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COMBUSTION-DERIVED NANOPARTICLES: MECHANISMS OF PULMONARY TOXICITY

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SUMMARY

1. The general term 'nanoparticle' (NP) is used to define any particle less than 100 nm in at least one dimension and NPs are generally classified as natural, anthropogenic or engineered in origin. Anthropogenic, also referred to as 'ultrafine' particles (UFPs), are predominately combustion derived and are characterized by having an equivalent spherical diameter less than 100 nm.

2. These particles, considered to be 'combustion-derived nanoparticles' (CDNPs), are of toxicological interest given their nanosized dimensions, with properties not displayed by their macroscopic counterparts.

3. The pulmonary deposition efficiency of inhaled UFPs, along with their large surface areas and bound transition metals, is considered important in driving the emerging health effects linked to respiratory toxicity.

4. The toxicology of CDNPs is currently used to predict the health outcomes in humans following exposure to manufactured NPs. Their similar physicochemistry would suggest similar adverse health effects (i.e. pulmonary (and perhaps cardiac) toxicity). As such, it is essential to fully understand CDNP nanotoxicology in order to minimize occupational and environmental exposure.

Key words: carbon black, combustion-derived particles, diesel exhaust, fly ash, nanoparticles, pulmonary toxicity.

PARTICULATE MATTER (PM_{10} AND $PM_{2.5}$) AND NANOPARTICLES

Epidemiological and toxicological research suggests that 'small', ambient, airborne particles cause 'big' health effects in people with pre-existing cardiopulmonary diseases.[1,2] These so-called 'ultrafine particles' (UFPs) are in the nanometer size range (1×10^{-9} m) and are considered to be nanosized particles (NPs). They are usually unintentionally produced byproducts of processes involving industrial, combustion and automobile activities (Fig. 1). The airborne particulate matter (PM) produced from these sources contains particles in three size categories, which are collectively referred to as PM_{10}. The metric PM_{10} is defined as particulate matter less than 10 μm in aerodynamic diameter, where particles less than 0.1 μm are regarded as being 'ultrafine' (UF), those between 0.1 and 2.5 μm are 'fine' in size and particles that are 2.5–10 μm are referred to as 'coarse'. Most of the particle mass is found in the fine or $PM_{2.5}$ size range and the largest number of particles is observed in the UF category. Matter in the PM_{10} category is highly heterogeneous in nature and it would be futile to define its mineralogy or structure. However, the composition of PM_{10} is controlled by factors such as weather, continental-scale influences and regional and local influences.

In urban and industrial areas, PM_{10} is dominated by road transport, industrial and construction particles, whereas in rural areas it is mainly composed of biogenic (e.g. pollen grains, fungal spores, plant material) and fugitive dust particles from erosion. Consequently, UFPs may possess a wide range of physicochemical properties (e.g. surface metal contaminants and aromatic compounds) owing to different emission sources and geographical locations. From a toxicological point of view, the most important class of these so-called NPs is that derived from traffic exhaust, which accounts for up to 80% of human exposure.[2,3]

Human exposure to NPs has increased markedly over the past century due to anthropogenic activities dominated by coal and diesel oil fuel combustion. Moreover, the advent of nanotechnology will most likely contribute yet another source of aerial PM pollution via engineered nanomaterials. Consequently, this rapidly progressing field has given rise to a new division of toxicology, namely 'nanotoxicology', which involves the safety evaluation of engineered nanostructures and nanodevices.

Current and historical epidemiological and toxicological investigations with airborne UFPs[4] are viewed as the basis for the expanding field of nanotoxicology and a number of key endocytotic and biokinetic concepts have been identified from the results of these studies: (i) the major portal of entry into the human body for NPs is via inhalation into the respiratory system (Fig. 2); (ii) NPs can transcytose epithelial/endothelial cells into the systemic circulation to reach sensitive target tissues (Fig. 3a); (iii) NPs are capable of generating reactive oxygen species (ROS), which have been linked to inflammatory lung diseases (Fig. 3b); and (iv) invasion of the systemic circulation by NPs has been implicated in cardiac dysfunctions (Fig. 3c,d).

