conversely, the toxicological research on NMs is motivated by a desire to define material

properties that are linked to adverse health effects, thus supporting effective risk management. To achieve engineering for safety goals, it is necessary to understand the toxicity of the NMs themselves while recognizing that toxicological assessment is only one part of an overall risk assessment process. However, it is impossible to determine safer exposure levels and safer materials design without first understanding dose-specific toxicological effects. Studies that address these questions have provided a wealth of knowledge that might now be useful to better understand the toxicology of PM.

Characterization of Test Materials

In the past, characterization of UFPs has focused on mass and number concentrations, chemical composition, and size distribution. Studies initially used existing methods obtained from PM and materials science research to characterize NMs, but over time, the methodology has been refined. This refinement has allowed NM toxicological researchers to generate a list of desired characterization information (https://www.iso.org/obp/ui/#iso:std:iso:ts:17200:ed-1:v1:en). A similar list of requested characterization end points was not developed in PM research owing to the lack of understanding of how different physical and chemical characteristics could interact to influence PM toxicity. However, a comparable list for PM health effect studies could be beneficial for understanding mechanisms as well as for enhancing comparability across fields. In the future, these techniques will provide an opportunity for improved monitoring of PM.

Lesson 4. A range of particle characteristics have been shown to influence their toxicity. These characteristics should also be considered in UFP research, where appropriate characteristics that can be used for the prediction of toxicity have not yet been identified. Furthermore, the variance of these characteristics in space and time should also be determined comparably to the methods used for NMs.

Before the development of NM toxicity testing, standard operating procedures (SOPs) for particle characterization were rarely in place. Development of SOPs is currently ongoing in the nanosafety communities as well as via standardization projects conducted by the European Committee for Standardization (CEN) and the International Organization for Standardization (ISO) (European Commission. 2010).

Especially relevant particle parameter protocols for standardization are those of dispersion, size, agglomeration, and aggregation in different environmental and biological media. Techniques that can (semi-)automatically obtain multiple size parameters using transmission electron microscopy will help to satisfy the challenges of regulatory NM definitions such as those proposed by the European Commission (2011). Advances in dynamic light scattering and coupling to inductively coupled plasma mass spectrometry (ICPMS) technologies such as field-flow fractionation multiangle light scattering ICPMS and single-particle ICPMS have been driven by requirements for particle sizing and behavior in liquid dispersions. Great improvements have also been made in understanding the applicability of different measurement devices for airborne particles. These improvements include knowledge of the care needed in using and interpreting data obtained from charge-based instruments, particularly instruments using unipolar charging such as surface area monitors and the Fast Mobility Particle Sizer™ (Asbach et al. 2009; Levin et al. 2016). These instruments can provide erroneous results when significant amounts of agglomerates and aggregates that are approximately 200 nm or more in size are present in the aerosol (Todea et al. 2015).

Advances have also been made in chemical analysis of NMs, where ICPMS is often the preferred method, using either the single-particle mode or particle extraction protocols (Lee et al. 2014). Nondestructive methods such as Instrumental Nuclear Activation Analysis (INAA) and X-ray fluorescence (XRF) may be preferable for bulk chemical characterization to avoid challenges in developing material-specific extraction techniques.

New developments also include procedures to identify and quantify specific surface coatings/functionalization of NMs using combinations of differential thermal gravimetric/differential thermal analysis–gas chromatography and chemical-specific methods such as high-performance liquid chromatography–mass spectrometry/optical emission spectroscopy or gas chromatography–mass spectrometry. Combinations of these methods are particularly important for second- and third-generation NM analysis, and methods have recently been developed as part of the EU FP7 NANoREG project (European Commission 2013). Further knowledge transfer of techniques between materials science, environmental, and nanosafety researchers continues and is highly likely to be applicable to PM research.

Analysis of surface charge via zeta potential measurements is straightforward in simple systems (e.g., a pure NM in pH-controlled water with moderate ionic strength) but becomes challenging in multicomponent complex systems such as PM in air pollution. From a toxicological perspective, because zeta potential varies significantly with pH and with the composition of the test medium, a full assessment should consider all likely media and biological compartments of interest.

