



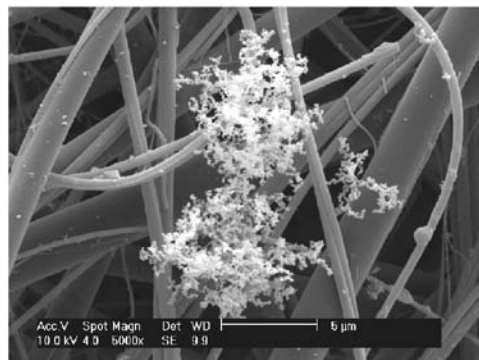
Environmental Particles: A Breath of Fresh Air?

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and Chris Jones*

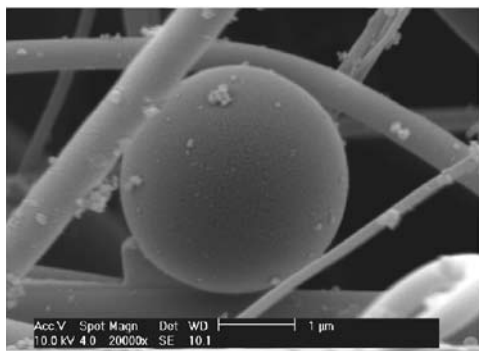
Our lungs are exposed to airborne particles in all aspects of everyday life. Research from around the world suggests that 'small' aerial particles can cause 'big' health problems in persons with pre-existing lung and heart disease. The Lung & Particle Research Group at Cardiff University are investigating the biological effects of environmental particles in the lung. They are trying to seek links between the distribution and biopersistence (i.e. solubility) of particles in the lung with mechanisms of cell damage or compromised lung biochemistry. This is a challenge facing all respiratory toxicologists today, since there is no 'biological plausibility' to explain how ultrafine particles, such as diesel exhaust, fly ash and nanoparticles, induce adverse health effects. The major portal of entry into the human body for environmental particles is via inhalation into the respiratory system. Therefore, the current research suggests that ambient particles have direct effects on the lung, which in turn, may lead to disruption of cardiac events. The greater pulmonary deposition efficiency of a given particle, with a large surface area and transition metals bound to it, is considered important in cardiopulmonary toxicity. The greatest risk to human health is now thought to come from inhaled nanoparticles, since they appear to be able to evade the body's natural defence mechanisms, with a consequence of redistribution into other sites of the body, causing systemic health effects.

What is Air Pollution?

The term 'air pollution' is used to describe the presence of chemicals or materials in the atmosphere that produce poor air quality. Air pollutants may be classified into four principal categories (Figure 1), which include anthropogenic (man-made) activities [e.g. combustion products], biogenic (biological) processes [e.g. pollen, allergens], technogenic (technology) production [e.g. metal aerosols or smelter] and geogenic (geological) occurrences [e.g. erosion of earth, i.e. minerals, volcanic ash]. From these categories are derived the seven main pollutants of human health concern, i.e. carbon monoxide, nitrogen dioxide, ozone, sulphur dioxide, hydrocarbons, lead, and particulate matter (PM). The common provenance of these emissions is from the combustion of fossil fuels (e.g. coal, petrol, diesel fuel; Figure 2). PM is now considered to be the most precarious of pollutants and responsible for nearly 10,000 premature and excess annual deaths in the



(a)



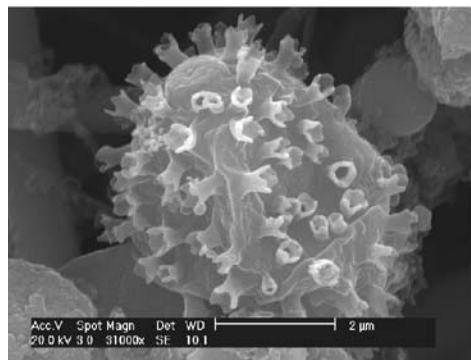
(c)

Fig. 1. The four principal classes of particle species found in ambient air: (a) anthropogenic [DEP collected on a glass fibre filter]; (b) biogenic [pollen grain]; (c) technogenic [metal oxide or 'smelter' particle]; (d) geogenic [calcium sulphate or 'gypsum' crystals]. Note: all particles were imaged on a Philips XL30 FE SEM.

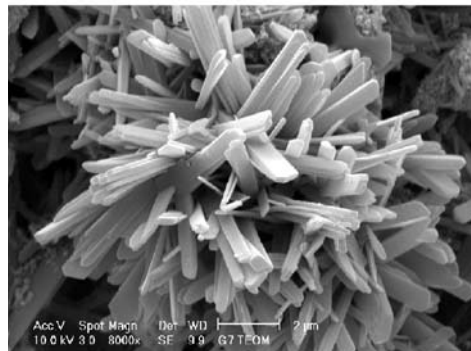
UK (Donaldson *et al.*, 2005; Oberdorster *et al.*, 2005; COMEAP, 2006; Bérubé *et al.*, 2007).

