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Short running title: Comparing nanomaterial and ultrafine particle toxicology

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Abstract

Background: A rich literature exists that has demonstrated adverse human health effects following exposure to ambient air particulate matter (PM), with strong support for an important role for ultrafine (nano-sized) particles. At present, relatively little human health or epidemiology data exists for engineered nanomaterials (NM) despite clear parallels in their physicochemical properties and biological actions in *in vitro* models.

Objectives: NMs are available in a range of physicochemical characteristics which allow 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 NM, while the study of NM provides an opportunity to facilitate the understanding of the mechanism of toxicity of UFP.

Methods: A workshop of experts systematically analysed the information available 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 where additional research prioritisation 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.

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 years, it has become firmly 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 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. National Nanotechnology Initiative in the US and the Interagency Working Group on Nanotechnology), with risk assessment and regulation (e.g. EU NanoSafety Cluster and associated projects such as NANoREG). Nanotoxicology, the study of the toxicity of nano-scale materials, has advanced in line with nanotechnology in terms of the amount of literature being published. Indeed, unlike 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 PM in air pollution (Figure 1). 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 project hosted a workshop in order to exchange and merge knowledge in PM and nanoparticle toxicology. The following outlines the systematic comparison of these overlapping research fields, and identifies lessons (in boxes) for advanced understanding as well as *priority research gaps* (in italics) that must be addressed.

What can be learnt 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 smaller than 10 micrometers in aerodynamic diameter (PM_{10}) can be inhaled by humans and deposit in the respiratory tract (ICRP-66 1994)(Appendix I), with smaller particles having higher fractional deposition in the alveoli. Consequently, ambient PM is frequently regulated as PM_{10} and $PM_{2.5}$ (smaller than 2.5 micrometers aerodynamic diameter), the latter of which reflects the fine fraction of PM_{10}. The composition of PM is complex and variable (Appendix I). Although not contributing substantially to the (regulated) mass, UFP have also been identified as one component responsible for the adverse health effects observed at typical outdoor levels. Evidence also exists for an involvement of other components in the toxicity, such as metals (Frampton et al. 1999; Jimenez 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 hypothesised 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 TiO_2 particles (20 nm) were more likely to cross the lung barrier and induce inflammation than larger TiO_2 particles (250 nm) (Ferin et al. 1992; Oberdorster et al. 1994). The hypothesis was soon after supported by epidemiological evidence (Peters et al. 1997). Due 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 UFP, 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, NM are made deliberately at the nano-scale because they exhibit properties that provide technological advantages compared to the bulk form of the same material (The Royal Society 2004) Appendix I). For example, elemental (graphitic) carbon has semi-conductor properties at the nano-scale (e.g. carbon nanotubes). This expands the number of possible products and applications, offering great opportunities and economic gains. While UFP and NM are often derived from very different sources and processes, their physicochemical characteristics can overlap (Appendix I), suggesting that their properties, behaviours and, importantly, 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 recognised the potential risks (e.g. SCENIHR 2005; The Royal Society 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, especially 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 rehospitalisation in patients with prior myocardial infarction (Von Klot et al. 2005).

For hospitalisation 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, raised 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 or $PM_{2.5}$ 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 IL-6 and 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 UFP. For example, field studies have observed associations between UFP and carbon with reductions in lung function among asthmatics (McCreanor et al. 2007), while asthmatic and healthy adolescents in New York exhibited an increase in indicators of inflammation (Patel et al. 2013). The majority of preclinical and clinical studies on UFP have been conducted with diesel exhaust and diesel exhaust particles (DEP), an especially rich

source of UFP. 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 UFP modify numerous aspects of cardiac function, e.g., reduced 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, UFP in urban air (Weichenthal 2012) or diesel engine emissions (Mills et al. 2007) exacerbate myocardial ischaemia (Cascio et al. 2007; Robertson et al. 2014). Blood vessels finely regulate blood flow through changes in the tone of vascular smooth muscle, and UFP generally alter the balance in favour of constriction (Moller et al. 2011). The resulting increased blood pressure (Bartoli et al. 2009) and reduced ability of arteries to relax are usually detrimental. Vascular dysfunction can be caused by loss of mediators such as nitric oxide released by the vascular endothelium (Courtois et al. 2008; Miller et al. 2009; Moller et al. 2011), increased sensitivity to vasoconstrictor factors (Langrish et al. 2009), and alterations in baroreceptor/neuroregulatory feedback (Rhoden et al. 2005; Robertson et al. 2012). Blood components are also dysregulated, with UFP tending to increase blood coagulability (Kilinç et al. 2011; Nemmar et al. 2004), encouraging platelet activation (Cascio et al. 2007; Lucking et al. 2011) and reducing 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 UFP in animal models (Araujo et al. 2008; Miller et al. 2013) has been shown to worsen atherosclerotic vessels disease.

Other target organs

Although research has predominantly focused upon the inhalation of UFP 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 UFP to the blood-stream allowing for non-specific interaction with other essential organs such as the brain and kidneys. UFP exposure and mucociliary clearance from 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 about 15 years 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, cognitive and behavioural changes in humans and animals (Calderón-Garcidueñas et al. 1999; Calderón-Garcidueñas et al. 2011). Other studies using a myriad of PM collection techniques upheld the early findings related to PM induced brain-centric inflammatory processes, including 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, nose-brain transport following olfactory mucosa deposition (Balasubramanian et al. 2013; Elder et al. 2006), or via the spill-over of systemic inflammation to the CNS; a combination of these processes is also possible. While 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 behaviour and cognition. For example, Fonken et al. 2011 showed that mice exposed to $PM_{2.5}$ (which includes UFP) had deficits in spatial learning and memory. Using mice

exposed to concentrated UFP as neonates, (Allen et al. 2014a; Allen et al. 2014b) showed that males had behavioural 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 children (Freire et al. 2010; Suglia et al. 2008). Two US-based case-control studies have also reported increased odds ratio 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. *PM research therefore provides a basis to develop a strategy to identify potential neurological effects of NM in which physicochemical characteristics could be responsible.*

Epidemiological studies have also related $PM_{2.5}$ and PM_{10} air pollution to reproductive toxicity and 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, pre-term birth and small-for-gestational-age birth. van Rossem et al. 2015 found that maternal exposure to $PM_{2.5}$ and black carbon were associated with increased blood pressure in the new-born child. The effect seems to be mediated by altered placental vascular structure induced by $PM_{2.5}$ (Veras et al. 2008). Preclinical studies indicate that adverse health effects of UFP exposures cannot be excluded, though 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 respiratory, cardiovascular, neurological and reproductive adverse effects. For cardiovascular studies this extends to evidence for UFP. Direct evidence for the role of UFP in the induction of the other disease targets is in general is still lacking.