Particle reactivity is currently not well defined, perhaps understandably given that it is unlikely to be a single parameter. "Simple" methods include measurement of reactive oxygen species, pH and redox potential, and band gap. In recent years, band gap has been shown to be related to the toxicity of metal oxide NMs (Zhang et al. 2012). *In vitro* dissolution can also be an important indicator of reactivity for some NMs insofar as it is indicative of biodurability/biopersistance, methods which are under development (CEN/ISO). Recent work has shown that great care must be taken in the design and harmonization of such experiments to achieve reproducible results (Tantra et al. 2016). These developments are relevant for both NM and PM research, although the weight has been strongly tilted towards NM research in the last decade.

Lesson 5. A range of new and improved techniques for assessing the physicochemical and nanoscale characteristics of NMs have been developed. These techniques should be applied appropriately to inhalation exposure assessment in population studies to better determine the relationship between particle characteristics and health effects.

It is worth noting that the procedures used for sample preparation and the mode of exposure used in toxicology studies determine the requirements for characterization of exposure and fate. For example, quantification and characterization of aerosolized particles might include aerosol monitors and filter samples, whereas particles used as dispersions for exposure would require analysis via hydrodynamic size distribution, agglomeration state, sedimentation, and reactivity in the dispersion medium. NMs are often dispersed in protein-rich media, which can affect both the biokinetics and the toxicity, whereas ambient UFPs can be dispersed to some extent without these additives (Moore et al. 2015).

The improvements in characterization have revealed the need for correct storage of NM test items over time. For NMs analyzed by the Organisation for Economic Co-operation and Development (OECD) working party of manufactured nanomaterials (WPMN), this has resulted in storage under argon, in single-use vials, in the dark. Previously, both nanosafety and PM researchers have stored dry powders, filter-bound particles, and wet suspensions under many different conditions. For many ambient PM samples, storage under argon at <0°C could potentially

prevent oxidation/loss of toxicologically relevant (semi)volatile substances.

Exposure Characterization

Since the emergence of nanotoxicology as a discipline, it has become increasingly recognized that particles can be modified upon interactions with cells and tissues, for example, owing to the influence of the surrounding media (e.g., proteins). Such biomolecule interaction is likely to impact the fate of the particles by modifying the surface properties and the behavior of the particle (e.g., agglomeration, solubility, bioavailability, biodurability) as well as the adsorbed protein properties (Brown et al. 2010a; Deng et al. 2011). These modifications could, in turn, alter how particles are taken up into cells, how they trigger signaling pathways, and in what physicochemical format they are translocated between cells and to distal organs.

Characterization of NMs at various stages throughout the life cycle of the material is far from simple. For PM, this is further complicated by the complex mixtures present in ambient air. Furthermore, it is important to note that consumer exposure to NMs may be different than occupational exposure depending on the state of the material (e.g., native NMs, NMs embedded in a product, NM degradation and disposal). In toxicology studies, many NMs have been studied as dispersions (usually of agglomerates) in biological or culture media. Several protocols for NM dispersion have been developed using different dispersion principles and media (Hartmann et al. 2015); first attempts have also been made to establish harmonized dispersion for regulatory testing (e.g., HA Jensen, H Crutzen, A Dijkzeul, unpublished work, 2014). In contrast, harmonized dispersion protocols have largely been lacking in PM research.

Lesson 6. Harmonized dispersion protocols can be transferred to PM research to increase harmonization and comparability between test methods and results.

There is, however, a major obstacle for PM research, in that much smaller amounts of PM (obtained via collection) are normally available than for NM research, where direct synthesis is often possible. For ambient PM, it is necessary to collect and extract PM from the collector (e.g., scraping, sonicating, or chemically extracting from a filter), which may alter the state of the PM before its use in toxicology studies. Transformation and loss of toxicologically important semivolatile compounds during sampling is also an issue that must be taken into account when testing collected PM.

Lesson 7. The need to extract PM from filters can be avoided by moving the laboratory to the field, for example, using *in vivo* or air–liquid interface (ALI) systems. In addition, systems such as particle concentrators (Gupta et al. 2004; Kim et al. 2001) have been developed; these systems help to ensure sufficient doses over the period of the experiment. However, such toxicological studies in the field can be expensive and additionally complicated.

