The biological effects of nanoparticles

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1. Introduction

This article will attempt to describe the effects of nanoparticles on the different organs and tissues of the intact individual and also describe what is known from cell studies performed in tissue culture. It does not look at the biological effects of drug delivery systems or the use of nanoparticles in the treatment of disease. The aim has been to describe the effects of nanoparticles in the environment or generated in the body.

There exists a large literature on the cellular and tissue responses to particles in man, experimental animals and *in vitro*. Particles in general are a source of local irritation initiating an inflammatory response because of their physical, chemical or biological properties. The inflammation may be acute and followed quickly by resolution and healing, or it may pass into a chronic phase. Chronic inflammation is well described as continuing inflammation in the presence of an attempt at healing. A detailed description is not relevant here. Suffice it to say that acute inflammation is characterized by a polymorphonuclear leucocyte infiltration of tissue which shows also extravasation of red cells and an increase in extracellular fluid. Chronic inflammation usually shows the presence of macrophages and lymphocytes, with plasma cells under some circumstances, depending on the type of immunological response that is occurring. The attempt at healing may be manifest as increased vascularity and fibroblast proliferation, known as granulation tissue when seen together. Depending on the organ or tissue, there may be healing with restoration of the normal local architecture, or there may be fibrous tissue forming a scar. None of these is a separate process; rather, there is a continuum as the defensive response to foreign material evolves.

The literature and the following account refer frequently to the function of specialized phagocytic cells, the macrophages, in various sites, but it is necessary to bear in mind the broader context in which the cellular reactions are occurring. Thus, the cells exposed to foreign particles initiate an immunological response, which may involve antibody production by B lymphocytes, or be mediated by macrophages under the influence of T lymphocytes in the so-called cell-mediated or delayed hypersensitivity response. Also, particles may have an effect on the cellular transcription processes and changes at the molecular level potentially leading to alterations in the nucleus with carcinogenicity and genotoxicity.

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Particles may gain access to the body by inhalation, ingestion, through the skin or following introduction of material in some medical procedure, for example implantation of a prosthetic joint that subsequently wears, generating debris. The dust-related diseases of the lungs (pneumoconioses) provide an example of the sort of factors involved. Size and shape are important as particles over 5 μm in diameter are filtered out in the airways proximal to the small terminal bronchioles, while those between 0.5 and 5 μm are able to reach the more distal respiratory part of the lung, though some of them may be deposited higher up in the nose or large conducting airways. Asbestos in the serpentine chrysotile (white) form causes little in the way of significant changes, while the amphibole types, particularly crocidolite (blue), which exist as long thin straight fibres, cause severe fibrosis, lung cancer and mesothelioma. Solubility of particulate matter is a factor, so that silica, which is insoluble, causes local, and marked, lung fibrosis, while beryllium, whose compounds are more soluble, has systemic as well as local effects. Other factors that need to be kept in mind are the predisposition to develop lung involvement in any individual, which might be genetic (allergic diathesis), environmental (cigarette smoking), or related to the presence of some other disease (rheumatoid arthritis and coal worker's pneumoconiosis with Caplan's syndrome).

This Introduction has dealt with particles in general and the response to those particles in broad terms of conventional biological science. The question arises as to whether these basic ideas apply to particles at the nanometre level or whether there is a paradigm shift when the biological effects of such particles are under consideration. Under these circumstances, are physical characteristics such as shape and chemical factors of importance? What are the differences between nano- and micro-particles of the same apparent chemistry?

It is necessary to define clearly the terms that will be used, as there remain a number of papers which discuss nanoparticles but they do not fall within the definitions which are finding general acceptance. "Nano-" has a longstanding use in science to mean one billionth (the American billion; 1×10^9). The prefix "nano" comes from the Greek word "nanos" meaning a dwarf. A nanometre (nm) is exceedingly small, amounting to about 10 atoms across. Nanoparticles are therefore also extremely small, having one or more dimensions of 100 nm or less [1]. Nanoparticles are chemically highly active because of their large surface area proportional to their volume. This causes them to form *agglomerates* in which particles are held together by relatively weak forces, including van der Waals forces, electrostatic forces and surface tension. Nanoparticles may also form a group of strongly associated particles that cannot easily be redispersed by mechanical means, and in this case the collection is known as an *aggregate* [1]. The use of these terms has been inconsistent in the literature even in the recent past. An alternative term found in the literature is ultra-fine particle (UFP). Nanoparticles smaller than 30 nm have markedly altered properties and are often referred to as "quantum dots" because their size controls the separation (or quantization) of energy levels within them.

Nanoparticles are unique because their physical behaviour when measuring less than 100 nm changes from classical to becoming dominated by quantum physics with decreasing particle size. The surface area of the particles is extremely high, so that a nanoparticle of 2.5 nm radius with a density of 5 g/cm^3 will have a specific surface of 240 m^2/g assuming it has a spherical shape. Twenty per cent of the atoms of such a particle are present at its surface, making it highly reactive, aggregating with its neighbours and associating with other molecules such as proteins.