Investigation of NM impacts on human health

A few studies are now emerging that demonstrate effects of nanomaterial's on human health, especially in an occupational setting. For multiwalled carbon nanotubes, Lee et al (2015) investigated workers manufacturing this material and found that while there was no impact on haematology and blood biochemistry, they did see an increase in a range of markers of lipid peroxidation in exhaled breath condensates of workers, including malondialdehyde, 4-hydroxy-2-hexenal and n-hexanal. Multiwalled carbon nanotubes have also been reported to impact on a range of endpoints in workers exposed for at least six months. These endpoints include the targeting of genes associated with the 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 carcinogenic outcomes in humans.

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

A study by Liou et al (2015) reviewed 15 studies that have investigated the effects of engineered nanomaterial's on workers. Of these 15 studies, 11 were cross-sectional, 4 were longitudinal and 1 was a descriptive pilot study. For the 11 cross-sectional studies all of them showed a positive relationship between various biomarkers and the exposure to engineered nanomaterials. For the longitudinal studies 3 of the 4 studies demonstrated a negative

relationship, with the fourth providing a positive relationship after one year follow-up. In general the exposure levels identified were not very high compared to those used in human inhalation chamber studies, however the were some exceptions with higher exposures. The studies in general were found to be limited by small numbers of participants, a lack of consistent exposure information, the detection of generally low exposures and finally 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 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 suggest an association between exposure to nanomaterial's and human health, relatively little clinical or epidemiology data exists, to date.

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 endpoints.

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 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 the particles) stimulate alveolar sensory receptors (Ghelfi et al. 2010; Hazari et al. 2011; Robertson et al. 2014), providing a signal to the central nervous system. This manifests through alterations in autonomic nervous system activity, which directly regulates cardiac function, and, indirectly, other aspects of the cardiovascular system (Pope Iii et al. 1999; Rhoden et al. 2005). The identification of the UFP fraction of PM paved the way for a third hypothesis: that the minute size of UFP allows them to translocate across the thin alveolar-capillary wall (by an as-yet undetermined mechanisms) and enter the circulation themselves to directly affect cardiovascular function (Nemmar et al. 2001; Oberdorster et al. 2002).

There is a wealth of evidence for and against each of these theories, but in truth all three are likely to occur, with the contribution of each dependent on the physicochemical properties of the UFP, the cardiovascular endpoint 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*. Intricacies of these processes may encompass non-classical inflammatory/oxidative biological mediators such as acute phase proteins (Saber et al. 2014) or oxidised phospholipids (Kampfrath et al. 2011); the release and accumulation of chemical particle surfaces and constituents within biological compartments (Murphy Jr 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 (Schaeffler et al. 2014). Reports are rapidly emerging from preclinical models that demonstrate similar cardiovascular effects for NM to that shown for UFP, e.g. altered autonomic function (Harder et al. 2005), impaired vasodilatation (Leblanc et al. 2010; Moller 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 elucidated 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 NM.

Genotoxicity/carcinogenicity

Markers of genotoxicity, such as elevated levels of oxidized DNA nucleobases, bulky DNA adducts and clastogenic endpoints 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 animal experimental models (Møller et al. 2014), as well as a lack of studies on neoplastic lesions in the respiratory tract*. Studies including exposure to traffic-related outdoor air pollution or DEP (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 (PAH), 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 latter mechanism has been discussed as a major mechanism contributing to the mutagenic and carcinogenic properties of DEP, as well as poorly soluble

nanoparticles like 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*. Recent reviews on comparisons of genotoxicity between DEP and NM 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 revealed that single- and multi-walled 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 UFP in the genotoxicity, mutagenicity and carcinogenicity of PM.

Lessons learnt from NM toxicology that could be applied to PM

Regulatory standards for ambient PM are promulgated from a rich literature that has demonstrated adverse human health effects following exposure. Conversely, the toxicological research with NM 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 NM themselves while recognizing that toxicological assessment is only one part of an overall risk assessment process. On the other hand, it is impossible to determine safer exposure levels and safer material 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 UFP has focused on mass and number concentrations, chemical composition and size distribution. For NM characterization, studies initially used existing methods obtained from PM and material science research, but over time, the methodology has been refined. This 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 due 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 potentially be beneficial for understanding mechanisms as well as enhancing comparability across fields. In future, these techniques provide an opportunity for improved monitoring of PM.

Lesson 4: A range of particle characteristics have been shown to influence their toxicity. These 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 NM.

Prior to 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 Standardisation (CEN) and International Organization for Standardization (ISO) (EC Mandate M461 to CEN).

Especially relevant particle parameters protocols for standardization are that 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 (EU, 2011 http://ec.europa.eu/environment/chemicals/nanotech/faq/definition_en.htm). Advances in dynamic light scattering and coupling to inductively coupled plasma mass spectroscopy (ICPMS) technologies such as 'field flow fractionation multi-angle light scattering ICPMS' or 'single particle ICPMS' have been driven by requirements for particle sizing and behaviour in liquid dispersions. For airborne particles, great improvements in understanding the applicability of different measurement devices has also been reached. This includes knowledge of the care needed in using and interpreting charge based instruments, especially instruments using unipolar charging such as surface area monitors and 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 with ca. >200 nm are present in the aerosol (Todea et al. 2015).

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

New developments also include procedures to identify and quantify specific surface coatings/functionalization of NM using combinations of Differential Thermal Gravimetric/ Differential Thermal Analysis - Gas Chromatography and chemical specific methods such High Performance Liquid Chromatograph – Mass Spectroscopy/Optical Emission Spectroscopy or Gas Chromatography – Mass Spectroscopy. Combinations of these methods

are particularly important for 2nd and 3rd generation NM analysis and methods have recently been developed as part of the EU FP7 NANoREG project (http://www.nanoreg.eu/images/2015_12_03_NANoREG_Factsheet_D2.4.pdf). Further knowledge transfer of techniques between material 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 straight-forward in simple systems (e.g. a pure NM in pH-controlled water with moderate ionic strengths), but becomes challenging in multi-component complex systems such as PM in air pollution. From a toxicological perspective, since zeta-potential significantly varies due to pH and composition of the test medium, a full assessment should consider all likely mediums and biological compartments of interest.