Inhalation Exposure and Deposited Dose

The experimental data for total lung deposition of particles are highly consolidated (ICRP 1994); however, the regional deposition of NMs is weakly supported by direct experimental data to validate the models (a priority research gap).

The deposition of NMs depends on three sets of parameters: particle dynamics, lung geometry, and gas flow dynamics. Owing to their size, the primary region for the deposition of NMs is the alveolar region of the human lung (a priority research gap), which means that the first biological matrix encountered is lung surfactant (Gasser et al. 2010). Interaction with NMs may alter the structure and function of the surfactant proteins (Beck-Broichsitter et al. 2014; Valle et al. 2015) and subsequently influence the specific mammalian cell interactions (Schleh et al. 2013).

Lesson 8. NM and parallel UFP inhalation studies can provide important information on pulmonary deposition and surfactant interactions and would facilitate the investigation of comparability between both types of particles.

For PM and NM toxicology studies, consideration of relevant particle doses is required. A daily inhalation mass dose for PM that relates to maximal air-quality standards has been suggested [WHO Global Air Quality Guidelines (WHO 2016): $10\,\mu g/m^3$ for $PM_{2.5}$]. A daily inhaled volume for a moderately active adult human (75 kg) is typically $20\,m^3/d$. If the mean deposited fraction is 0.3 (Price et al. 2001), the suggested daily mass dose would be $60\,\mu g/d$. However, PM mass is often dominated by coarse particles, so only a fraction of the $60\,\mu g/d$ would actually represent UFPs.

Therefore, dose might be better expressed as particle number for such small particles because of their low mass. There is great variability in ambient UFP numbers, ranging from 500–10,000 particles/cm^3 in rural areas to 7500–25,000 particles/cm^3 in urban background (Putaud et al. 2010), and with a European mean concentration of 31,500 particles/cm^3 at hot spots (busy streets). To estimate *in vivo* exposure conditions based on particle number concentrations, a healthy adult breathing during moderate exercise in ambient air with an assumed moderate concentration of 30,000 particles/cm^3 (of which 80–90% of the particle count is assumed to be UFPs) will inhale 6×10^{11} particles/cm^3. Assuming a mean deposition probability for UFPs of 0.5, this corresponds to a dose of 3×10^{11} particles deposited per day or 1.2×10^{10} particles deposited per hour (Geiser and Kreyling 2010).

To obtain a more relevant assessment of health effects *in vitro*, the UFP and NM dose per cell number or area should reflect real inhalation. The initial use of high UFP or NM doses may be justified by the need to be able to detect effects of exposure, but such high doses need to be accompanied or followed up with studies using realistic doses in relation to current information concerning occupational and ambient exposures to UFPs or to specific NM types.

The respiratory zone of the lung represents by far the largest compartment for NM deposition. Estimates of lung physiological data (Stone et al. 1992), together with the above-mentioned European mean ambient exposure concentration of 31,500 particles/cm^3, suggest that on average 8 nanoscale particles deposit per day per cell of the alveolar epithelial surface (Geiser and Kreyling 2010). According to limits imposed by thermodynamic conditions, the highest possible NM aerosol number concentration is approximately $10^6/cm^3$, which translates to 700 particles/d/cell or 30 particles/h/cell of the alveolar epithelium. Because mass is a more frequently used metric, these numbers must be converted using the effective density of the particles. This information is useful when verifying relevant *in vitro* doses. Models have been published (including experimental verification) that can estimate particle deposition onto a monolayer of cells *in vitro* (Cohen et al. 2013; Hinderliter et al. 2010; Teeguarden et al. 2007). These studies identify NMs, and the effective density of NMs, as important factors in modeling cellular dose. In essence, only submicrometer and larger NM agglomerates will deposit within one hour, whereas NMs <100 nm may remain suspended for >24 h.

Lesson 9. The models of *in vitro* NM deposition should be more widely used for estimation of NM dose in cultured cells to refine models so that they better reflect anticipated airborne

exposures. Their application to PM would be more difficult owing to the size and density diversity of such a mixed particulate sample. However, such dosimetry models (DeLoid et al 2015) can in principle deal with size distribution data as well as with mean size data in cases where such distribution data are measured. Thus, the lack of a narrow size distribution for PM should not preclude the more effective use of such modeling advances to improve dosimetry in both the NM and PM fields.