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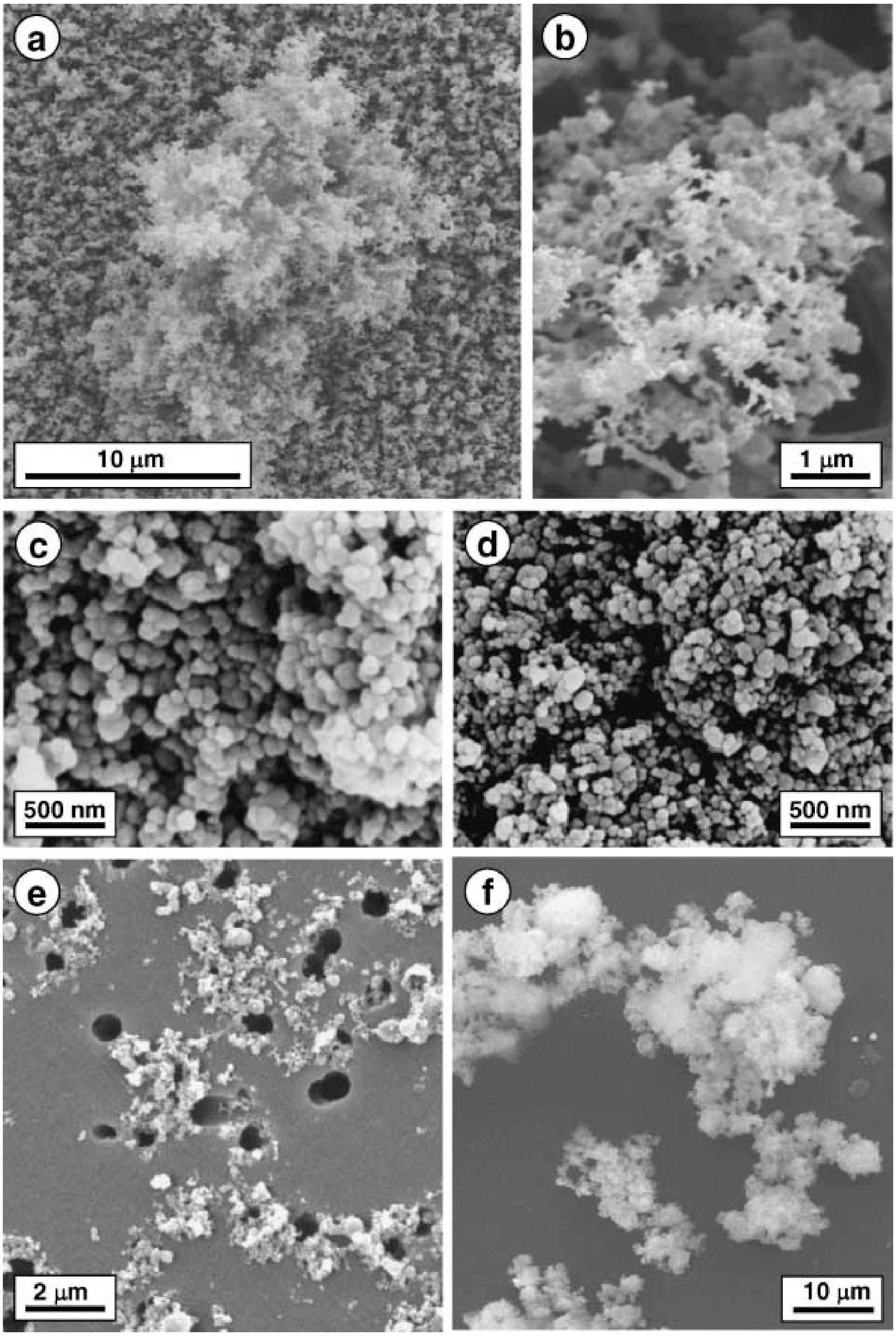

Fig. 1 Field emission scanning electron micrographs of combustion-derived nanoparticles: (a) large soot nano-structured particle, lying on a dense bed of soot nanoparticles; (b) detail of the flocculated ('bunch-of-grapes') structure of soot; (c) carbon black sample denoting a mixed population of fine and nanosized particles; (d) carbon black sample from a population of strictly nanoparticles; (e) urban nanoparticles collected from Cardiff, Wales, UK; (f) residual oil fly ash (ROFA) particles.