Particle reactivity is currently not well-defined, perhaps understandably considering this 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 NM (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 designing and harmonization of such experiment to achieve comparable results (Tantra et al. 2015). 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 NM have been developed. These 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 determines 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 toxicity, whereas ambient UFP can be dispersed to some extent without these additives (Moore et al. 2015).

The improved characterization knowledge has revealed the need for correct storage of NM test items over time. For NM in the 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 both dry powders, filter-bound particles and wet suspension under many different conditions. For many ambient PM samples, storage under argon at $<0^{o}C$ 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, due to the influence of the surrounding media (e.g. surfactant proteins). Such biomolecule interaction is likely to impact the 'fate' of the particles by modifying the surface properties, the behaviour of the particle (e.g. agglomeration, solubility, bioavailability, biodurability) and the adsorbed protein properties (Brown et al. 2010a; Deng et al. 2011). This could, in turn, alter how a

particle is taken up into cells, triggers signalling pathways, and in what physicochemical format they are translocated between cells and to distal organs.

Characterization of NM 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 NM may be different than occupational exposure depending on the state of the material (e.g. native NM, embedded in a product, degradation and disposal). For toxicology studies, many NM 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 mediums (Hartman et al., 2015). But first attempts have been also made to establish harmonized dispersion for regulatory testing (e.g. Jensen et al. 2014). In contrast, harmonized dis several persion protocols have largely been lacking in PM research.

Lesson 6: Harmonised 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 (i.e from collections) are normally available compared to 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 chemical extraction from a filter), which may alter the state of the PM prior to its use in toxicology studies. Transformation and loss of toxicologically important semi-volatile compounds during sampling is also an issue that must be taken into account when testing collected PM.

Lesson 7: This 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 which help to ensure sufficient dose 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-66 1994)), however, *the regional deposition of NM is weakly supported by direct experimental data to validate the models.*

The deposition of NMs depends on three sets of parameters: particle dynamics, lung geometry and gas flow dynamics. *Due to their size, the primary region for the deposition of NM is the alveolar region* of the human lung, which means that the first biological matrix encountered is lung surfactant (Gasser et al. 2010). The interaction with NM may alter the structure and function of the surfactant proteins t (Beck-Broichsitter et al. 2014; Valle et al. 2015), and subsequently influence the specific mammalian cell interaction (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: 10 μg/m³ for $PM_{2.5}$). A daily inhaled volume for a moderately active adult human (75 kg) is typically 20 m³/day. If the mean deposited fraction is 0.3 (Price et al. 2001), the suggested daily mass dose would be 60

μg/day. However, PM mass is often dominated by coarse particles, so only a fraction of the 60 μg/day would actually represent UFP.

Therefore dose might better be expressed as particle number for such small particles, due to their low mass. There is a large variability in ambient UFP numbers, ranging from 500-10,000 in rural areas, to 7500- 25,000 particles/cm³ in urban background (Putaud et al. 2010), and with a European mean concentration at 31,500 particles/cm³ at hot spots (busy streets). To estimate *in vivo* exposure conditions based on particle number concentrations, a healthy adult breathing at moderate exercise in ambient air with an assumed moderate number concentration of 30,000 particles/cm³ (of which 80-90% of the particle count is assumed to be UFP) will inhale 6×10^{11} particles/cm³. Assuming a mean deposition probability for UFP of 0.5, this corresponds to a particle number 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 studies using realistic doses in relation to current knowledge concerning the occupational and ambient exposures of UFP or 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 at 31,500 particles/cm³, suggest that on average 8 nano-scale particles deposit per day per cell of the alveolar epithelial surface (Geiser and Kreyling 2010). According to limits caused by thermodynamic conditions, the highest possible NM aerosol number concentration is around 10^6/cm^3, which relates to 700 particles/d/cell or 30 particles/h/cell of the alveolar epithelium. As mass is a

more frequently used metric, these numbers have to be converted using the effective density of the particles. This information is useful when verifying relevant *in vitro* doses. Models have now been published (including experimental verification) which 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 NM, and the effective density of NM, as important factors in the modelling of cellular dose. In essence, only sub-micrometer and larger NM agglomerates will deposit within one hour, while NM less than 100 nm may remain suspended for more than 24 hours.

Lesson 9: The models of *in vitro* NM deposition should be more widely used for estimation of NM dose in cultured cells in order to refine models so that they better reflect anticipated airborne exposures. Their application to PM would be more difficult due to the size and density diversity of such a mixed particle sample. However, such dosimetry models (eg., DeLoid et al 2015) can in principle deal with size distribution data as well as mean size data, in cases where such distribution data is 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

Using rodent models, within 1-2 days of exposure, NM clearance is relatively low compared to micrometer-sized particles, and is associated with a 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 does not reflect well clearance in humans. For humans, there is evidence that both NM 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 of rodents (Kreyling 2013). *For the human distal regions of the lungs, neither macrophage-mediated long-term clearance kinetics data nor translocation data of NM into the circulation are currently available.*

Particle clearance from the lungs can also occur via transport towards 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.

Early pioneering studies in the 1990's first demonstrated detectable translocation of NM TiO_2 particles into the lung interstitium, while translocation is lower for larger particles (e.g. 21 nm *vs.* 250 nm diameter) (Ferin et al. 1991; Oberdorster 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 NM 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, primary 7 nm gold particles translocating more than the primary 20 nm gold

particles, suggesting deagglomeration of the 45 nm agglomerates in the lung. *Based on the observations described in the previous section, that in humans NM retention in the lung is likely to be longer than for 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 NM can reach and accumulate in secondary organs (Geiser and Kreyling 2010; Kreyling 2013). Quantitative biokinetics analysis of NM applied via the lungs of rodents, demonstrated small fractions of NM (iridium, carbon, gold, TiO_2) in all secondary organs studied, including the brain, heart, and even in the foetus (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 1h 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 total dose that reach such 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 impacts biodistribution (Kreyling et al. 2014). As a corollary to biodistribution studies, *in vitro* studies with NM have helped to define the ability of particles to breach cellular membranes (Bachler et al. 2015), interactions with subcellular structures, and, therefore, toxicological mechanisms related to particle uptake.