Particulate Pollution

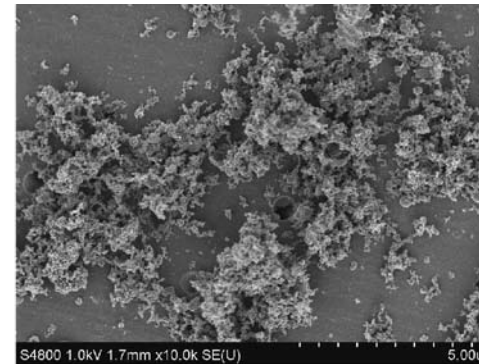
The metric used to measure ambient PM is PM10 (PM with aerodynamic diameter less than 10 microns) and is sub-divided into 3 fractions: (1) 'ultrafine' (UF) particles (UFPs) less than 0.1 microns in diameter and highly respirable, i.e. penetrate into the alveoli in the lung where gaseous exchange of oxygen and carbon dioxide takes place; (2) 'fine' particles that extend over an intermediate size range of 0.1 to 2.5 microns in diameter and thoracic in nature, i.e. will not pass into the distal region of the lung; and (3) 'coarse' particles that are 2.5 to 10 microns in diameter and are trapped in the upper respiratory tract, i.e. nose and mouth. Particle translocation within the tissues and cells of the human body is clearly dictated by size and the smaller the particle, the more efficient



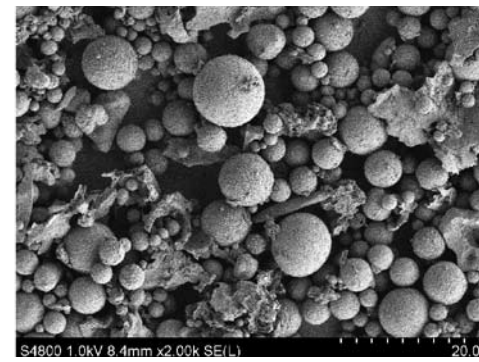
(b)



(d)



(a)

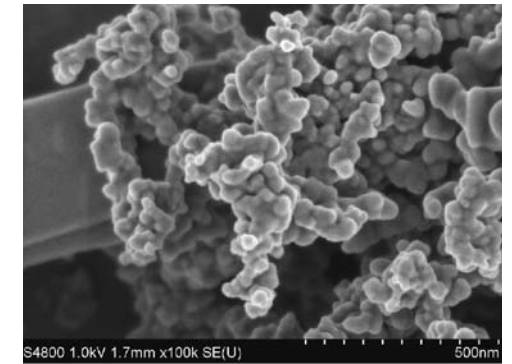


(c)

Fig. 2. The common provenance of ambient particle emissions is the combustion of fossil fuels: (a) DEP exhibiting their characteristic flocculated, chain-like aggregates; (b) CB displays a similar morphological organisation to diesel particles; (c) FA maintains discrete spherulites, unlike DEP and CB PM. Note: all particles were imaged on a Hitachi S-4800 FE SEM.

its distribution (Figure 3). Anthropogenic, UFPs within the PM10 metric are now considered to be 'nanoparticles' (NPs; particles less than 100nm in at least one dimension) but the anthropogenic NPs are further defined as having an equivalent spherical diameter of less than 100nm (Bérubé *et al.*, 2007). These different PM10 size fractions, when deposited into the human respiratory system, exacerbate a variety of pre-existing lung conditions, with the UF or the so-called NPs, potentially driving carcinogenesis (Figure 3).

Combustion-derived NPs (CDNP) comprise 80% of the particle species collected within urban PM10 samples in the UK (Bérubé *et al.*, 2006). Theoretically, CDNP should have free-reign to travel everywhere in the human body and the comparison of the relative size of man versus atoms is a useful



(b)

working scale to demonstrate this parallel (Figure 4). Man is typically measured in metres and atoms in angstroms. In between this range are insects (millimetres) and animal cells at the micron level. At the sub-micron level, i.e. nanometres, are the viruses (e.g. 20nm is smallest viral size known) and DEP (individual particles are 7nm in diameter; Bérubé *et al.*, 1999). Given that viruses are the smallest pieces of biological material able to penetrate all regions of the body, then NPs like DEP, which are 3 times smaller than viruses, will have unlimited deposition in the human body. Now that's small!

Exposure to Environmental Particles

People are exposed to PM10 via both the indoor and out door environments. In general, people spend the majority of their time indoors mostly in the domestic environment, where their health may be effected by significant airborne particulate pollution (Bérubé *et al.*, 1999; Bérubé *et al.*, 2004). Indoor particulate matter can be derived from contaminated air from outdoors, such as, heavily trafficked roads, presence of industry, fugitive dusts from the erosion of the earth and from specific indoor sources such as cigarette smoking (Figure 5). The composition of the indoor PM10 is controlled by outdoor sources, and to a lesser extent, by anthropogenic activities, except in the presence of tobacco smokers (Bérubé *et al.*, 2004). Furthermore, there are significantly higher concentrations of indoor PM10 when compared