Uptake, Clearance, and Fate following Pulmonary Exposure

In rodent models, within 1–2 d of exposure, NM clearance is relatively low compared with clearance of micrometer-sized particles, and it is associated with more rapid and extensive uptake into epithelial cells (Kreyling et al. 2002; Semmler-Behnke et al. 2007; Semmler et al. 2004). However, the rodent model is not a good reflection of clearance in humans. For humans, there is evidence that both NMs and micrometer-sized particle clearance from the conducting airways is less extensive, leading to long-term particle retention (Möller et al. 2008). Interestingly, in dogs (Kreyling et al. 1999) and monkeys (Nikula et al. 1997), long-term retention increases substantially in conducting airways with decreasing particle size. Considering long-term clearance predominantly from the alveolar region in these species, macrophage-mediated clearance occurs at a rate that is one order of magnitude lower than that in rodents (Kreyling 2013).

Priority research gap. For the human distal regions of the lungs, neither macrophage-mediated long-term clearance kinetics data nor translocation data for NMs into the circulation are currently available.

Particle clearance from the lungs can also occur via transport toward lymph nodes and translocation into the blood circulation, leading potentially to accumulation in secondary organs and tissues. These pathways will have a limited or lesser relevance for the clearance of rapidly or moderately soluble particles, respectively (Oberdörster et al. 2005).

Lesson 10. NM inhalation studies provide details of the potential for UFP biopersistence and transport based upon their solubility.

Pioneering studies in the 1990s first demonstrated detectable translocation of nanoscale TiO_2 particles into the lung interstitium and that translocation is lower for larger particles (e.g., 21 nm vs. 250 nm diameter) (Ferin et al. 1991; Oberdörster et al. 1994). More recently, a comprehensive list of inhalation studies and instillation studies using various NMs has provided consensus that, in rodents, relatively small fractions (approximately 1%) of inhaled NMs are translocated across the air–blood barrier, leading to accumulation in secondary organs, including the liver and spleen (Balasubramanian et al. 2013). Notably, this rat inhalation study used gold NM of different primary particle sizes but agglomerated to give the same diameter in air of 45 nm. The authors demonstrated size-related translocation with the smaller 7 nm primary gold particles translocating more than the 20 nm primary gold particles, suggesting deagglomeration of the 45 nm agglomerates in the lung.

Priority research gap. Based on the observation that in humans, NM retention in the lung is likely to be longer than in rodents, it is necessary to consider whether the relatively small translocation proportions identified in rodents might be greater in humans.

NM research has facilitated understanding of the biokinetics and biodistribution of particles such that there is now clear evidence that inhaled NMs can reach and accumulate in secondary organs (Geiser and Kreyling 2010; Kreyling 2013). Quantitative biokinetics analysis of NMs applied via the lungs of rodents demonstrated small fractions of NM (iridium, carbon, gold, TiO_2) in all secondary organs studied, including the brain and heart, and even in fetuses (Kreyling et al. 2002; Semmler-Behnke et al. 2007; Semmler-Behnke et al. 2014). An inhalation study using 20-nm iridium NM was extended to six months after a single 1-h inhalation and yielded significant retention in the liver, spleen, kidneys, heart, and brain (Semmler-Behnke et al. 2007; Semmler et al. 2004). Although the fractions of the total dose that reach these tissues are very small in rodents, the studies have highlighted the importance of epithelial barrier health (Heckel et al. 2004) and that the particle's protein corona affects biodistribution (Kreyling et al. 2014). As a corollary to biodistribution studies, *in vitro* studies with NMs have helped to define the ability of particles to breach cellular membranes (Bachler et al. 2015), their interactions with subcellular structures, and therefore, the toxicological mechanisms related to particle uptake.

Lesson 11. NM translocation studies provide clear evidence of the potential for UFPs to translocate from the lung surface into blood and to distribute throughout the body, accumulating in a range of secondary organs. The knowledge gained from NM biokinetics and biodistribution studies provides an evidence base to predict the fate and health effects of UFPs in the body.