Lesson 11: NM translocation studies provide clear evidence of the potential for UFP to translocate from the lung surface into blood and to distribute around 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 UFP in the body.

In addition to translocation, rodent studies also suggest that NM 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 μg per lung) such as titanium dioxide, carbon black, gold, quantum dots, silver nanowires and CNT have identified accumulation of NMs in the lung-associated lymph nodes (Schinwald et al. 2012). *The development of this research area using different types of NM 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 NP in rodent lungs are schematically sketched in Figure 5.

Lesson 12: The differential clearance and uptake by NM 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 UFP and NM 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 oxidant species (ROS), oxidative stress (Miller 2014; Nel et al. 2006; Stone et al. 2007) feature widely in the literature for both NM and UFP (see below*)*. Endothelial cells and epithelial cells may also generate nitric oxide in response to NM and UFP 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), DEP (Miller et al. 2009) and many different NM 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) with an inherent ability to generate ROS, to amorphous nanosilica which exhibits no intrinsic capacity to generate oxidants (Napierska et al. 2012). Some NM do not exhibit intrinsic oxidant generating capacity but will generate ROS upon interaction with cellular targets causing changes in the intracellular redox status (Hussain et al. 2009).

However, studies with NM 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* (Thomassen et al. 2011). Increased epidermal growth factor receptor expression and phosphorylation have also been observed for DEP (Pourazar et al. 2008).

Lesson 13. The ability of PM, UFP and NM to generate reactive oxygen species (ROS) and to induce oxidative stress, intrinsically or via cellular sources, has been well documented and is frequently associated with mechanisms of toxicity. In addition, both NM and DEP studies demonstrate receptor activation, while NM research also identifies other potential mechanisms such as direct physical cell interaction, and unknown mechanisms which require further investigation.

Lesson 14. For NM and UFP which generate ROS, the amount of ROS production is likely to be associated with their physical and chemical properties. For UFP this is important, as it could result in different toxicity as the composition varies with time and location.

The induction of oxidative stress by NM and by PM has been linked to pro-inflammatory intracellular signalling responses and cytokine production (Baulig et al. 2009; Brown et al.

2004a and b) as well as 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 pre-existing diseases (e.g. asthma, cardiovascular disease) (Donaldson et al. 2000), or increases in the incidence of autoimmune, allergic and other immune related diseases (Hussain et al. 2012). Evidence exists that environmental PM and DEP 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). While such observations have also been made with 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 pro-inflammatory effects of UFP and NM may exacerbate existing disease and increase the incidence of other immune related diseases. These effects are likely to relate to multiple physicochemical characteristics of the particles.

The relationship between the NM physicochemical characteristics and the observed responses is a key research endeavour in Nanotoxicology. Quantitative Structure Activity Relationship (QSAR) models have been developed to identify these key characteristics (e.g Puzin et al). The role 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; Duffin et al. 2007; Johnston et al. 2013; Kermanizadeh et al. 2016; Oberdorster et al. 1994; Poland et al. 2008; Prach et al. 2013; Schinwald et al. 2012). QSAR research is an important component of Nanotoxicology (www.modena-cost.eu). Since PM is a complex mixture, a comprehensive characterisation 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 modelling 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 these to physicochemical characteristics. The relationship between these mechanistic endpoint and the physicochemical characteristics of the NM or UFP will be essential in the further development of QSAR and modelling type approaches.

In the last decade NM surface-biomolecule (proteins, lipids, etc.) corona interactions have been characterised in different media and body fluids to investigate their actions/fate of NM should they enter the circulation. These interactions, when concerning intracellular proteins, can alter the effects of NM as already 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: Understanding of the composition of the molecular corona for NMs can be applied to UFP as this 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 bacteria and virus infections, trauma, etc.. 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 the upregulation of the gene *Serum Amyloid A3 (Saa3)* in the lung (Halappanavar et al. 2011). Similarly, inhalation and intratracheal instillation of NM and carbon nanotubes also increased expression of *Saa3* (Saber et al. 2013; Saber et al. 2014). Lung Saa3 mRNA levels were shown to correlate with deposited surface area of carbon black and TiO_2 NM and to neutrophil influx into the bronchioalveolar lavage fluid (Saber et al. 2013; Saber et al. 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). Diesel exhaust particles have also been shown to induce a pulmonary acute phase response such as C-reactive protein and serum amyloid A (Saber et al. 2014). *Both PM and NM research studies need to consider a wider array of biological mediators, e.g. 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; Worle-Knirsch et al. 2006) and mutagenicity assays (Clift et al. 2013).

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

Over the past decade, there has been a progressive approach towards standardized protocols to provide a better understanding of the biological impact of NM (e.g. International Alliance towards NanoEHS Harmonisation, EU FP7 projects ENPRA and NanoTest). 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: Standardised protocols for assessing biological responses to NMs, once wholly available, could be applied to both UFP 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 in order to better understand both fields of research. In fact, this review identifies that UFP and NM toxicology are not two distinct fields, but 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 UFP, whilst 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 and other materials, however more work is required to confirm that. It seems safe to conclude that UFP and NM 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 is important because this means that not all NM are created equal in terms of their toxic potential and, likewise, not all

ambient PM or UFP have the same potential to induce health effects. *While more work could be conducted to compare the mechanism of toxicity of UFP with NMs, a more effective use of resources might be to translate the techniques for physicochemical characterization into the PM field in order 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.

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Appendix I. Ambient UFP and engineered NM physicochemical characteristics

Ambient UFP
• Ambient air PM composition is complex, including coarse (2.5-10 um), fine (<2.5 um) and UF (<100 nm) particles. • Urban UFP derive mainly from combustion processes (e.g. traffic) and subsequent particle nucleation, coagulation and vapour condensation. • Urban UFP often contain transition metals or organic chemicals, i.e. complex composition (See Figure 2). • Mixture of insoluble to soluble particles and droplets, possibly leading to the release of several constituents from one particle in lungs. • Vary over time and place in size distribution, particle morphology, chemical composition and concentration. • Although relatively large in terms of number, UFP contribute relatively little to the mass of PM compared to coarse particles. • Controlled exposures are impeded by the temporal variability, which complicates mechanistic studies. • Are always surrounded by gaseous pollutants.