Technology using nanoparticles is being applied for the development of drug delivery systems, genetic engineering and other therapeutic or investigative procedures. These smart materials may be targeted to different sites in the body. They are not the subject of this review and it is clear that sometimes the term nanoparticle is being used in this context more loosely than defined above. In a number of reports in this area the particles are between 0.1 and 1 μm in size and have lipid, polymer, protein or other molecules attached to them.

Nanoparticles might enter the body by a variety of different routes and this makes the assessment of the risks in relation to any material difficult. As will be seen, particles may enter the body by one route but be widely disseminated to various organs and tissues. The most significant method of exposure to nanoparticles is by inhalation, though ingestion for example with food, or application to the skin, either deliberately or inadvertently, are other means of access to the body. Nanoparticles also may be generated *de novo* within the body, for example, at the load-bearing surfaces of a prosthetic joint replacement. Nanocoatings on implanted medical devices that could shed nanoparticles as they wear should also be included. This review will deal with each of these areas in turn, discussing the main nanoparticles involved in that form of exposure.

2. Airborne nanoparticles and inhalational exposure

The chief source of primary nanoparticles in the atmosphere is road transport (60%) with a further 23% produced by other forms of combustion processes in industrial, energy production and domestic contexts [2]. Diesel and petroleum combustion produces an aerosol that is nanoparticulate in terms of individual particle size (5–20 nm), though the particles are aggregated [3, 4]. Diesel produces more particles than petrol for the same unit volume combusted. It is difficult to be sure as to the numbers of particles in the outdoor environment since the aggregates will not be detected as nanoparticles and may be measured as larger microparticles. Background levels of nanoparticles are likely to be from 5000–10 000 particles per cm^3, though they may reach 3 000 000 particles/cm^3 at times of heavy pollution [5]. The factors which are thought to make nanoparticles toxic are large surface area, adsorbed organic molecules and metals [6, 7] and it is difficult to separate the part played by each of these. Low solubility particles have been shown to induce inflammation in direct relation to their surface area. Inhalation of nanoparticles from diesel exhaust results in inflammation and this leads on to fibrosis. The inflammation occurs after a short time of exposure (hours) in man and experimental animals [8–10]. Combustion nanoparticles having a low organic and metal content have been shown to cause inflammation through their surface characteristics and not by any soluble material. In terms of toxicity, such combustion-derived nanoparticles (diesel soot, welding fume, carbon black and coal fly-ash) have been considered together [11] even though they originate in different working environments. Because of the large surface area of these nanoparticles, they are considered to provide maximum opportunity for dissolution of soluble molecules from the insoluble particle core. The insoluble core in turn may provide a surface on which catalytic processes may occur, including free radical chemistry [11]. Thus, oxidative stress may result from the presence of free radicals and an inflammatory response in the region of the nanoparticles may be generated. It is also possible that the soluble organic molecules and transition metals released may cause inflammation.

Diesel nanoparticles caused an increase in pro-inflammatory cytokines and growth factors, an effect which was lost when the organic component was removed [12]. Diesel exhaust particles have been shown to activate intracellular signalling pathways for pro-inflammatory cytokine expression in cultured respiratory epithelial cells, namely MAP kinase [13–16], and NFkB [13, 17]. Among the cytokines expressed by cultured lung epithelial cells or alveolar macrophages in the presence of diesel nanoparticles are IL1[18], TNF [18], IL6 [19], IL8 [19, 20] and GM-CSF [20].

In welding, two metals are joined together using another metal, the welding material, at high temperature. The metal is vaporized to produce a fume containing oxides of metal which occur mostly as nanoparticles containing aluminium, cadmium, chromium, and copper [21]. *In vitro* cell studies and human bronchoalveolar lavage samples have both shown the expression of pro-inflammatory cytokines in the presence of nanoparticulate welding fume [22–24]. Carbon black is a well established material valued for over one hundred years for its optical properties, being incorporated into paints and inks, and more recently in plastics. Nanoparticulate carbon black causes NFkB expression by lung epithelium [25].

3. Distribution of nanoparticles to other sites after inhalation

3.1. The heart

There is evidence from epidemiological studies in man that increases in particulate pollutants in the atmosphere are accompanied by increased incidence of cardiovascular disease [26–28], including acute myocardial infarction [29, 30]. The question arises as to whether this is through the effects on the lung, causing increased strain on the heart, or whether there might be a direct effect of nanoparticles on the cardiovascular system. It has been demonstrated in man and from animal studies using ^{99}Tc (< 100 nm) or ^{192}Ir (< 80 nm) that nanoparticles that are inhaled or instilled into the lungs gain access to the circulating blood [31–33].