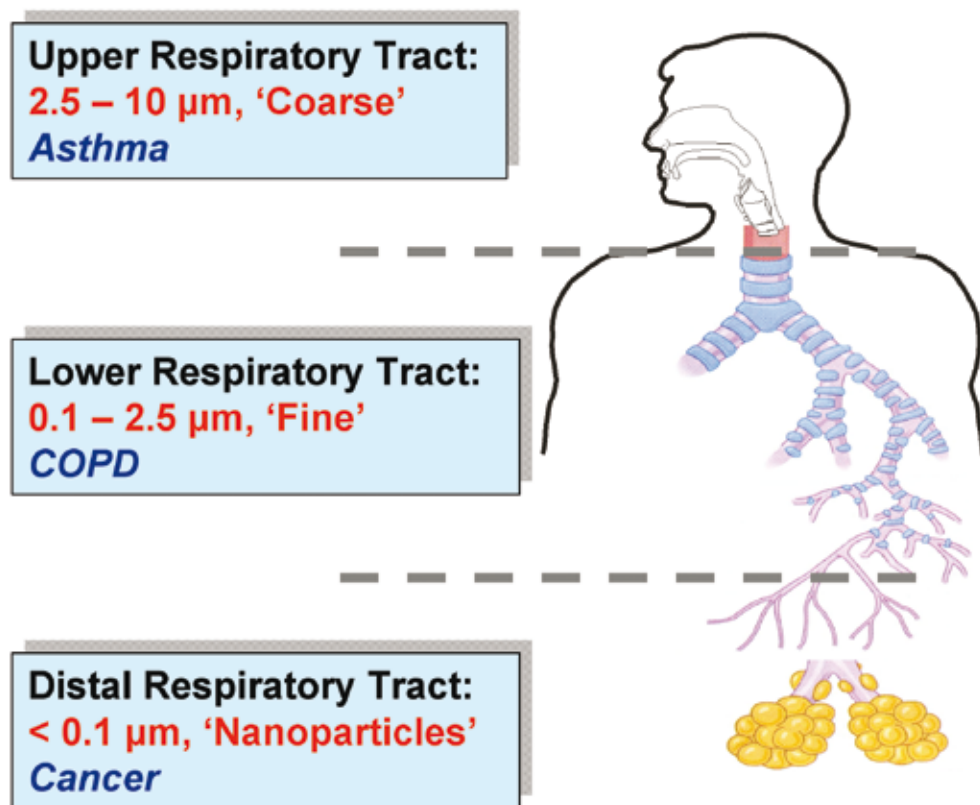


Fig. 3. A schematic diagram depicting the different regions of the human respiratory tract, with its related particle deposition sites and accompanying disease states.

to outdoor levels (Bérubé et al., 2004). Therefore, it is nearly impossible to avoid exposure to PM10, regardless of one's environment.

The greatest health risk from exposure to PM will arise from the single class of 'free' and 'poorly-soluble' UFPs and/or NPs. In other words, particles that are incorporated into a matrix or composite material should be of limited risk to human health. Given that the free NPs may pose a health risk, of the points of entry into the human body (i.e. the skin, gastrointestinal tract and lungs), the main danger will be for the lungs, which is the major portal of entry into the body via inhalation into the respiratory system. Current research suggests that NPs have direct effects on the lung, which in turn disrupt cardiac events (COMEAP, 2006).

The greater pulmonary deposition efficiency of NPs, with their large surface area (SA) is considered important in cardiopulmonary toxicity (COMEAP, 2006). Inhaled NPs appear to be able to translocate from the upper respiratory tract to its lower and distal regions by evading phagocytes (e.g. lung macrophages) that remove debris from the respiratory system, thus giving inhaled particles longer residency time in the lung (i.e. 'biopersistence'; Figure 6). PM may then be able to cross the air/blood barrier in the distal respiratory tract (Figure 7) where respiration takes place, i.e. 'breathing', and enter the blood circulation. The consequence of particle redistribution into other sites within the human body may include systemic health effects, such as inflammation, and ultimately, cardiac disruptions; events that may result in death.

Nanotoxicology

Nanotechnology is considered to be the biggest engineering innovation since the Industrial Revolution. Proponents of this new technology promise to re-engineer the man-made world, molecule-by-molecule, sparking a wave of novel revolutionary commercial products from machines to medicine. This so-called 'industrial revolution' in molecular manufacturing will alter the relationship of materials so profoundly that this change may produce both positive and negative effects on health and the environment. However, there is the caveat that the technologic progress during the Industrial Revolution enhanced quality of life but also resulted in a tremendous human health burden!

As in the case of asbestos, with its decades of long latency that still remain, especially in the UK, there are many legitimate concerns about the unknown human health consequences of ambient NPs, especially DEP, carbon black (CB) and fly ash

(FA). There is a unifying pathway of toxicity for the NP fraction of PM10. Their large SA present a platform for the dissolution of soluble species (e.g. transition metals, volatile organic compounds [VOCs], polycyclic aromatic hydrocarbons [PAHs] from the surfaces of their insoluble cores, and provides a substrate for the catalytic reactions which favour the formation of 'reactive oxygen species' (ROS; superoxide anion, hydroxyl radical). The free radicals are responsible for driving oxidative stress which is the underlying mechanism that promotes an inflammatory response, especially with the CDNP (Jones and Bérubé, 2007).