Nanomaterials
• A number of definitions exist which usually stipulate that at least one dimension is in the nano-scale (1-100 nm). Many NM have three dimensions in the nano-sclae, making them nanoparticles. • Often referred to as engineered or manufactured as they are designed and generated for a specific purpose. • Made in a wide variety of chemistries, consisting of single elements (e.g. carbon or metal), compounds (e.g. metal oxides or salts) or complex composites (e.g. core plus shell structure). • 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. • Can be handled in a standardised manner, facilitating studies of defined properties.

Figure Legends

Figure 1. Time line showing the increased interest in PM and NM over the last three decades, highlighting key studies and research trends in both areas. 'Number of references' per year (non-cumulative) based on Pubmed.gov search, without further limits applied.

Figure 2. Schematic providing an example of the complex composition of UFP (e.g. urban 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.

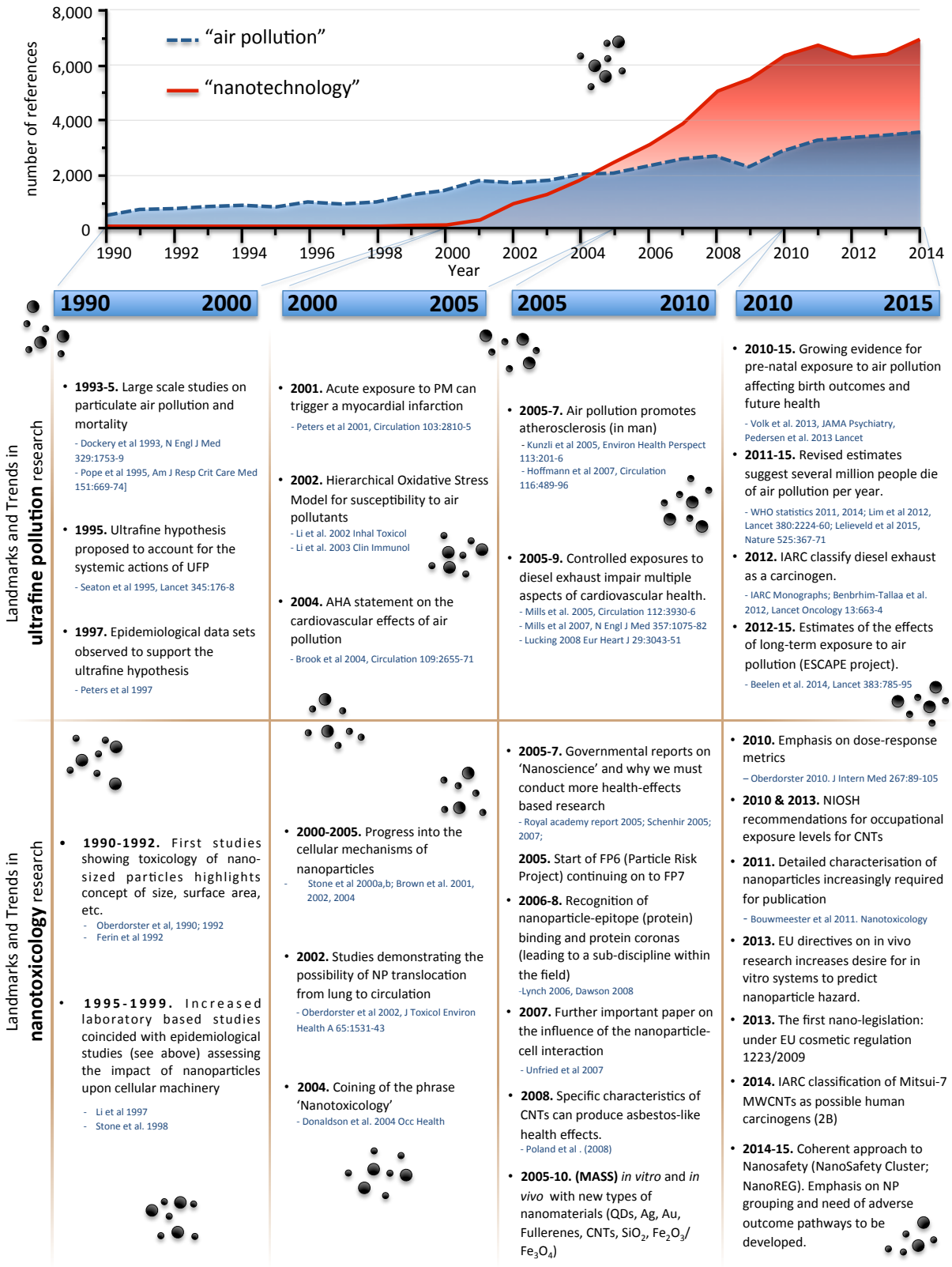
Figure 3. Schematic demonstrating some of the key mechanisms through which inhaled UFP may influence secondary organs and systemic tissues, with emphasis on the means through which inhaled particles may cause cardiovascular events. Note that there are three main pathways linking the pulmonary and cardiovascular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

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

Figure 5. Exposure to NM 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 II) and limited initial phagocytosis by alveolar macrophages. Pathways exist for transport of inhaled NM into the alveolar epithelium and interstitium of the rodent lungs

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 re-entrainment back onto the alveolar epithelial surface (via an unknown mechanism) for long-term macrophage-mediated transport toward ciliated airways and the larynx. Nano-sized NM may cross the epithelium, while larger aggregates/agglomerates are likely to be phagocytosed by alveolar macrophages.

Figure 1.