Radioactivity was detected in the blood within one minute and reached a peak between 10 and 20 minutes in a study with 5 volunteer humans [31]. The possible pathophysiological mechanisms for such cardiac involvement have been reviewed [34] with the suggestion that particles sequestered in the lung might lead to the release into circulation of prothrombotic and inflammatory cytokines. It should be pointed out that this particular article also considers sulphur dioxide, nitrogen dioxide and carbon monoxide effects as well as ozone, all of which have been shown to have adverse cardiovascular effects [34]. A recent human study gives no support to the hypothesis that short-term exposure to diesel nanoparticles is associated with systemic inflammation, activation of blood coagulation, endothelial dysfunction or lung epithelial injury in individuals at risk with obstructive airways disease [35].

3.2. The brain

Transfer of inhaled nanoparticles to the brain in rats has recently been demonstrated using radiolabelled carbon [36]. These workers concluded that there was transmission from the nasopharyngeal region through the olfactory mucosa and down the olfactory nerve to the brain. They have also shown similar transfer of inhaled manganese oxide particles [37]. This may not be the mechanism of dissemination of all particles to the brain. There is one further example of

a rat study in which the presence of manganese from stainless steel welding fume was demonstrated in the blood, liver and brain after a 60 day exposure [37]. More work is required to characterize nanoparticle translocation to the brain.

4. Nanoparticles and the skin

Theoretically nanoparticles might penetrate the skin by entering between or through epithelial cells (inter- and intra-cellular routes) or via the skin appendages (hair follicles, sebaceous and sweat glands) [39]. One of the best examples of the application of nanoparticles to the skin is in sun blocks, which contain a small proportion of ultrafine particles of TiO_2, other constituents being larger than 100 nm. These materials do not enter the skin but are localized to the superficial part of the stratum corneum where their optical properties provide an effective reflecting and ultraviolet absorbing layer. They are also found in hair follicles [40]. Since the particles in this study were coated, there could be some question as to whether overall they should be considered nano- or microparticles. A similar pattern is reported with so-called microfine particles of TiO_2 and ZnO using pig skin, though again it is difficult to be completely sure that these were in the "nano" range [41]. Another study with the same result used polymer particles but their range was unfortunately 20–200 nm [42]. Evidence from studies of clearly nanoparticulate cadmium sulfide suggest that this is definitely localized to the cornified layer with material also present in the orifices of hair follicles [43]. Three different types of nanoparticulate TiO_2 failed to penetrate deeper than the stratum corneum in a study in which electron microscopy was included as a technique [44].

5. The gastro-intestinal tract and nanoparticles

Papers dealing with the gastro-intestinal (GI) fate of ingested nanoparticles often can be criticized for the imprecision with which the particles are defined. Thus, many of the so-called nanoparticles are actually over 100 nm in size. It is estimated that 10^{12}–10^{14} microparticles (100–300 nm in this case) are ingested per person per day in the Western world [45]. They are said to be taken up by phagocytic cells in the intestinal mucosa and so do not pass through the intestinal epithelium itself. Such phagocytosed particles find their way into the gut-associated lymphoid tissue (Peyer's patches). Rats fed a suspension of iron particles, which were microsized (6–9 μm) down to nanosized (5–30 nm), showed the presence of iron within the duodenum, by light and electron microscopy, the latter including X-ray microanalysis. Metallic iron nanoparticles were found in the brush border, lateral intercellular spaces of the mucosal cells, mitochondrial cristae and cytoplasm of both mucosal and stromal cells, indicating passage of the particles across the epithelial barrier [46] The absorption and translocation of polystyrene latex "nanoparticles" (50 nm–3 μm) was related to size and constitution, uptake increasing with decreasing particle diameter. Adsorption of hydrophilic block copolymers onto the polystyrene markedly reduced the uptake by intestinal lymphoid tissue, and surface modification with lectin molecules producing binding to and internalization by enterocytes [47].

Other workers have failed to show significant translocation of ultrafine radioactive metal particles from the GI tract to other organs through the blood [33]. After oesophageal administration of an ultrafine ^{192}Ir particle suspension by gavage, virtually all the isotope was found in

faecal excretion within 2–3 days, no detectable ^{192}Ir was observed in urine any day during the observation period, and at termination (6 days) no ^{192}Ir was detected in any organ or tissue.

6. Nanoparticles and the brain

Access by nanoparticles to the brain by a transneuronal route along the olfactory nerve has already been described in §3.2. There are also important questions about the blood-brain barrier, a highly specialized endothelial cell and basal lamina junction that minimizes the passage of all but the smallest molecules to the brain. Transport to the brain of large molecules such as proteins is by a transcellular route. It seems likely from studies using magnetic nanoparticles and MRI* that this physiological barrier protects the brain from exposure to blood-borne nanoparticles.