The Devil in the Diesel

DEP accounts for up to 80% of the mass of PM10 collected in urban areas (Figure 8). It has a propensity to form flocculated aggregates, referred to as 'spherules' (Bérubé et al., 1999) less than 100nm in equivalent spherical diameter (ESD), hence nano-sized PM. Individual particles or 'spherulites', are

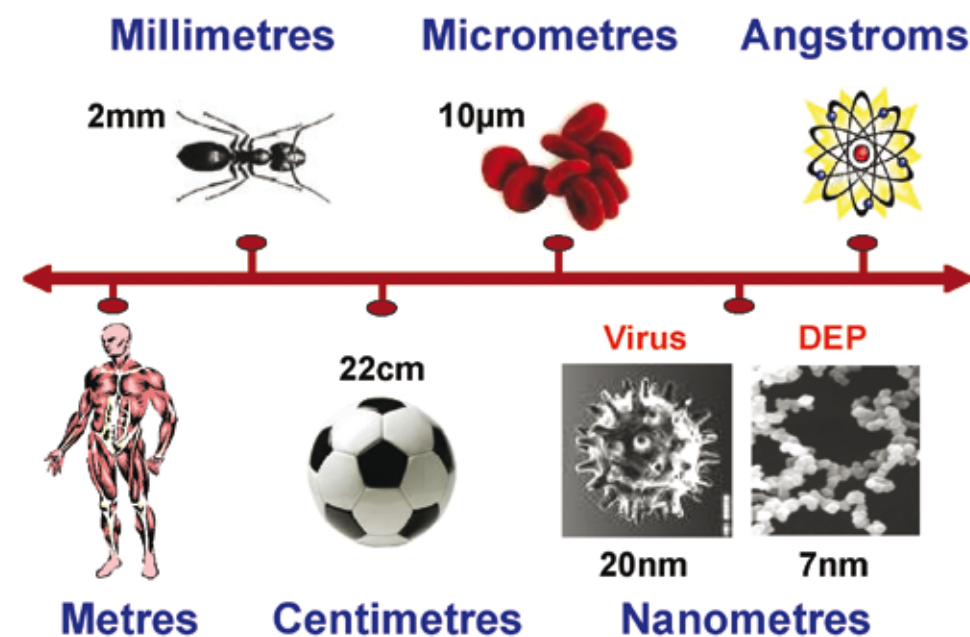


Fig. 4. The size of common objects in relation to the size of nanoparticles, beginning with man (in metres) and concluding with environmental particles (in nanometres).

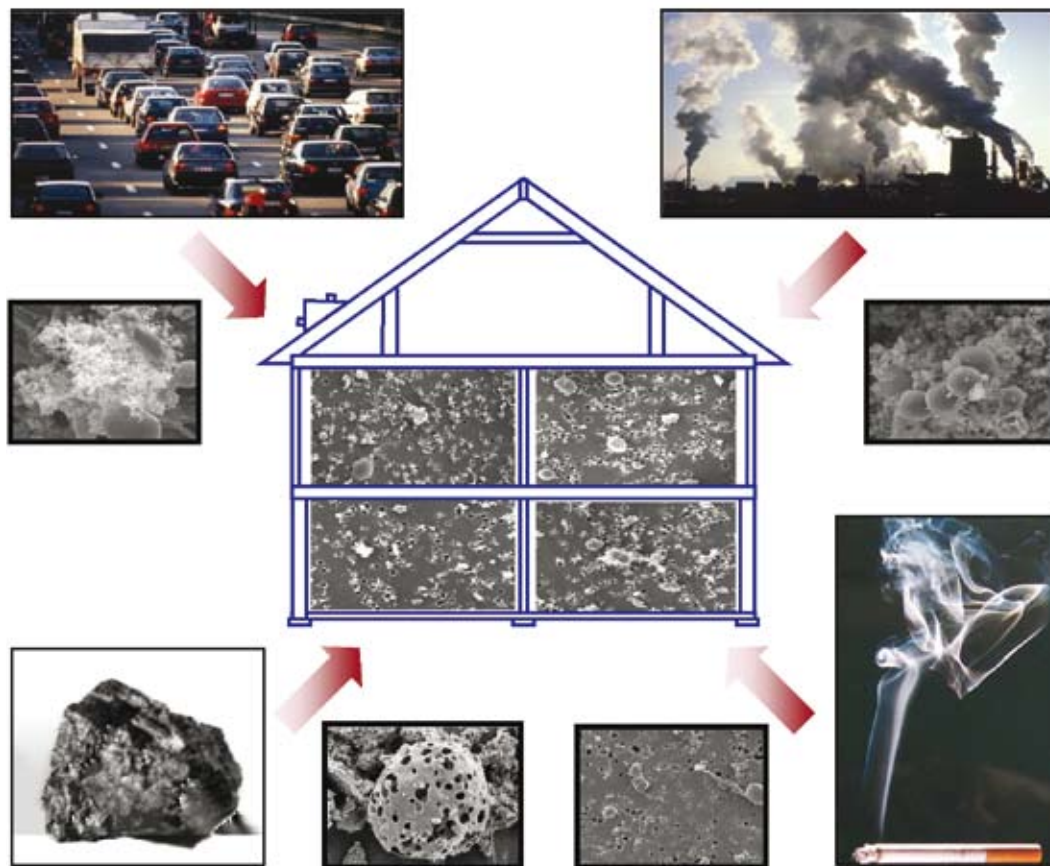


Fig. 5. The different sources of anthropogenic activities that contribute to the composition of ambient air in the indoor and outdoor environments: (clock-wise) motor vehicles, power-generation stations, smoking cigarettes and coal burning. Note: particles imaged on a Philips XL 30 FE SEM.

less than 10nm in ESD (Bérubé *et al.*, 1999). DEP NPs differ from soot, in the form of CB, by the presence of transition metals, VOCs and PAHs that undergo cycling-chemical reactions in the lung lining fluids to generate ROS. DEP is also an 'immunogenic'

particle, when in a synergistic relationship with inhaled biogenic PM (e.g. pollen grains, fungal spores and other allergens), where it exacerbates asthma and related respiratory allergies (Jones and Bérubé, 2007).