PARTICLE PHYSICOCHEMISTRY

Manufactured nanoparticles

The principal patterns of particle endocytosis and biokinetics are largely dependent on their physicochemical properties, which include particle size and distribution, agglomeration state, shape, crystallinity, chemical composition, surface area (SA), surface chemistry, surface charge and porosity.[1,2] Nanoparticles have been broadly defined as microscopic particles with dimensions less than 100 nm; however, 'manufactured' NPs are more precisely defined as having one dimension less than 100 nm (Fig. 4). Many nanoparticles, whether manufactured, combustion derived or natural (e.g. emissions from volcanic activity, earth erosions and sand storms), are prone to rapid agglomeration, forming larger 'particles' with dimensions much greater than 100 nm. These are termed 'nano-structured particles' (NSP), as long as their activity is governed by their nanoparticle components (Fig. 1). For example, an agglomerate of a TiO_2-manufactured NSP (Fig. 4b), forming a single NSP much larger than 100 nm in diameter, has significantly greater biological activity than a single crystal TiO_2 particle of the same diameter.[2] The physicochemical properties of NPs have been associated with pulmonary toxicity in humans.[5]

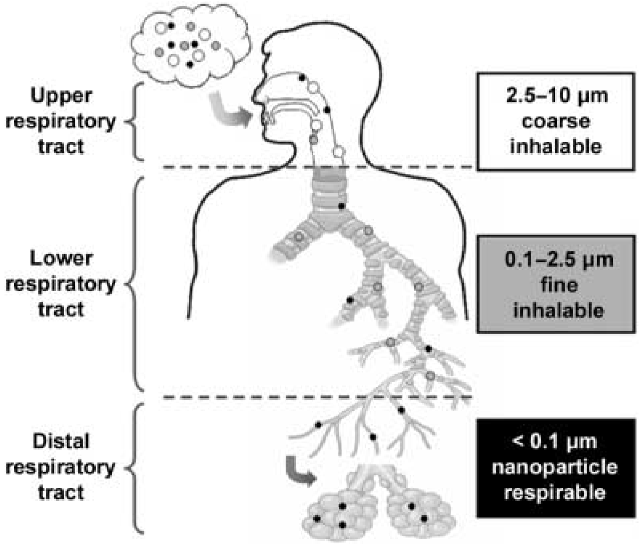

Fig. 2 Diagrammatic representation of the translocation patterns of coarse (inhalable), fine (thoracic) and ultrafine/nanosized (respirable) particles in the human respiratory system.

COMBUSTION-DERIVED NPs

Combustion-derived NPs (CDNPs), such as diesel exhaust particles (DEP), carbon black (CB) and fly ash (FA; e.g. residual oil fly ash (ROFA)) are all occupational and environmental hazards (Fig. 1).[2] For this class of poorly soluble particles, their large SA alone is enough to drive lung inflammation; when a given mass of material is divided into an increasing number of units, the total SA of those units increases. The CDNPs are 'primary' emitted particles in the sense that they arise directly from combustion processes. However, as they age, and when mixed with other ambient pollutants, their chemical composition can change. Their chemistry is derived from the combustion and pyrolysis processes, whereby combustion concentrates transition metals and pyrolysis generates organic compounds, along with elemental organic carbon particles.

Diesel exhaust particles

Diesel exhaust particles account for up to 80% of the mass of PM_{10} collected in urban areas (Figs 1a,b,4c). They differ from soot, in the form of CB, because they contain toxic metals and organics and this, in combination with their large SA, drives the production of ROS (Fig. 5).[2,3] For the formation of ROS, the large SA presents