7. Dissemination to other organs

It has been known for over 30 years that particles and dyes introduced into the circulation are taken up in what was called the reticulo-endothelial system, now more often referred to as the macrophage system. This consists of cells that have the ability to phagocytose foreign material, cellular debris, pathogens and foreign substances. These cells reside mainly in the liver, spleen and bone marrow though uptake in other organs such as the adrenal also occurs. The liver acts as a filter for the blood draining from the gastro-intestinal tract via the portal vein, while the spleen has a similar function for the general circulation. (Incidentally, lymph nodes serve the same function as filters of lymphatic fluid passing through them in the lymphatic vessels.) Greater numbers of particles are present in the spleen and liver at autopsy of coal workers than in non-coalworkers, implying the circulation of particles in the bloodstream of the former [50] (see §3.1). In man, there is a condition known as idiopathic granulomatous liver disease, in which generalized symptoms are present and small granulomas are seen in the liver parenchyma. In a small study of six patients with this diagnosis who had biopsy-proven granulomas present in the liver, traces of metals in nanoparticlulate form were found in the livers by environmental scanning electron microscopy (Revell and Gatti, unpublished findings). The metals detected could all be related to some event in the medical history of the individuals. Blood-borne carbon black particles (14 nm) induced platelet accumulation in the hepatic microvasculature of healthy mice [51]. This platelet adhesion was associated with fibrin deposition and increased von Willebrand factor (vWF) expression but other features of inflammation such as P-selectin expression on endothelial cells were not increased.

There is little information available about the localization of nanoparticles in the lymphoid organs apart from work by the group of the author, which has shown the localization of nanoparticles of hydroxyapatite implanted in the femoral bone marrow to the spleen of rabbits [52] and of microparticles of CoCr (0.3–5 μm) from the same site also to the spleen in guinea pigs [53]. These particles were also localized to the liver (Revell and Gatti, unpublished findings) (Fig. 1). Dissemination of particles from loosened prosthetically replaced joints to lymph nodes and spleen is well recognized [54, 55], but these are not at the nanoparticle level as far as is known.

* Magnetic resonance imaging.

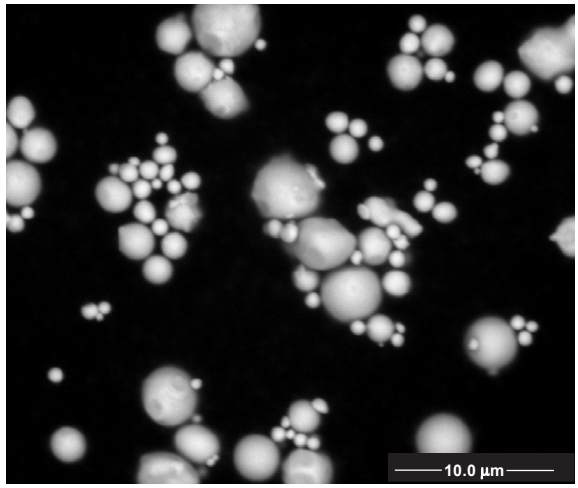

Figure 1a. Electron micrograph by SEM of CoCr particles (0.3–5 µm) retrieved from tissues adjacent to a human implant at revision surgery.

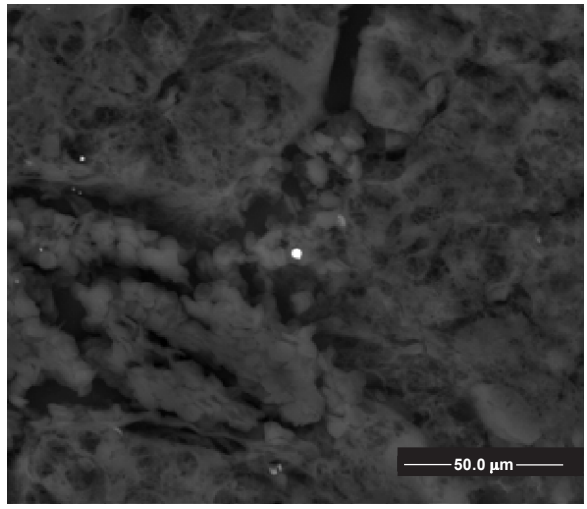

Figure 1b. Electron micrograph by ESEM of a microparticle (centre) and nanoparticles (top left, bottom centre) in the liver of a rabbit following implantation in the femoral bone marrow. The particles used are those shown in Figure 1a.