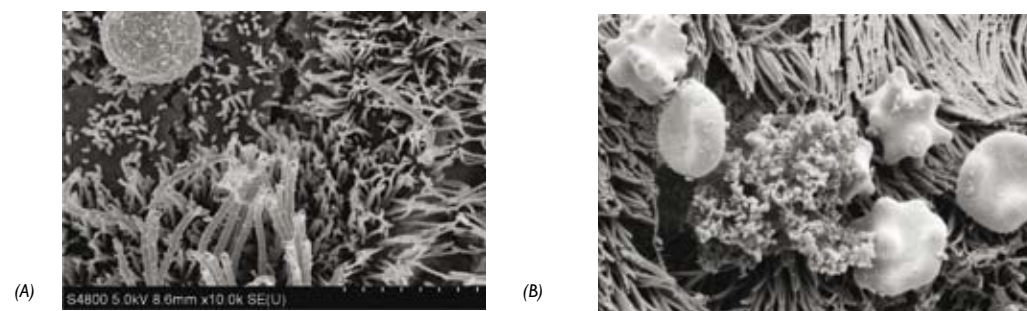


Fig. 6. The deposition of DEP in the bronchial epithelium (lower respiratory tract). (A) Overview of the lung tool kit for defence against inhaled PM: Mucous (balls) are used to trap the particles along with long finger-like projections (cilia) and short cell surface protrusions (microvilli) to propel particles upwards and out of the lungs. Note: lung tissue imaged on a Hitachi S-4800 FE SEM; (B) The deposition of DEP in the bronchial epithelium. Red blood cells suggest an injury to the tissue following exposure to PM. Note: lung tissue/DEP imaged on a Philips XL 30 FE SEM. Blood cells have a diameter of approximately 6-8 micrometers.

The Carbuncle in the Carbon

CB is a low-solubility particle produced from incomplete thermal decomposition of hydrocarbons in which the process is controlled to achieve pre-defined and reproducible particle sizes and properties suitable for a diverse range of industrial applications (Figure 9; Jones & Bérubé, 2007). It exhibits the same behaviour of DEP, in that its spherulite particles (ESD less than 20nm) form aggregates of spherules (less than 100nm ESD) that can be highly respirable. CB differs from DEP in that its surface is devoid of soluble metals, organics and

hydrocarbons, leaving the large reactive SA to drive toxicity, i.e. permit redox-reactions to take place. Consequently, CB is an 'inflammogenic' particle species, where the toxicological response in the lung is due to a non-specific irritant effect from the burden of excessive dust exposure.

The Flail in the Fly Ash

The term 'fly ash' is a generic word coined to describe PM derived from mineral and metal contaminants of solid (e.g. coal) and liquid (residual oil) organic fuels (Figure 10). FA derived from solid fuel, i.e. coal and