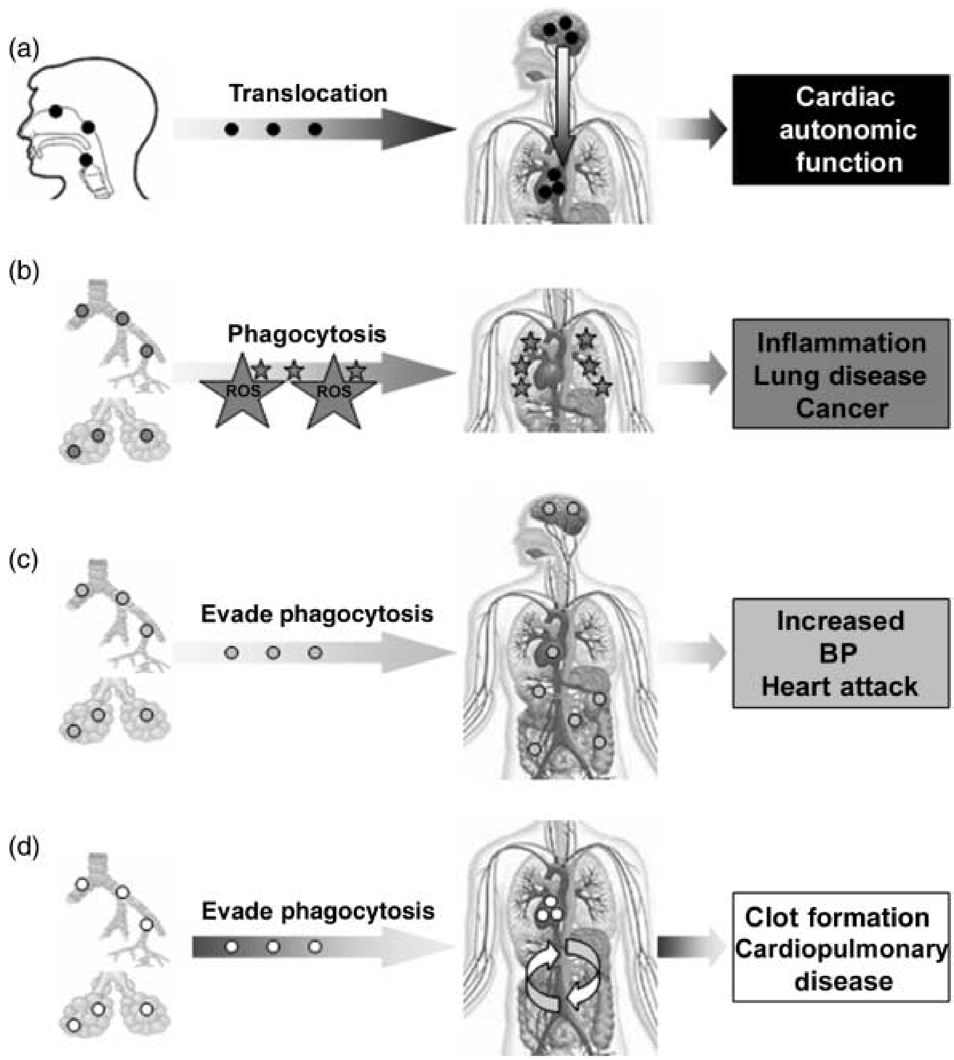

Fig. 3 The fate of nanoparticles in the human body: (a) translocation into the brain via transcytosis into the cells of the nasal and tracheo-bronchial epithelia; (b) phagocytosis by macrophages within the mucociliary escalator and resultant reactive oxygen species generation; (c) evasion of defence mechanisms and translocation into the systemic circulation (blood); (d) translocation into the blood and disruption of atheromatous plaques.