8. Nanoparticles generated within the body

It has been known for over 40 years that prosthetic replacement joints wear and that particles are generated from the load-bearing surfaces This wear debris is shed into the joint space from where it is cleared by the phagocytic synovial lining cells and synovial fluid macrophages, being moved off to the local lymph nodes [56, 57]. Particles find their way down between the bone and the implant and when there is a large accumulation of them they are responsible for substantial peri-implant bone loss. The particles provoke a macrophage and

multinucleate giant cell (MNGC) response, which encroaches onto bone giving rise to the resorption and bone loss known to surgeons as osteolysis [57]. The nature of the particles clearly depends on the materials used at the bearing surfaces. They may be metal alloy, ultra-high molecular weight polyethylene (UHMWPE), ceramic (zirconia or alumina), or acrylic from "bone cement"(polymethylmethacrylate, PMMA). Although more than 95% are less than 0.5–1 μm in size, regardless of material type [58], nanoparticles have not been identified with any regularity in the past. Our own group has observed 15 nm titanium particles by TEM in macrophages adjacent to loosened joints [58], and similar findings of metal nanoparticles (< 50 nm) have been reported by others where metal is rubbing on metal [59]. Nanoparticulate UHMWPE was generated in joint simulator studies though this has not been found in tissues where particles have been > 100 nm [60]. Particles from prosthetic joints have been shown in the liver, spleen, kidney, lung and lymph nodes [54, 55, 61], but there are no reports to date of nanoparticles in these sites from an orthopaedic implant, with the exception of the lymph node of one individual reported in an autopsy series [55]. Metal levels for the spleen, liver and lymph nodes were also described in that paper showing the presence of implant-derived materials, but no ultrastructural images were shown for those sites.

9. The effects of nanoparticles on cells in culture

Cell culture enables the dissection of complex biological events into small component parts, albeit in an artificial environment divorced from the multifactorial environment occurring in the organs and tissues of the body. Comparisons may be made between the effects of nanoparticles in different systems and commonalities sought. It is not known at present whether work with a particular type of cell, for example a human macrophage cell line, is relevant to other cell types, for example alveolar macrophages. The interpretation of such *in vitro* studies is fraught with questions about the validity of such comparisons, but the same cell, lines are now being used in different laboratories as well as comparisons being made with primary cells so that more confidence in the interpretation of such *in vitro* work should be possible.

The effect of diesel exhaust nanoparticles on lung epithelial cells and alveolar macrophages has been mentioned above (see §2). The pro-inflammatory cytokines that are produced (IL1,IL6, TNFα, GM-CSF) turn out to be among those which are well-recognized as being up-regulated when macrophages or phagocytic cell lines are incubated with various types of orthopaedic particulate wear debris [56, 57, 61]. Moreover, the cell signalling molecules that are activated (MAPkinase, NFkB) in lung cells with diesel nanoparticles are also the same as those upregulated in macrophages with those nano- and microparticles that are relevant to the replacement joint [62,63]. A direct comparison between nano- and microparticles when incubated with the monocyte/macrophage cell line U937, as well as with primary peripheral blood monocytes, has shown the close similarity between these two cell types with respect to the response [63]. Also, microparticles had a more marked effect than nanoparticles on the expression of all the molecules examined. Thus, there was greater costimulatory molecule expression with microparticles of diamond (0.15–0.81 μm) than with two different nanodiamond particles (both 1–5 nm). CoCr particles (0.3–1.0 μm) of comparable size to the larger diamond particles resulted in effects like those found with those particles [64]. Diesel nanoparticles and an organic extract of them caused increased Ia and costimulatory molecule

expression on peripheral blood monocytes but not alveolar macrophages in other work [65]. Nanoparticles of hydroxyapatite have also been shown to upregulate costimulatory molecule expression on macrophages *in vitro* [66]. Particles were found in both aggregated and single nanoparticulate form after incubation with macrophages (Revell and Lewin, unpublished findings) (Fig. 2). The expression of IL1 and TNFα was also less with the two nanoparticles of diamond than it was with the microparticles in our own laboratories [63, 64]. The nanoparticles were difficult to keep separate from their aggregated form and the aggregates were between 0.19 μm and 0.57 μm, i.e. certainly not nanosized. Nevertheless there were clear indications of a difference in behaviour from the microparticles in this size range. An increasing amount of evidence shows that aggregates do not behave like microparticles of the same apparent geometrical shape and overall size, because of the greater surface area of the individual particles forming the the aggregate and present at its surface. Aggregates seem to have the same effects in terms of toxicity and upregulation of inflammatory mediators as the nanoparticles of which they are made.

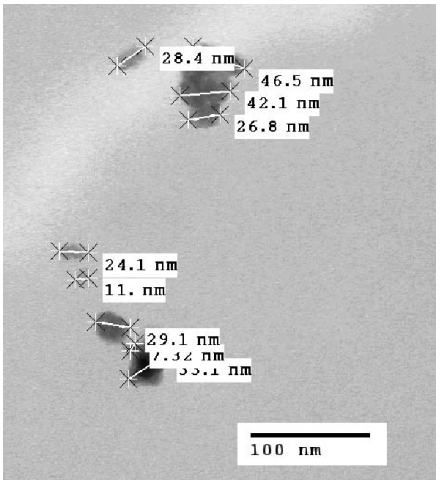

Figure 2a. Electron micrograph by TEM of nanoparticles of hydroxyapatite, occurring as single particles or possibly in pairs.