8,000
6,000
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number of references
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1990
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2010 2015
Landmarks and Trends in ultrafine pollution research
• 1993-5. Large scale studies on particulate air pollution and mortality
- Dockery et al 1993, N Engl J Med 329:1753-9
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• 1995-1999. Increased laboratory based studies coincided with epidemiological studies (see above) assessing the impact of nanoparticles upon cellular machinery
- Li et al 1997
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• 2000-2005. Progress into the cellular mechanisms of nanoparticles
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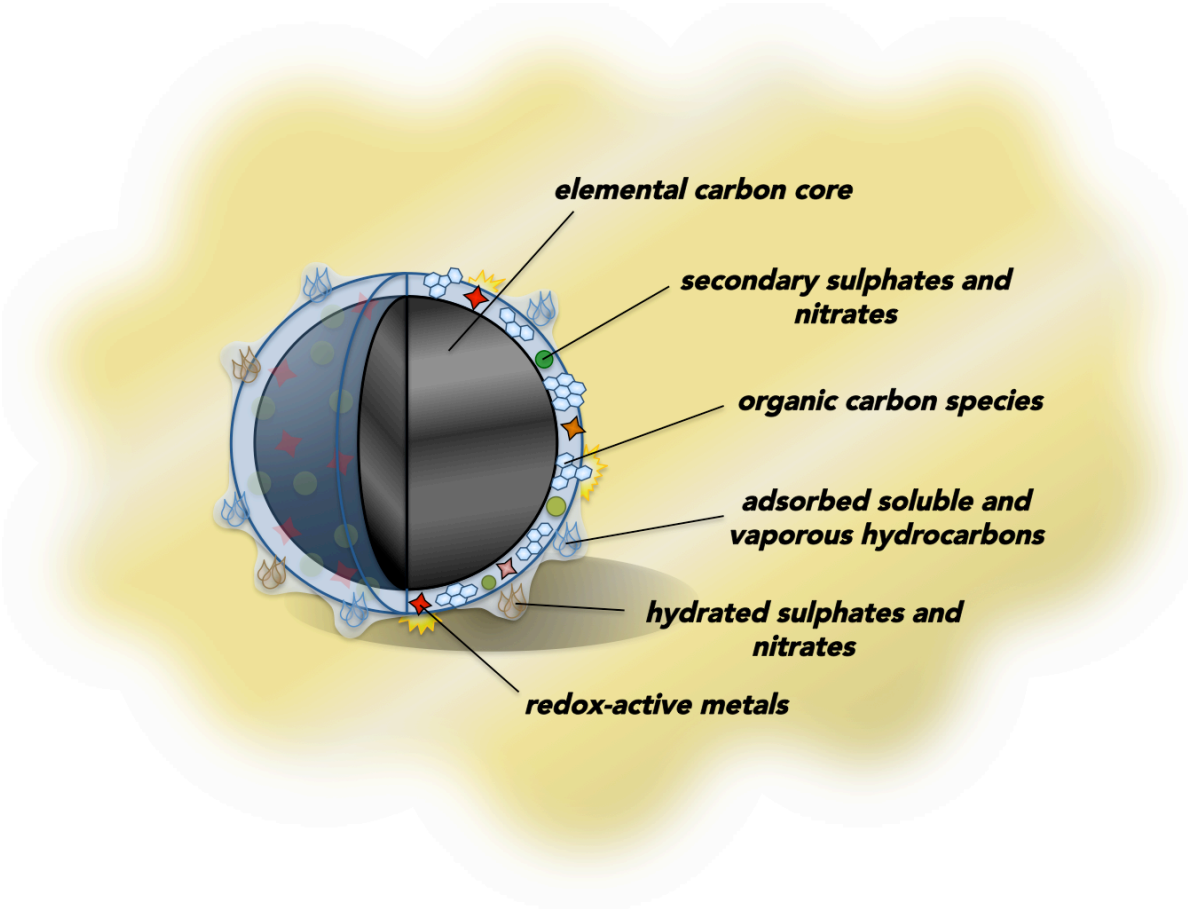
• 2002. Studies demonstrating the possibility of NP translocation from lung to circulation
- Oberdorster et al 2002, J Toxicol Environ Health A 65:1531-43
• 2004. Coining of the phrase 'Nanotoxicology'
- Donaldson et al. 2004 Occ Health
• 2005-7. Governmental reports on 'Nanoscience' and why we must conduct more health-effects based research
- Royal academy report 2005; Schenhir 2005; 2007;
2005. Start of FP6 (Particle Risk Project) continuing on to FP7
• 2006-8. Recognition of nanoparticle-epitope (protein) binding and protein coronas (leading to a sub-discipline within the field)
-Lynch 2006, Dawson 2008
• 2007. Further important paper on the influence of the nanoparticle-cell interaction
- Unfried et al 2007
• 2008. Specific characteristics of CNTs can produce asbestos-like health effects.
- Poland et al . (2008)
• 2005-10. (MASS) in vitro and in vivo with new types of nanomaterials (QDs, Ag, Au, Fullerenes, CNTs, SiO_2, Fe_2O_3/ Fe_3O_4)
• 2010. Emphasis on dose-response metrics
– Oberdorster 2010. J Intern Med 267:89-105
• 2010 & 2013. NIOSH recommendations for occupational exposure levels for CNTs
• 2011. Detailed characterisation of nanoparticles increasingly required for publication
- Bouwmeester et al 2011. Nanotoxicology
• 2013. EU directives on in vivo research increases desire for in vitro systems to predict nanoparticle hazard.
• 2013. The first nano-legislation: under EU cosmetic regulation 1223/2009
• 2014. IARC classification of Mitsui-7 MWCNTs as possible human carcinogens (2B)
• 2014-15. Coherent approach to Nanosafety (NanoSafety Cluster; NanoREG). Emphasis on NP grouping and need of adverse outcome pathways to be developed.

Figure 2.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secondary sulphates and nitrates
organic carbon species
adsorbed soluble and vaporous hydrocarbons
hydrated sulphates and nitrates
redox-active metals

Figure 3.