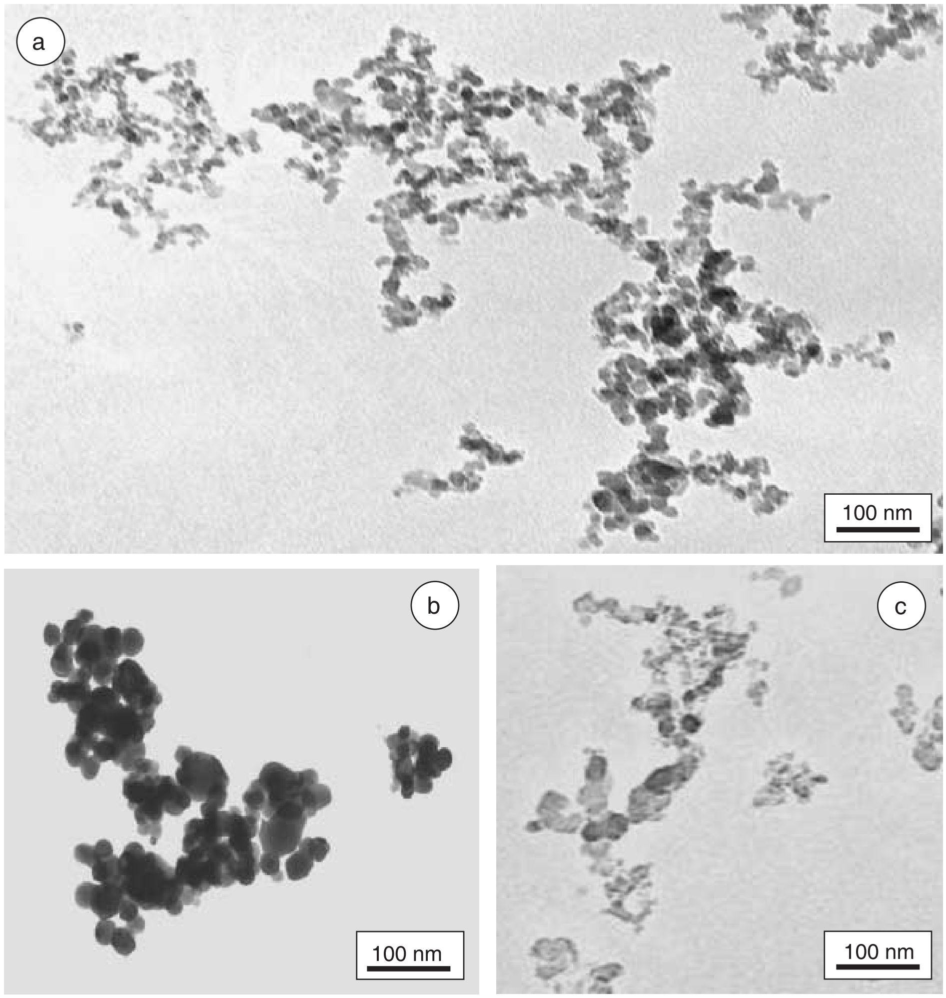

Fig. 4 Transmission electron micrographs of manufactured and combustion-derived nanoparticles (anthropogenic): (a) 'Cab-O-sil' (Cabot, Cardiff, UK); (b) TiO_2 (Sigma, Poole, UK); (c) diesel exhaust particles (Cardiff, Wales, UK). The manufactured and anthropogenic particles share a common morphological organization characterized by chain-like aggregates of spherical particles.

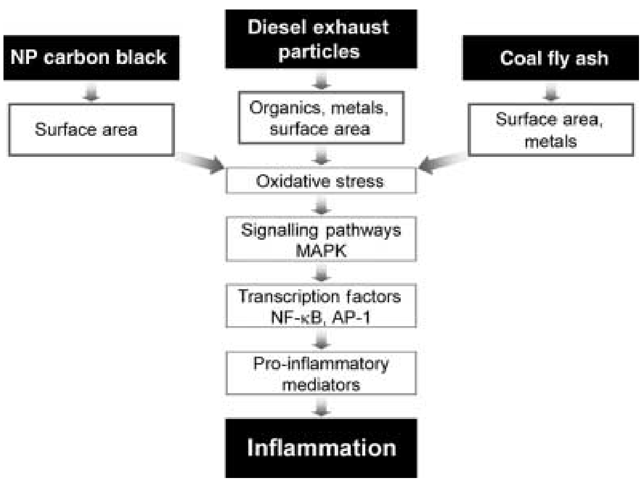

Fig. 5 The common molecular pathways of combustion-derived nanoparticles (CDNPs). MAPK, Mitogen-activated protein kinase; NF-κB, nuclear factor-κB; AP-1, activator protein-1.

an opportunity for dissolution of soluble species and provides a substrate on which catalytic chemistry can occur.

Soot particles are typically organized as chain-like aggregations of primary, spherical particles. The aggregations can consist of just a few through to many thousands of spheres. The mechanism of soot formation involves an initial particle nucleation from fuel pyrolysis forming polycyclic aromatic hydrocarbons (PAHs), addition to the nucleus by gas molecules, coagulation by particle–particle collisions, removal of functional groups and dehydrogenation and structural rearrangements of the condensed material.[2] The core of the sphere, typically with a diameter of approximately 10 nm, is composed of concentrically piled, thermodynamically unstable turbostratic, carbon networks. The consequence of this organization is a potential to hold materials such as volatile organic compounds (VOCs), sulphur and transition metals in the large intranetwork spaces.[2] The outer part of the sphere is better ordered, more stable and composed of graphitic microcrystallites of orientated carbon sheets. Vapor-phase hydrocarbons condense on the sphere surface during the cooling stage of the combustive process. Many toxicological studies have shown that chemically 'pure' soot particles, such as CB, have significantly less bioreactivity than common, urban PM_{10} soot particles generated by diesel engines.[2]