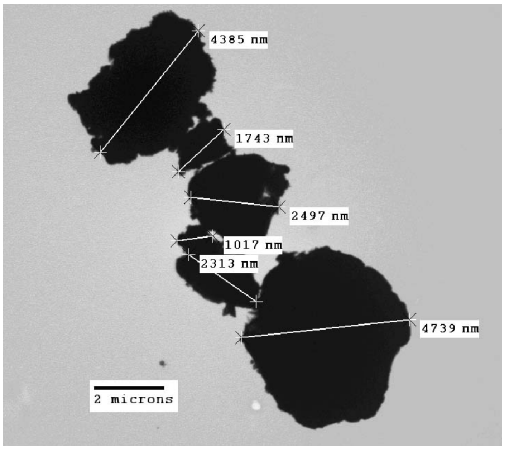

Figure 2b. Electron micrograph by TEM of the same nanoparticles as shown in Fig. 2a. These particles are formed into aggregates between 1 and 4.7 μm in size in this sample.

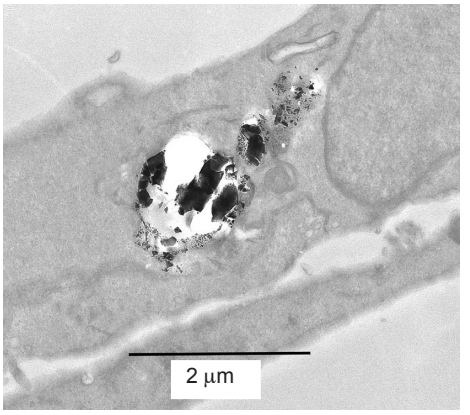

Figure 2c. Electron micrograph by TEM of a macrophage which has internalized the nanoparticles shown in Figure 2a and b after incubation with them in culture. All sizes of aggregate including small ones are present within a membrane-bound phagolysosome. The smallest particles present may be single nanoparticles though they are difficult to resolve at this magnification in a biological preparation. The smaller aggregates on the upper right are associated with some other membrane-containing structure, which may be a mitochondrion.

Particles of nanometre size have been observed in cells both *in vivo* and after cell culture studies. The question arises as to how they are internalized by the cell. Microparticles in the range 0.3–10 μm are the optimum size for phagocytosis [67]. Large particles give rise to multinucleate giant cell formation by the fusion of macrophages. Substances at the molecular level are taken in by pinocytosis. The mechanism for nanoparticles might therefore also be pinocytosis. Pinocytosis involves the ingestion of fluid and solutes via vesicles of about 100 nm in diameter. However, when a human epithelial cell line was cultured with nanoparticles of TiO_2, the particles that had been phagocytosed were in aggregates and separated nanoparticles were present as a layer at the cell surface [68]. Ultrafine particles (< 100 nm) and microparticles (0.2–1 μm) of polystyrene have been found within macrophages after culture but the uptake of the former was blocked by cytochalasin D while that of the microparticles was not [69]. That the nanoparticles were not membrane-bound also suggested that their uptake was not phagocytotic. Possible more "passive" ways in which nanoparticles may be taken up by cells are discussed by these authors [69]. "Nanoforms" of Cr have been described in association with macrophages incubated with 50 ppm of Cr^{3+} *in vitro* [70]. These were formed as chromium phosphate with some protein according to elemental analysis, and were both intracellular and on the surface of the cell membrane. Actin-based mechanisms are involved in phagocytosis of particulate wear debris in the micrometre range and in the internalization of nanoparticles [62].

There are differences in opinion as to whether nanoparticles are, in general, more or less toxic than microparticles of the same material. Apart from our own studies quoted above, there is evidence from various other sources that nanoparticles of materials that are finding use in implants and drug delivery systems are not toxic. Thus SiC, which is an interfacial material for diamond-like coatings of orthopaedic implants, has not been found to be toxic in nanoparticulate form to macrophages *in vitro* [71]. SiC and particles derived from a diamond-like coating also failed to cause inflammatory changes in a rabbit bone chamber model [72]. Using endothelial cells, uptake of a series of nanoparticles into phagolysomes has been demonstrated. No evidence of a cytotoxic effect was found for SiO_2, TiO_2, or Ni nanoparticles and toxic effects were only seen with Co nanoparticles at high doses [73].

10. The possible effects of nanoparticles on the genome

It will by now be clear that nanoparticles readily enter the cytoplasm of cells and have effects on intracellular signalling molecules (see §§2 and 9). Both MAPkinase and NFkB are activated not only in inflammatory but also in neoplastic processes. Whether they are important factors in genotoxicity in relation to particles, including nanoparticles, has yet to be determined. What is clear is that chronic irritation leads on to the development of cancer in a number of different systems, for example the lung and the bladder.