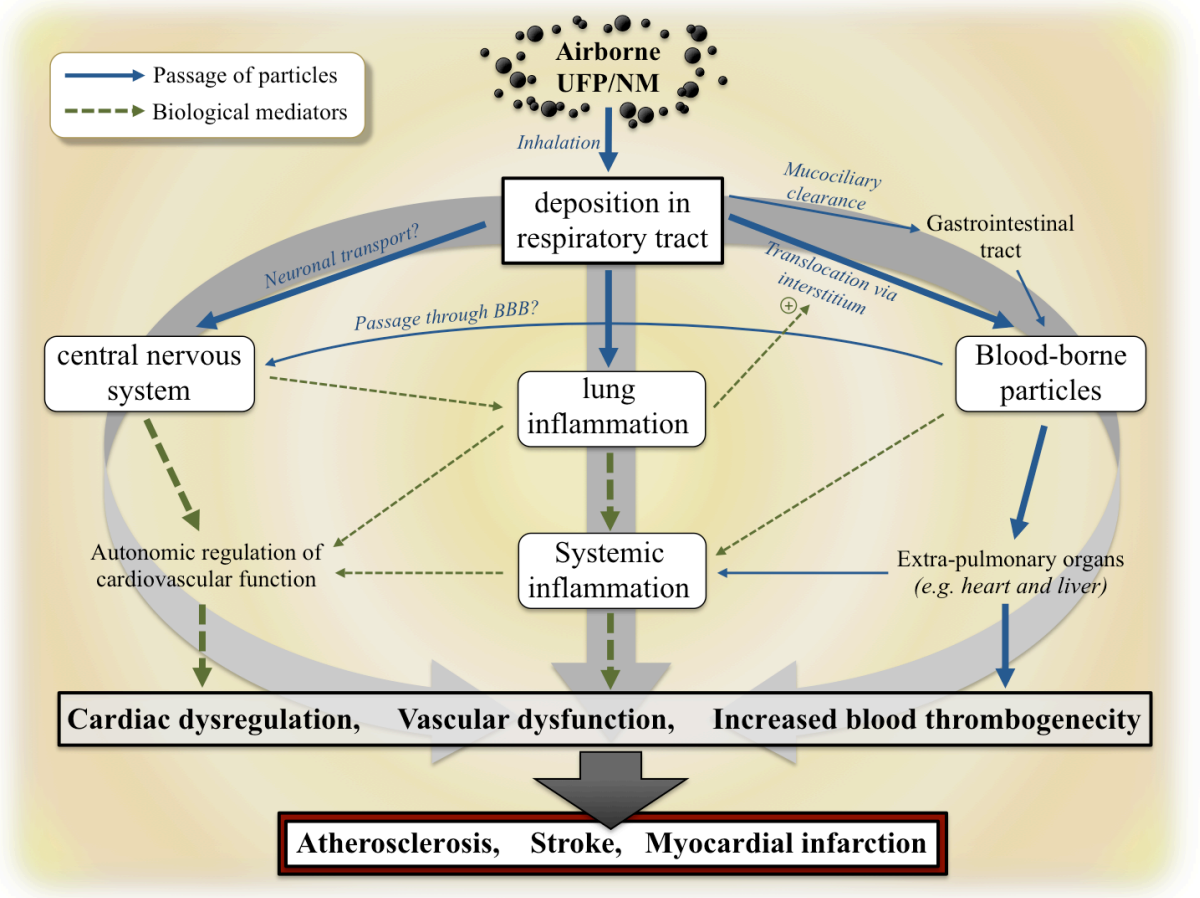

Passage of particles
Biological mediators
Airborne UFP/NM
Inhalation
deposition in respiratory tract
Mucociliary clearance
Gastrointestinal tract
Neuronal transport?
Translocation via interstitium
Passage through BBB?
central nervous system
lung inflammation
Blood-borne particles
Autonomic regulation of cardiovascular function
Systemic inflammation
Extra-pulmonary organs (e.g. heart and liver)
Cardiac dysregulation, Vascular dysfunction, Increased blood thrombogenecity
Atherosclerosis, Stroke, Myocardial infarction

Figure 4.