Carbon black particles

Carbon black is a low-solubility particle (Fig. 1c,d) produced industrially from the incomplete thermal decomposition of hydrocarbons. This process is controlled to achieve predefined and reproducible

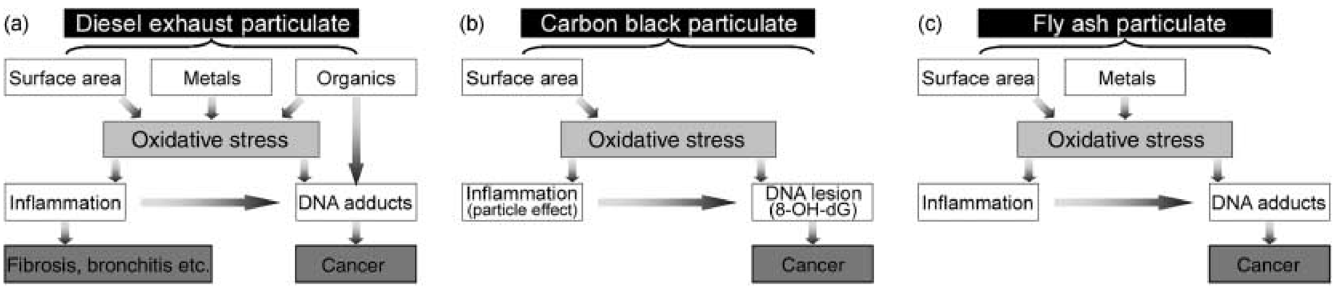

Fig. 6 Suspected genotoxic pathways of combustion-derived nanoparticles: (a) diesel exhaust particles; (b) carbon black; and (c) fly ash. 8-OH-dG, 8-hydroxydeoxyguanosine.

particle sizes and properties suitable for a diverse range of industrial applications, such as photocopier toner. Carbon black exhibits the same behaviour as DEP, in that its spherical particles aggregate into agglomerates that can be highly respirable. In long-term studies, CB has been shown to be a carcinogen, but the phenomenon of 'particle effect' (i.e. large SA drives toxicity)[6] plays a likely role in this outcome (Fig. 5).[3]

The acute effects of CB exposure in humans are similar to effects caused by other insoluble particles. In general, long-term occupational exposure to CB may cause slight radiological changes; workers may develop chronic bronchitis and a slight reduction in lung function. These effects can be attributed to a non-specific irritant effect of heavy dust exposure. Fibrosis has been reported in early studies under excessive dust conditions, but epidemiological studies have not established excess human cancer risk with CB exposure.[7–9]

Fly ash particles

Fly ash is a generic term for particulate matter from mineral and metal contaminants of organic fuels. The two main groups of FA are ROFA (from liquid fuel) and solid fuel. The ROFA contains transition metals, sulphates and acids incorporated into a particulate carbonaceous core. The solubility and bioavailability of the transition metals associated with these particles is of interest. Occupational exposure resulting in adverse respiratory health effects in humans has been recorded.[10–12]

Fly ash produced by coal or waste combustion is derived from mineral, sulphur or other contaminants in the fuel. Especially in industrial towns, this type of FA is a common air-borne component. It is composed of predominantly spherical or subspherical glassy particles (60–90% glass), from the melting of the minerals. Most FA is found in the PM_{10}–$PM_{2.5}$ coarse fraction. The spheres are either solid or hollow and broken fragments of the spheres are commonly collected. Fly ash particles generated by the metal industries often have characteristic granular or spherical morphologies.

Toxicological studies using unfractionated FA from solid fuels have shown it to be of low toxicity, but its bioavailable metals have been implicated in ROS generation (Fig. 5).[3,13,14] Upon fractionation of FA, the NSPs exhibited increased potency compared with its fine and coarse fractions.[14] The NSP fraction contained carbonaceous aggregates (20–50 nm) and surface-bound metals (approximately 10 nm particles of Fe, Al and Ti). In general, FA has a low metal concentration and it is believed that the bioreactive NSP fraction is due to increased SA, which allows redox reactions to take place. This is a similar mechanism to that proposed for CB and, hence, in the absence of soluble transition metals and VOCs associated with a particle, a large SA can drive the inflammation that causes a variety of lung and heart diseases.