In addition to translocation, rodent studies also suggest that NMs can relocate from the interstitium and epithelium back onto the epithelial surface via an unknown mechanism (e.g., via macrophages) (Semmler-Behnke et al. 2007). The fraction removed via the lymphatic or cardiovascular system is relatively small in contrast. Studies using NMs (sometimes at doses higher than a few hundred micrograms per lung) such as TiO_2, carbon black, gold, quantum dots, silver nanowires, and carbon nanotubes have identified accumulation of NMs in the lung-associated lymph nodes (Schinwald et al. 2012).

Priority research gap. The development of this research area using different types of NMs and different investigative protocols will be useful in determining the importance of this potential route of uptake and hence potential translocation within the body. Pathways of relocation of inhaled NMs in rodent lungs are schematically sketched in Figure 5.

Lesson 12. The differential clearance and uptake of NMs and micron-sized particles could also apply to the varied size fractions of PM, adding to the plausibility of a difference in their toxicity.

Toxicological Mechanisms

There are a number of mechanisms by which UFPs and NMs may have an impact on cells, and these mechanistic studies provide a great opportunity for comparison or alignment of our understanding of NM and UFP toxicity. Mechanisms including reactive oxygen species (ROS) and oxidative stress (Miller 2014; Nel et al. 2006; Stone et al. 2007) feature widely in the literature for both NMs and UFPs (see below). Endothelial cells and epithelial cells may also generate nitric oxide in response to NMs and UFPs via stimulation of NOX4 (e.g., in addition to the respiratory burst generated by inflammatory cells exposed to particles, it appears that particles can also generate ROS directly, including PM_{10} (Gilmour et al. 1996), DEPs (Miller et al. 2009), and many different NMs including carbon black (Stone et al. 1998; Wilson et al. 2002), polystyrene beads (Brown et al. 2001), and a range of metal/metal oxide particles (Dick et al. 2003; Rushton et al. 2010). Different material compositions vary in their potential to induce ROS production, ranging from copper (Rushton et al. 2010), which has an inherent ability to generate ROS, to amorphous nanosilica, which exhibits no intrinsic capacity to generate oxidants (Napierska et al. 2012). Some NMs do not exhibit intrinsic oxidant-generating capacity but will generate ROS upon