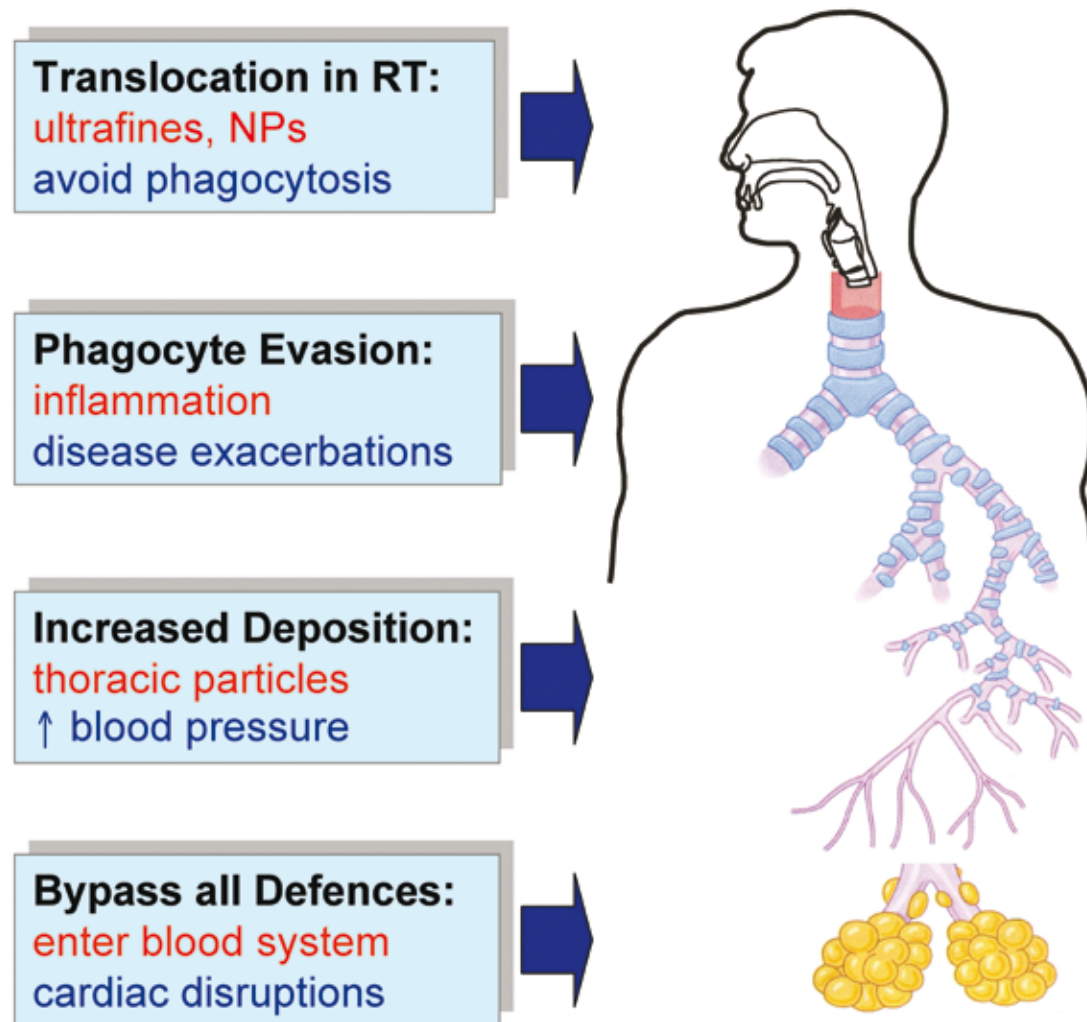


Fig. 7. TA schematic diagram depicting the key stages of inhaled UFP/INP translocation in the respiratory tract and the concomitant pulmonary and cardiac reactions within the various regions of the lung.

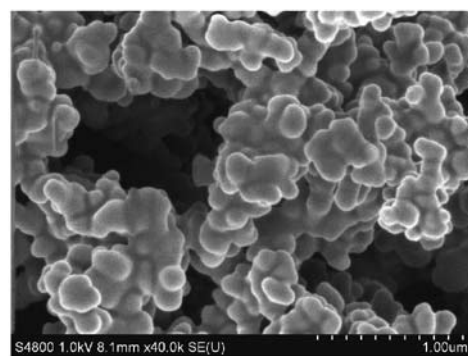
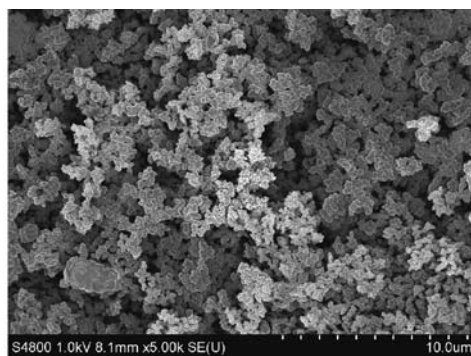


Fig. 8. A sample of DEP (overview and close-up) exhibiting its characteristic dispersion of chain-like aggregates of spherulites. Note: all particles were imaged on a Hitachi S-4800 FE SEM.

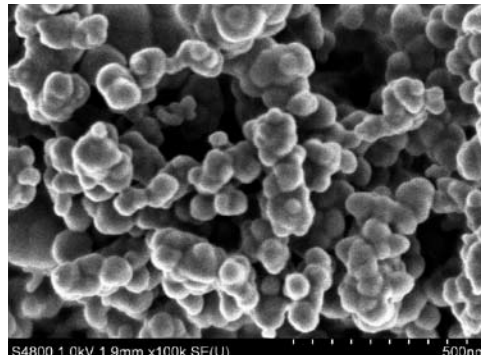
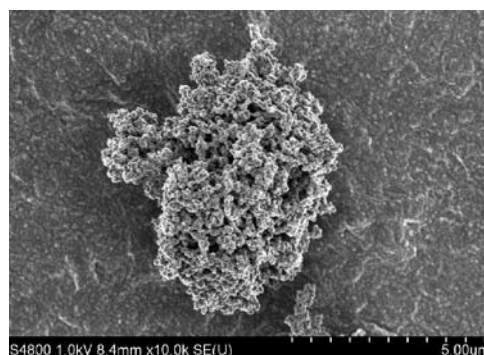


Fig. 9. A sample of CB (overview and close-up) demonstrating a flocculated spherule. Individual particles (spherulites) also form chain-like aggregates, in the same manner as DEP. Note: all particles were imaged on a Hitachi S-4800 FE SEM.

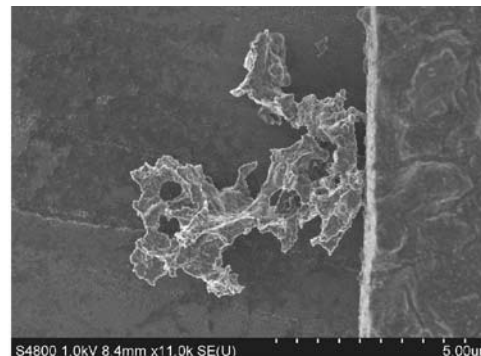
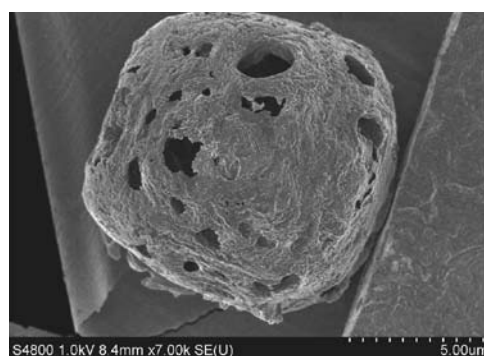


Fig. 10. A sample of FA from solid and liquid fuels: (a) hollow spherulite formed during the combustion of solid organic fuels; (b) Fragment of a hollow spherulite produced during the combustion of residual oil (liquid fuel). Note: all particles were imaged on a Hitachi S-4800 FE SEM.