NANOPARTICLE TOXICOLOGY

Given that inflammation is a common response to inhalation of CDNPs, in both animals and human epidemiology, there is now a unifying hypothesis for their toxicity.[3] They have a generic ability to cause inflammation via oxidative stress and activation of redox-sensitive transcription factors (i.e. mitogen-activated protein kinase and nuclear factor-κB; Fig. 5)[15–17] that can lead to the observed adverse health effects (e.g. fibrosis, chronic inflammatory lung disease, cancer). The physicochemical properties that drive these effects differ greatly between these exemplar CDNPs. That is, DEP have a soluble component and release transition metals or organics as their primary pro-inflammatory mechanisms, a combination of large SA and soluble metals determine the pro-oxidant activity of FA, and the SA effect alone is responsible for the bioreactivity of CB particles. When the transition metals and PAHs interact with the lining fluids of the lung, they undergo cycling redox reactions that produce ROS (e.g. superoxide anion, hydroxly radical).[3]

The mechanisms involved in particle-induced genotoxicity remain poorly understood, because the particles are uniquely complex owing to their physicochemical characteristics. There is some piecemeal evidence that DEP,[19,19] CB[21,21] and FA[23,23] are carcinogenic in humans (Fig. 6).[3] Diesel exhaust particles consist of a carbon core with adsorbed PAHs and transition metals.[24] Genotoxicity may be induced by the direct interaction of PAHs, which are known to cause DNA adducts.[25,26] Alternatively, the transition metals may induce ROS, which results in DNA strand breakage.[27] Carbon black is generally devoid of adsorbed organics and metals and, thus, its genotoxicity is most likely an effect of the particle overload phenomenon, but some research has revealed the formation of the oxidative DNA lesion 8-hydroxydeoxyguanosine (8-OH-dG).[20,21,28] Studies investigating the genotoxicity of FA have determined a role for particle size and iron release leading to radical generation and oxidative stress.[29]

A few key mechanistic hypotheses (Fig. 7)[5] have been advanced to explain the associations observed in the epidemiological studies and have provided the foundation upon which current toxicological research is now focused.

1. Cardiac effects are a consequence of pulmonary inflammation, which interferes with coagulability and stability of atheromatus plaques.
2. Inhalation of particles triggers pulmonary reflexes that disrupt cardiac rhythm.