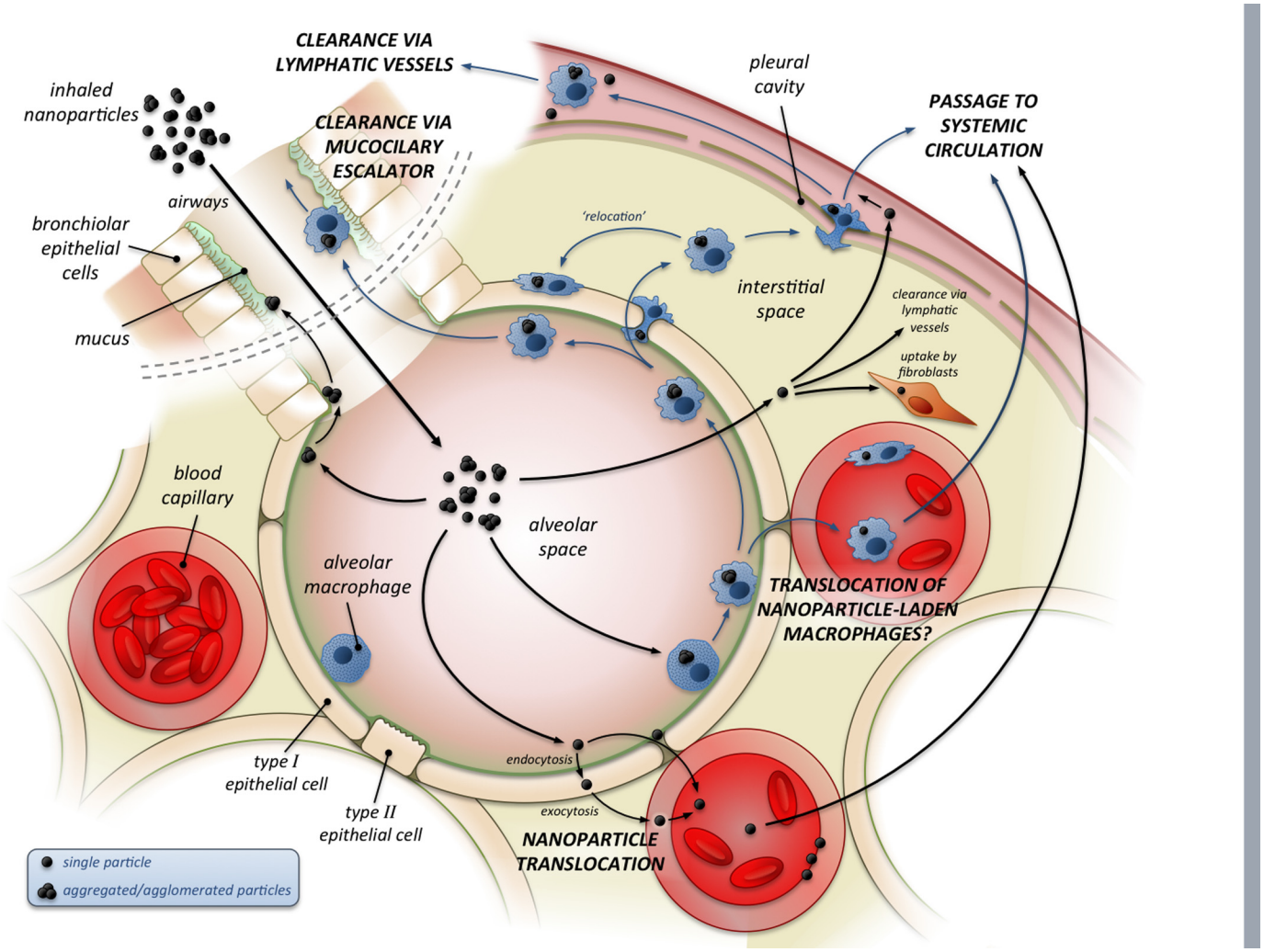

Figure 5. Exposure to nanomaterials (NMs) via the lungs results in rapid transport into the epithelium and interstitial spaces and long-term retention as a result of substantial endocytosis by epithelial cells (Type I and Type II) and limited initial phagocytosis by alveolar macrophages. Pathways exist for the transport of inhaled NMs into the alveolar epithelium and interstitium of rodent lung and further across the endothelial vascular membrane of blood circulation as well as into the lymphatic drainage system. Some evidence suggests that a predominant route of clearance from the lung tissue is then via reentrainment back onto the alveolar epithelial surface (via an unknown mechanism) for long-term macrophage-mediated transport toward ciliated airways and the larynx. Nanosized NMs may cross the epithelium, whereas larger aggregates/agglomerates are likely to be phagocytosed by alveolar macrophages.

interaction with cellular targets, causing changes in the intracellular redox status (Hussain et al. 2009).

However, studies with NMs suggest that the mechanisms of toxicity may be more diverse than via oxidants, including direct physical NM–cell interaction, receptor-mediated, or other unknown mechanisms (the last of which is a priority research gap) (Thomassen et al. 2011). Increased epidermal growth factor receptor expression and phosphorylation have also been observed for DEPs (Pourazar et al. 2008).

Lesson 13. The ability of PM, UFPs, and NMs to generate ROS and to induce oxidative stress, either intrinsically or via cellular sources, has been well documented and is frequently associated with mechanisms of toxicity. In addition, both NM and DEP studies have demonstrated receptor activation, and NM research has also identified other potential mechanisms such as direct physical cell interaction and unknown mechanisms that require further investigation.

Lesson 14. For NMs and UFPs that generate ROS, the amount of ROS production is likely to be associated with their physical and chemical properties. This is important for UFPs because different toxicities could result as compositions vary with time and location.

The induction of oxidative stress by NMs and by PM has been linked to proinflammatory intracellular signaling responses and cytokine production (Baulig et al. 2009; Brown et al. 2004a,b) as well as to cytoprotective intracellular molecules such as heat shock protein 70 (HSP70) (Xin et al. 2015) and nuclear factor (erythroid-derived 2)-like 2 transcription factor (Nrf2) (Brown et al. 2010b). Inflammatory cells are crucial to clearance; however, excessive inflammation can lead to exacerbation of preexisting diseases (e.g., asthma, cardiovascular disease) (Donaldson et al. 2000) or to increases in the incidence of autoimmune, allergic, and other immune-related diseases (Hussain et al. 2012). Evidence exists that environmental PM and DEPs can interact with allergens to act as an adjuvant, leading to allergic sensitization (Alessandrini et al. 2009; Hussain et al. 2011; Li et al. 2008). Although such observations have also been made for some NMs (e.g., TiO_2) (Larsen et al. 2010), the mechanism of NM–allergen interaction cannot be related to the particle size alone; instead, other physical and chemical factors such as surface reactivity and chemistry play a role (Smulders et al. 2015).