waste combustion, is derived from geogenic (e.g. minerals, sulphur) contamination in the fuel, and is a common component in urban and industrial conurbations. It forms solid or hollow spherulites (20nm to 50nm) of 'glassy', carbonaceous particles as a result of the melting, and subsequent, sublimation

of the metal contaminant during the combustion of the fuels. In comparison to DEP, its 'bioavailable' metals have been implicated in ROS generation. The 'NP' sized fraction of FA has increased potency when compared to its 'fine' and 'coarse' fractions (Jones & Bérubé, 2007). The NP-fraction is coated

with multi-elemental inorganic species, in the form of Fe-, Al-, Ti-rich, 10nm-sized particles. In general, FA has a low metal concentration and it is believed that the bioactive NP-fraction is due to increased SA, which allows redox reactions to take place (Jones & Bérubé, 2007); a similar mechanism proposed for CB.

Ultimate Human Health Effects

CDNP are a fact of modern life and they affect the air quality in the indoor, outdoor and workplace environments. DEP, CB and FA are unified by their combustive origins, NP-sized fractions, common mechanism of inflammatory injury, and pathways of translocation in the body. The lung may be the primary portal of entry into the human body for CDNP but it is now believed that the heart is the ultimate target organ. The greater pulmonary deposition efficiency of CDNP, along with large SA and soluble metals, is considered important in cardiopulmonary toxicity. The ultimate toxicological end-point of exposure to aerial PM for persons with pre-existing lung and heart disease is now

believed to be 'sudden cardiac death' (Donaldson et al., 2005; Oberdorster et al., 2005; COMEAP, 2006; Bérubé et al., 2007). Following inhalation of PM into the lung, the NPs cause inflammation that disrupts the autonomic nerve endings which regulate heart rhythm, and cause dysrhythmia. The additional effects on the pathways governed by the 'pulmonary reflexes' and 'systemic inflammation' culminate in a sudden cardiac death (Figure 11). The precise relationship between particle physicochemistry and resultant adverse biological reactions in the lung environment remain elusive but there is evidence to suggest that changes in peripheral blood dynamics (e.g. prothrombotic effects) following interference by NPs, triggers cardiac morbidity and mortality (Nemmar et al., 2004; Evans et al., 2006).

Nanoparticles in the 21st Century

With the advent of nanotechnology, the prospects for using engineered nanomaterials with diameters of <100nm in industrial, consumer and medical applications have progressed rapidly. The possible

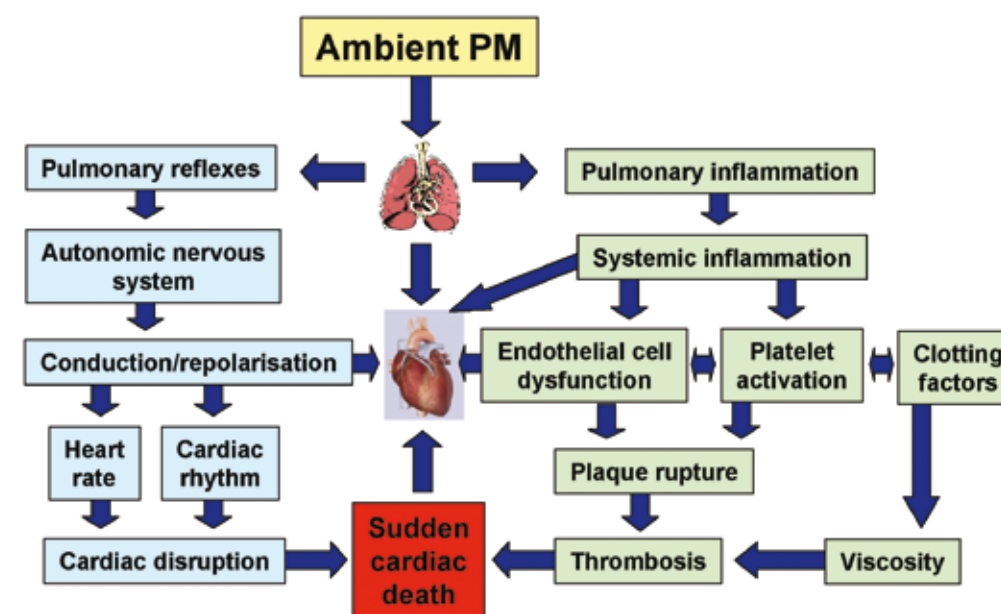


Fig. 11. A flowchart outlining the two pathways that may be triggered following inhalation exposure to UFPs/NPs: (a) (left-hand side of chart) Stimulation of the 'pulmonary reflexes' pathway would result in disruption of cardiac events; (b) (right-hand side of chart) If the 'pulmonary inflammation' pathway is engaged, then inflammation would drive prothrombotic events. The ultimate outcome for both pathways can be sudden cardiac death.



Fig. 12. Chris Jones (Hitachi High-Technologies Europe).

toxic health effects of these NPs associated with human exposure are not fully known, and as such, precautionary measures to inhibit their inhalation should be implemented. The comparability of engineered NPs to CDNP suggests that the human health effects are likely to be similar, and therefore, it would be prudent to elucidate their toxicological effects to minimize occupational and environmental exposure. Otherwise, the un-regulated release of NPs into the ambient air that we breathe may render them the 'asbestos' of the 21st century!