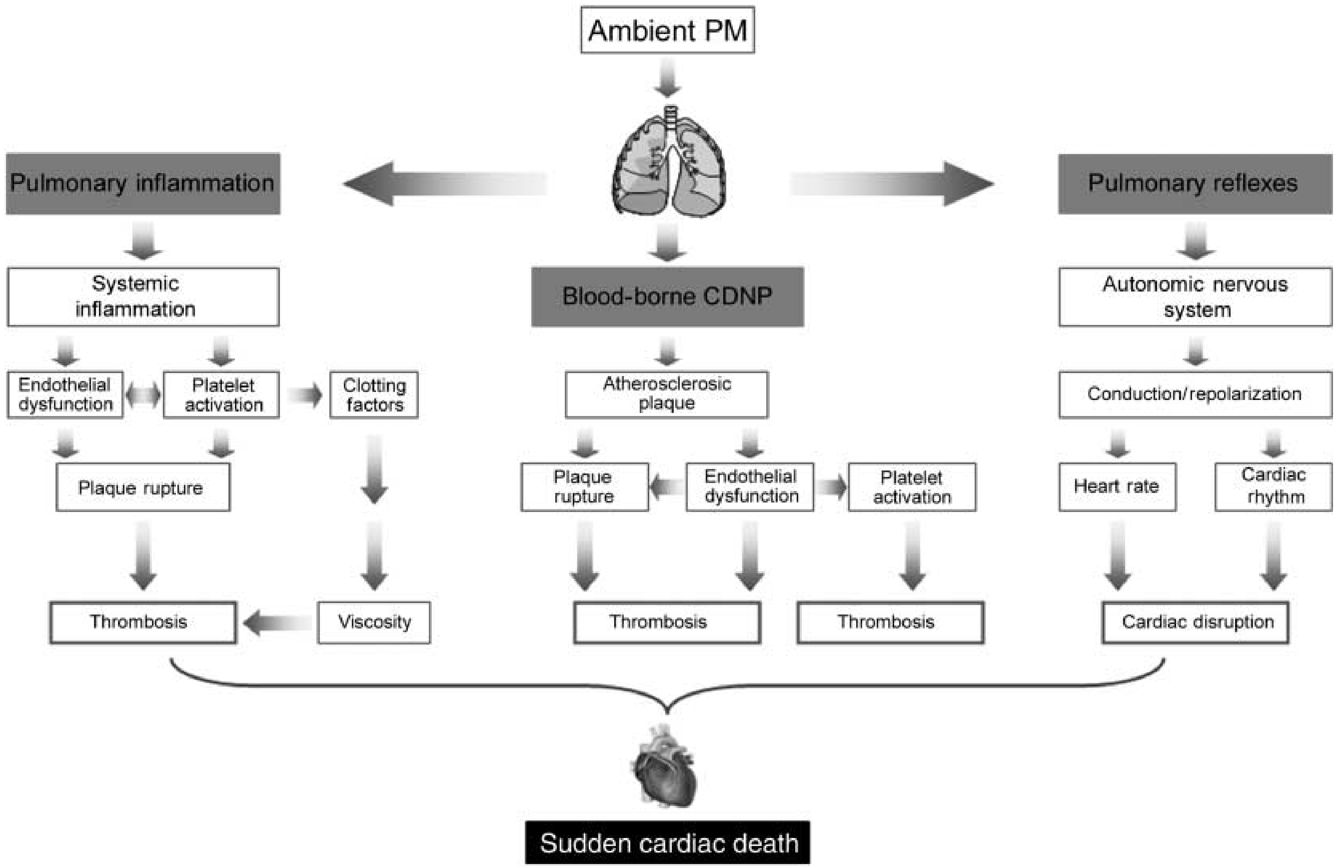

Fig. 7 Principal hypotheses of the cardiopulmonary toxicity of nanoparticles: pulmonary inflammation, pulmonary reflexes and blood-borne combustion-derived nanoparticles (CDNP). PM, particulate matter.

3. Lung inflammation is a consequence not of the mass, but of the number of particles.

The first hypothesis suggests that inhaled particles, especially NPs, establish pulmonary inflammation that triggers changes in the control of blood clotting.[5,30–34] The concomitant changes in chemical factors in the blood can affect the stability of the atheromatus plaques (fatty deposits) found in the walls of arteries that supply blood to the muscle of the heart itself. If this is true, then a link between inhalation of particles and the likelihood of, for example, heart attacks will have been established.

The UFPs/NPs could also have effects on cardiac physiology if they gain access to the bloodstream (Fig. 7).[5] The possibility of transfer of particles by blood to the heart causing a direct effect has been proposed.[31–34] The circulating particles may interact with vascular endothelium/atherosclerotic lesions, causing local oxidative stress that could destabilize plaques, setting off a chain reaction (rupture, thrombosis) with resultant acute coronary syndrome.[5] Furthermore, particles may interact with circulating coagulation factors to promote thrombogenesis. To date, there are no published data demonstrating that the CDNP gain access to the blood in humans.

The second hypothesis suggests that inhaled particles may act directly, or perhaps as a result of, a local inflammatory response on nerve endings in the walls of the airways throughout the respiratory system.[5] Activation of such receptors initiates changes in the autonomic control of the heart and, thus, changes in the heart's rhythm (e.g. fatal arrhythmias). This hypothesis links with the one above and, hence, inflammation may be involved in the early stages of both.[5]

A third and final theory purports that lung inflammation is a consequence not of the mass, but of the number of particles, particularly those in the UF size range.[5] This hypothesis has the potential to explain both short-term morbidity and also longer-term atherogenesis. The human lung is subjected to daily low mass concentrations of particles, but high number concentrations.[35] It is estimated[5] that on a so-called 'low pollution' day (over 24 h), an adult human will inhale approximately 200 billion particles, half of which will be deposited in the lung, without apparent harm. These huge numbers of particles are contained in a very small mass (400 μg). During pollution episodes, where the average particle mass rises to 50 $\mu g/m^3$, the mass of particles inhaled associated with adverse cardiac effects may contain 2000 billion particles. It is therefore self-evident that the mass does not represent the number of particles.

CONCLUSIONS

The CDNPs are a fact of modern life and contribute substantially to poor air quality, especially in the urban environment.[36] The lung is the primary portal of entry into the human body for CDNPs, but it is the heart that is the final sensitive target organ. The CDNPs are unified by their combustive origins, small size and universal mechanism of injury and common pathways of translocation in the body. Consequently, they should be recognized as a cardiopulmonary hazard.

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