The difference between particles and fibres at the nanometre level on the one hand and non-particulate chemical carcinogens on the other has been emphasised [74], though the differences may not be so clear-cut. Consider, for example, the combined effects of a city dweller who smokes tobacco and inhales fine dusts as part of an occupational exposure. Nanoparticles may themselves form complexes with harmful chemicals, for example diesel exhaust particles comprise a carbon core which may be associated with organic materials like polycyclic aromatic hydrocarbons and transition metals. These cause DNA damage partly by oxidative

mechanisms, some of which may in turn be primarily related to inflammatory processes [75]. DNA damage caused by hexavalent Cr inhalation has been considered to be due to the generation of reactive oxygen species [76]. Carbon black particles have been used as a means of investigating carcinogenetic mechanisms in the rat. Unfortunately, there are special effects from overloading the rat lung with carbon black, but bearing this in mind, lung tumours have been produced by chronic inhalation and instillation in this species [77]. That diesel exhaust fumes are carcinogenic in man and rat seems well documented [11]. The exact mechanisms are less certain. Recently the development of sarcomatous (malignant connective tissue tumours) lesions has been reported in rats implanted subcutaneously with nanoparticles of metals [78, 79]. It has been known for many years that long term subcutaneous implantation of materials in solid sheets or in particulate form gives rise to sarcoma development in rats. This phenomenon is known as the Openheimer effect—tumours developed in the rat with many different materials for which there is not evidence of their promoting malignancy in man or other species. A source of further references on genotoxicity of nanoparticles is available in the review by Donaldson and colleagues [11], which also provides a large amount of other information on combustion-related particles.

11. Summary and conclusions

While there is a large body of evidence about the effects of dusts and larger (micrometre-sized) particles on the different body systems and cells from which they are constituted, there is less known about the biological consequences of exposure to nanoparticles. It is unlikely that the effects will be exactly the same as those with microparticles since the whole physics and chemistry involved changes below a particle size of 100 nm. This "quantum" level of activity means that the particles aggregate readily, and also become associated with other molecules and elements, factors which make the analysis and understanding of mechanisms all the more difficult.

There is a substantial body of material on airborne pollution that includes nanoparticles. The effects of their inhalation in terms of lung disease including carcinoma are described, and relevant cellular biology studies have been performed examining intracellular signalling molecules and the expression of pro-inflammatory cytokines. Nanoparticles may also gain access to the body by ingestion or be generated within joints as wear particles from the bearing surfaces of a prosthetic replacement. That nanoparticles can penetrate the skin is less certain. Direct transneural spread from the nose to the brain has been described. Dissemination around the body in the bloodstream occurs with nanoparticles that have been inhaled, ingested or derived from wear of replaced joints. The sites of deposition of these particles include the liver and spleen, in which organs there may be general consequences of exposure, for example the stimulation of immune processes.

Cellular studies *in vitro* have shown evidence for the up-regulation of antigen presentation in the form of increased costimulatory molecule expression, both for alveolar macrophages and lung epithelial cells with relevant nanoparticles of carbon and for macrophages in general as these relate to nanoparticulate wear. This provides some evidence for the idea that immune stimulation may occur. Whether mutagenesis is also induced in cells exposed to nanoparticles is more difficult to determine. Malignant tumours are readily induced in rat models, both in the lung and after soft tissue implantation, but there are reasons to believe that the rat may not be representative of the situation in other species, including man.

In conclusion, nanoparticles represent a new challenge to those involved with toxicology and biocompatibility since evidence suggests that they behave differently from particles of larger size. Relatively little is known of the biological consequences of exposure to nanoparticles. The increasing availability of sophisticated methods of evaluating biological phenomena, including molecular biology especially as it is applied in immunology and genetics, present opportunities for unfolding knowledge in this exciting and important area.