Lesson 15. The proinflammatory effects of UFPs and NMs may exacerbate existing disease and increase the incidence of

other immune-related diseases. These effects are likely to be related to multiple physicochemical characteristics of the particles.

The relationship between an NM's physicochemical characteristics and the observed responses is a key research endeavor in nanotoxicology. Quantitative structure–activity relationship (QSAR) models have been developed to identify these key characteristics (Puzin et al.). The roles of some dose metrics such as particle surface area, solubility (and the ability to release ions), and aspect ratio have already been confirmed (Brown et al. 2001; Duffin et al. 2002, 2007; Johnston et al. 2013; Kermanizadeh et al. 2016; Oberdörster et al. 1994; Poland et al. 2008; Prach et al. 2013; Schinwald et al. 2012). QSAR research is an important component of nanotoxicology (MODENA MPNS COST 2013). Because PM is a complex mixture, a comprehensive characterization of PM samples is needed, and QSAR methods can be used to determine which physicochemical characteristics of PM drive the observed adverse responses. Thus, the QSAR modeling approach currently being developed for nanotoxicology can also be relevant to air pollution research.

Lesson 16. There is now an opportunity to review in detail this rather large mechanistic body of research to look for synergies and differences between NM and UFP modes of action and to relate them to physicochemical characteristics. Clarifying the relationships between these mechanistic end points and the physicochemical characteristics of the NMs or UFPs will be essential in the further development of QSAR- and modeling-type approaches.

In the last decade, NM surface–biomolecule (proteins, lipids, etc.) corona interactions have been characterized in different media and body fluids to investigate their actions and the fate of NMs should they enter the circulation. These interactions, when concerning intracellular proteins, can alter the effects of NMs as has already been shown for xenobiotic-metabolizing enzymes (Sanfins et al. 2011). In addition, results suggest that the NM properties can influence the composition of the corona and that its composition changes over time and with passage through different tissue and subcellular compartments (Wang et al. 2013).

Lesson 17. Our understanding of the composition of the molecular corona for NMs can be applied to UFPs because it is likely to influence their uptake, fate, and effects within the body.

The acute-phase response has been proposed as a mechanism of particle-induced cardiovascular disease. The acute-phase response is a general alarm response of the body to various assaults including bacterial and viral infections, trauma, and so on. The most widely studied acute-phase protein is C-reactive protein (CRP), the serum levels of which are associated with risk of cardiovascular disease in prospective epidemiological studies (Ridker et al. 2000). Serum Amyloid A (SAA) may also play a causal role in cardiovascular risk by promoting plaque progression and atherosclerosis.

Inhalation of TiO_2 nanoparticles has been shown to cause upregulation of the *Serum Amyloid A3 (Saa3)* gene in the lung (Halappanavar et al. 2011). Similarly, inhalation and intratracheal instillation of NMs and carbon nanotubes also increased expression of *Saa3* (Saber et al. 2013, 2014). Lung *Saa3* mRNA levels were shown to correlate with deposited surface area of carbon black and TiO_2 NMs and with neutrophil influx into bronchioalveolar lavage fluid (Saber et al. 2013, 2014), consistent with SAA being a neutrophil chemoattractant (Badolato et al. 1994). Moreover, *Saa3* mRNA levels in lung tissue were shown to correlate with SAA3 levels in plasma following pulmonary exposure to carbon nanotubes (Poulsen et al. 2015). DEPs have also been shown to induce a pulmonary acute-phase response such as upregulation of C-reactive protein and serum amyloid A (Saber et al. 2014).

Priority research gap. Both PM and NM research studies need to consider a wider array of biological mediators, for example, acute-phase proteins or new carriers of the oxidative signal.