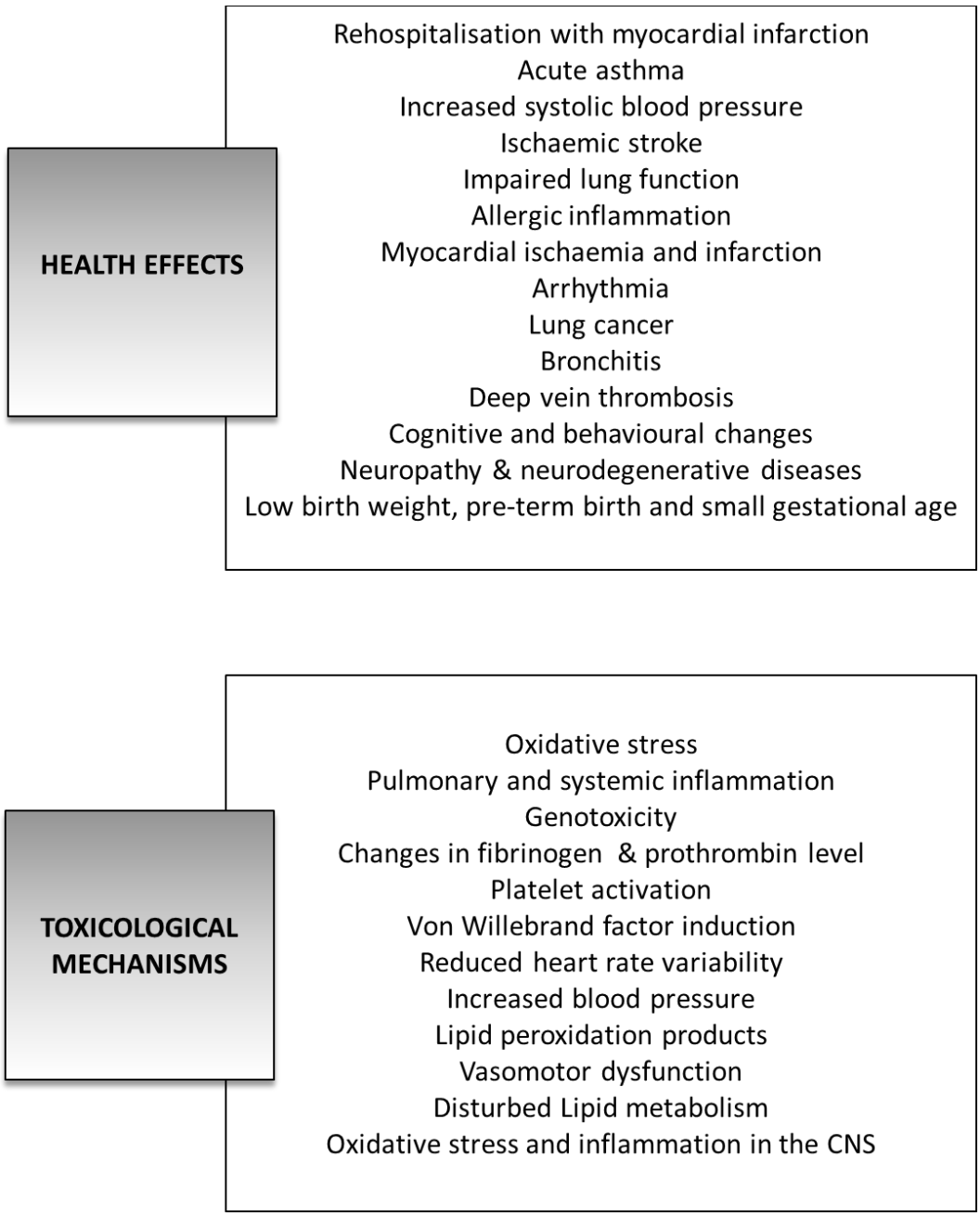
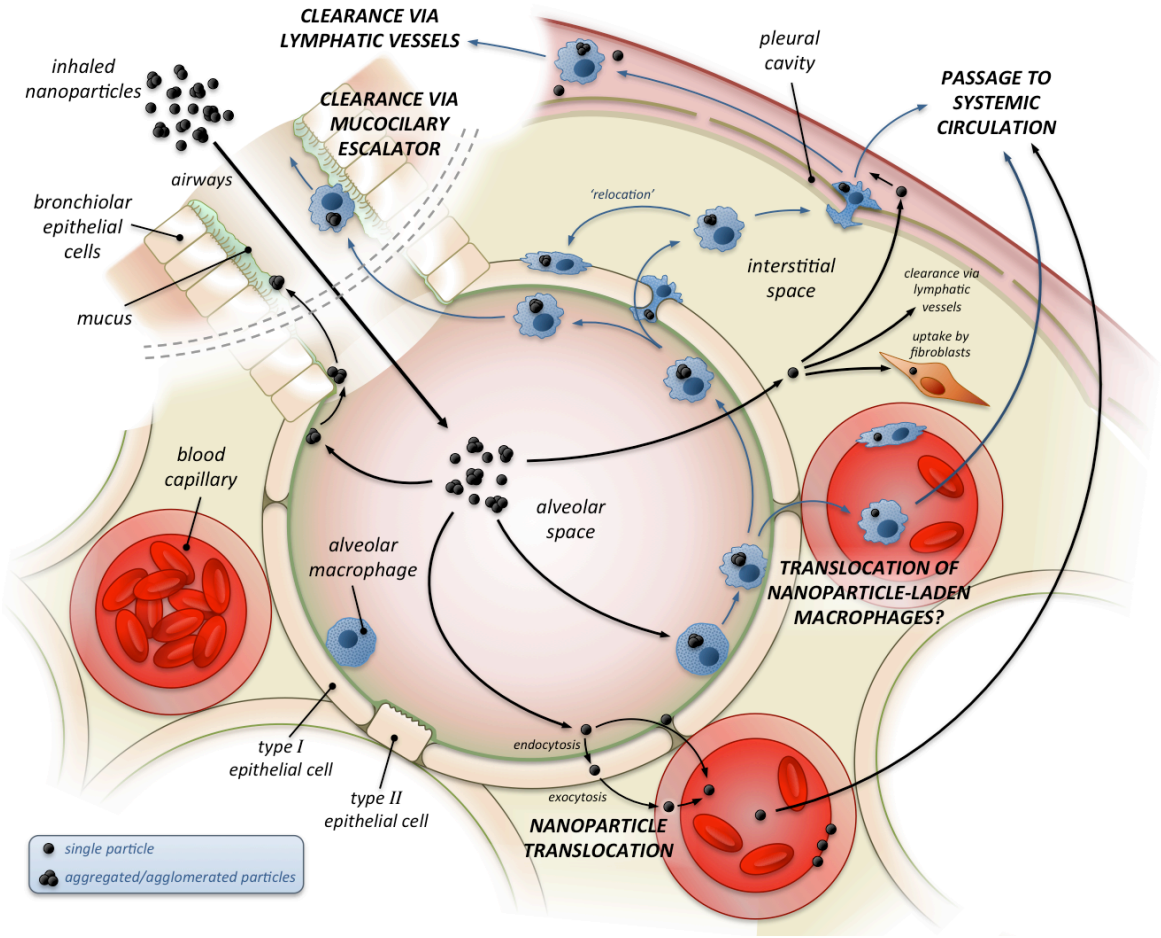

HEALTH EFFECTS
Rehospitalisation with myocardial infarction
Acute asthma
Increased systolic blood pressure
Ischaemic stroke
Impaired lung function
Allergic inflammation
Myocardial ischaemia and infarction
Arrhythmia
Lung cancer
Bronchitis
Deep vein thrombosis
Cognitive and behavioural changes
Neuropathy & neurodegenerative diseases
Low birth weight, pre-term birth and small gestational age
TOXICOLOGICAL MECHANISMS
Oxidative stress
Pulmonary and systemic inflammation
Genotoxicity
Changes in fibrinogen & prothrombin level
Platelet activation
Von Willebrand factor induction
Reduced heart rate variability
Increased blood pressure
Lipid peroxidation products
Vasomotor dysfunction
Disturbed Lipid metabolism
Oxidative stress and inflammation in the CNS

Figure 5.


CLEARANCE VIA LYMPHATIC VESSELS
pleural cavity
PASSAGE TO SYSTEMIC CIRCULATION
inhaled nanoparticles
CLEARANCE VIA MUCOCILARY ESCALATOR
airways
bronchiolar epithelial cells
mucus
'relocation'
interstitial space
clearance via lymphatic vessels
uptake by fibroblasts
blood capillary
alveolar space
alveolar macrophage
TRANSLOCATION OF NANOPARTICLE-LADEN MACROPHAGES?
endocytosis
exocytosis
type I epithelial cell
type II epithelial cell
NANOPARTICLE TRANSLOCATION
single particle
aggregated/agglomerated particles