Application Note on Imaging Particles for Research

Imaging nanoparticles can be challenging and the conventional scanning electron microscope (SEM) using thermionic electron sources (e.g. Tungsten or LaB6) may not give adequate results. Although thermionic SEMs can resolve in the region of 3nm or less, the microscope will only achieve good results at that extreme level of operation if the sample has sufficient electron yield. For example, a sample of

high electron yield such as gold or platinum will be easier to image than a lighter 'z' number material like carbon. When examining NPs it is always worth considering the morphology and composition of the sample to help determine the type of microscopy best suited to the purpose.

A 'cold' field emission (FE) SEM (Figure 12), uses a single crystal of tungsten (the cathode) at ambient temperature (hence the term 'cold') as its electron source. The tip of the tungsten source has been thinned to produce a source area in the region of just 100nm. The electrons are 'extracted' from this source by applying a strong electric field between the first of two anode plates and the cathode, to overcome the work function of the tungsten. The resulting beam of electrons has a much smaller spot size and greater intensity than a thermally assisted source like W, LaB6 or even a Schottky emitter. A cold FE SEM emitter will produce a flux of electrons much greater than a W source, in a much smaller area (spot size), resulting in readily achievable

resolution of less than 1nm.

The advantage of using this type of emitter for the examination of NPs can be seen from the results achieved at just 1 kV, reducing the risk of surface charging and the need to apply a conductive coating. However; sputter-coating with a conductive material will help improve the mechanical stability of the NPs under the electron beam. Another advantage of imaging at low kV is the reduced penetration of the beam at the sample surface. By reducing the depth of penetration more information is collected from the surface of the sample, in the form of secondary electrons (SEs), rather than images taken at higher kV, which have a much greater contribution of back scattered electrons (BSEs), giving 'z' number contrast.

FE scanning transmission electron microscopy (STEM) imaging (images not shown) in the FE SEM can be used as a useful tool for the collection of information from powders, NPs, biological material and fib-prepared samples at a thickness of <100nm. STEM imaging at 30kV in a FE SEM can give better contrast than imaging the same samples in a TEM at a much higher accelerating voltage. Many researchers are applying STEM FE SEM imaging as a method for routine 'first-screening' of samples, before using conventional TEM methods for higher resolution examination where necessary.

Acknowledgments

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Dr Kelly Bérubé trained as a cell biologist at the University of Massachusetts (USA), and gained specialist training as an electron microscopist at Royal Holloway, London University (UK) and lung toxicologist at the University of Vermont (USA). Kelly is the Director

of Nanoscience Initiatives at the School of Biosciences, Cardiff University, UK. With her background in electron microscopy and lung toxicology, she has built an international reputation in the field air pollution and human health and holds numerous appointments in the USA and UK on funding bodies, advisory councils, professional societies and journal editorial boards that focus on environmental health. Her research focuses on the determination of intelligent biomarkers of exposure and harm in the respiratory system, with a particular interest in understanding how pollutants compromise lung biochemistry and alter gene and protein expression to drive disease mechanisms. Dr Bérubé's research has been recognised with a number of awards, including the Institute for Science & Health (USA) 'Scientific Merit Award 2006' for toxicogenomics of inhaled xenobiotics and the honour of being the British Toxicology Society's 'Australasia Visitor 2006' to conduct a lecture tour on nanoparticle toxicology. Recent work on human tissue equivalents of respiratory epithelia, as viable *in vitro* alternatives for *in vivo* inhalation toxicology, was awarded the UK NC3Rs 'Replacement Prize' and 'Science & Technology Innovation Prize (Cardiff University)' in 2007. Kelly's field of study is of natural interest to the general public, and as such, she is a prolific science writer and popular invited-speaker, and has worked extensively on communicating her research through public engagements of science for primary schools, laymen groups and professional learned societies.



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Tim trained as a geologist at Royal Holloway, London University, and in his early career worked with analytical SEM and TEM, and reflected light microscopy. Later research interests focussed on smaller particles, including nanoparticles, and this required the use of high resolution analytical electron microscopy. The most recent research has concentrated on airborne geological or technogenic particulate matter. Examples of this include anthropogenic pollution, as well as naturally-occurring airborne particles such as volcanic ash or dust storm grains. Tim is co-leader of the Cardiff University Research Group on Airborne Particles and the Lung. As a geologist his role is primarily the collection and characterisation of airborne particles, before the material is assessed for respiratory toxicity. One of the most important methods used in this particle characterisation is analytical FE SEM.

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Chris trained as an earth scientist and started his career in the Geology Department at Oxford Brookes University working with both light microscopy and analytical electron microscopy. He

later joined the Mineralogy Department at the Natural History Museum in London, managing the Electron Microscope Unit. This facility utilises SEM, analytical VPSEM, FE SEM, TEM and Confocal microscopy for research of naturally occurring samples, along with research collaboration with institutions worldwide. Chris focused mainly on mineralogical fields, including naturally occurring minerals used as pigments, ultrastructure in bryozoans and some forensic projects. He also co-authored a Museum publication with Alex Ball titled *Close Up*. In 2004 Chris joined Hitachi as their UK and Nordic SEM applications specialist, with responsibility for SEM, VPSEM and FE SEM applications, development, demonstrations and training. Chris has continued collaborative research into areas as diverse as mineral-based pigments, FE SEM applications in the study of genetics of deafness and air-borne pollutants.