Methodological Considerations

NM research also offers methodological refinements for biological assessment of ambient PM, including means to account for particle-related interference (e.g., light absorbance, fluorescence quenching, protein binding), which may occur in some cellular (Pulskamp et al. 2007; Wörle-Knirsch et al. 2006) and mutagenicity assays (Clift et al. 2013).

Lesson 18. Evidence for the ability of NMs to interfere in various assays means that study designs for NM and UFP research require consideration of control procedures to limit the potential to confound result interpretation.

Over the past decade, there has been a progressive approach toward standardized protocols to provide a better understanding of the biological impact of NMs [the International Alliance for NanoEHS Harmonization, the EU FP7 (Risk Assessment of Engineereed Nanoparticles) ENPRA and NanoTest projects]. These projects have helped in understanding the pitfalls and advantages of the different biochemical test systems used within nanotoxicology (e.g., Guadagnini et al. 2015).

Lesson 19. Standardized protocols for assessing biological responses to NMs, once wholly available, could be applied to both UFPs and PM.

Conclusions and Recommendations

A comparison of the UFP and NM literature has identified at least 19 immediate unifying lessons, as well as a number of areas where further research is needed to better understand both fields of research. In fact, in this review, we identified that UFP and NM toxicology are not two distinct fields; rather, they overlap extensively with the potential to extrapolate from one to the other in many respects. Firstly, ambient PM research provided evidence of potential health impacts for UFPs, and NM toxicology has largely provided essential evidence of the mechanistic plausibility of these health effects. PM research provides indications of, at least in part, the potential disease effects to consider, and early initial human health studies involving workers suggest this may also be true for other materials; however, more work is required to confirm this hypothesis. It seems safe to conclude that UFPs and NMs share the same general biological mechanisms of adverse effects, such as oxidative stress and inflammation. However, NM toxicology has also provided a significantly better understanding of the role of physicochemical characteristics of particles regarding their toxicity, including factors in addition to size and surface area, such as solubility, charge, composition, coating, and agglomeration/aggregation. This information is important because it means that not all NMs are created equal in terms of their toxic potential, and likewise, not all ambient PM or UFPs have the same potential to induce health effects. Although more work could be performed to compare the mechanism of toxicity of UFPs with that of NMs, a more effective use of resources might be to translate the techniques for physicochemical characterization into the PM field to better enable identification of PM sources that are responsible for health effects, allowing their more effective management. Integration of both fields of research will provide greater potential for justification, interpretation, and application of the wealth of important knowledge that has been gathered over the last few decades.

Appendix I. Physicochemical characteristics of ambient ultrafine particles (UFPs) and engineered nanomaterials (NMs).

Ambient ultrafine particles

- Ambient air particulate matter (PM) composition is complex, including coarse (2.5–10 μm), fine (<2.5 μm), and UF (<100 nm) particles.
- Urban UFPs derive mainly from combustion processes (e.g., traffic) and subsequent particle nucleation, coagulation, and vapor condensation.
- Urban UFPs often contain transition metals or organic chemicals, that is to say, complex composition (See Figure 2).
- A mixture of insoluble and soluble particles and droplets may lead to the release of several constituents from one particle in the lung.
- Size distribution, particle morphology, chemical composition, and concentration vary over time and place.
- Although relatively large in terms of number, UFPs contribute relatively little to the mass of PM compared with coarse particles.
- Controlled exposures are impeded by temporal variability, which complicates mechanistic studies.
- UFPs are always surrounded by gaseous pollutants.

Nanomaterials

- A number of definitions exist that usually stipulate at least one dimension is in the nanoscale (1–100 nm). Many NMs have three dimensions in the nanoscale, making them nanoparticles.
- NMs are often referred to as engineered or manufactured because they are designed and generated for a specific purpose.
- NMs are made in a wide variety of chemistries, consisting of single elements (e.g., carbon, metals), compounds (e.g., metal oxides, salts), or complex composites (e.g., core-plus-shell structure).
- NMs can vary significantly in particle morphology and chemical composition but are well defined at production and close to production levels.
- Spatial and temporal variance in airborne concentration may vary significantly.
- Controlled exposures are possible, enabling detailed mechanistic studies.
- NMs can be handled in a standardized manner, facilitating studies of defined properties.

Editor's Note: In the Advance Publication of this article, João Paulo Teixeira was missing from the author list. The author and his affiliation have been added in this version of the article.

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