References

1. BSI. Publicly available specification: Vocabulary—nanoparticles. PAS 71:2005.
2. Airborne Particles Expert Group (APEG) Source apportionment of airborne particulate matter in the United Kingdom. HMSO. 1999.
3. Health Effects Institute Working Group. Diesel exhaust: a critical analysis of emissions, exposure and health effects. Special Report of the Institute's Diesel Working Group. 1995.
4. Kubo, S.; Nakano, M.; Chyatani, S.; Fukumoto, A.; Yamazaki, S. Formation of diesel nanoparticles. International Workshop on Vehicle Exhaust Nanoparticles—implications in the future research (Abstract). 2003.
5. Utell MJ, Frampton MW. Acute health effects of ambient air pollution: the ultrafine particle hypothesis. J Aerosol Med. 2000;**13**:355–359.
6. Lighty JS, Veranth JM, Sarofim AF. Combustion aerosols: factors governing their size and composition and implications to human health. J Air Waste Manag Assoc. 2000;**50**:1565–1618.
7. Neumann HG. Health risk of combustion products: toxicological considerations. Chemosphere. 2001;**42**:473–479.
8. Salvi S, Blomberg A, Rudell B, Kelly F, Sandstrom T, Holgate ST, et al. Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. Am J Respir Crit Care Med. 1999;**159**:702–709.
9. Salvi SS, Nordenhall C, Blomberg A, Rudell B, Pourazar J, Kelly FJ, et al. Acute exposure to diesel exhaust increases IL-8 and GRO-alpha production in healthy human airways. Am J Respir Crit Care Med. 2000;**161**:550–557.
10. Ishihara Y, Kagawa J. Chronic diesel exhaust exposures of rats demonstrate concentration and time-dependent effects on pulmonary inflammation. Inhal Toxicol. 2003;**15**:473–492.
11. Donaldson K, Tran L, Jimenez LA, Duffin R, Newby DE, Mills N, MacNee W and Stone V. Combustion-derived nanoparticles: A review of their toxicology following inhalation exposure. Part Fibre Toxicol 2005;**2**:10–24.
12. Nel AE, Diaz-Sanchez D, Li N. The role of particulate pollutants in pulmonary inflammation and asthma: evidence for the involvement of organic chemicals and oxidative stress. Curr Opin Pulm Med. 2001;**7**:20–26.
13. Bonvallot V, Baeza-Squiban A, Baulig A, Brulant S, Boland S, Muzeau F, Barouki R, Marano F: Organic compounds from diesel exhaust particles elicit a proinflammatory response in human airway epithelial cells and induce cytochrome p450 1A1 expression. Am J Respir Cell Mol Biol 2001;**25**:515–521.
14. Marano F, Boland S, 67 Marano F, Boland S, Bonvallot V, Baulig A, Baeza-Squiban A: Human airway epithelial cells in culture for studying the molecular mechanisms of the inflammatory response triggered by diesel exhaust particles. Cell Biol Toxicol 2002;**18**:315–320.
15. Hiura TS, Kaszubowski MP, Li N, Nel AE: Chemicals in diesel exhaust particles generate reactive oxygen radicals and induce apoptosis in macrophages. J Immunol 1999;**163**:5582–5591.
16. Hashimoto S, Gon Y, Takeshita I, Matsumoto K, Jibiki I, Takizawa H, Kudoh S, Horie T: Diesel exhaust particles activate p38 MAP kinase to produce interleukin 8 and RANTES by human bronchial epithelial cells and N-acetylcysteine attenuates p38 MAP kinase activation. Am J Respir Crit Care Med 2000;**161**:280–285.

17. Takizawa H, Ohtoshi T, Kawasaki S, Kohyama T, Desaki M, Kasama T, Kobayashi K, Nakahara K, Yamamoto K, Matsushima K, Kudoh S: Diesel exhaust particles induce NF-kappa B activation in human bronchial epithelial cells *in vitro*: importance in cytokine transcription. J Immunol 1999;**162**: 4705–4711.
18. Yang HM, Ma JC, Castranova V, Ma JH: Effects of diesel exhaust particles on the release of interleukin-1 and tumor necrosis factor-alpha from rat alveolar macrophages. Experimental Lung Research 1997;**23**:269–284.
19. Steerenberg PA, Zonnenberg JAJ, Dormans JAMA, Joon PT, Wouters IM, vanBree L, Scheepers PTJ, VanLoveren H: Diesel exhaust particles induced release of interleukin 6 and 8 by (primed) human bronchial epithelial cells (BEAS 2B) *in vitro*. Experimental Lung Research 1998;**24**:85–100.
20. Terada N, Hamano N, Maesako KI, Hiruma K, Hohki G, Suzuki K, Ishikawa K, Konno A: Diesel exhaust particulates upregulate histamine receptor mRNA and increase histamine-induced IL-8 and GM-CSF production in nasal epithelial cells and endothelial cells. Clin Exp Allergy 1999;**29**:52–59.
21. Antonini JM: Health effects of welding. Crit Rev Toxicol 2003;**33**:61–103.
22. McNeilly JD, Heal MR, Beverland IJ, Howe A, Gibson MD, Hibbs LR, MacNee W, Donaldson K: Soluble transition metals cause the pro-inflammatory effects of welding fumes *in vitro*. Toxicol Appl Pharmacol 2004,**196**:95–107.
23. Blanc PD, Boushey HA, Wong H, Wintermeyer SF, Bernstein MS: Cytokines in metal fume fever. Amer Rev Respir Dis 1993;**147**:134–138.
24. Kuschner WG, D'Alessandro A, Wintermeyer SF, Wong H, Boushe HA, Blanc PD: Pulmonary responses to purified zinc oxide fume. J Investig Med 1995;**43**:371–378.
25. Shukla A, Timblin C, BeruBe K, Gordon T, McKinney W, Driscoll K, Vacek P, Mossman BT: Inhaled particulate matter causes expression of nuclear factor (NF)- kappaB-related genes and oxidant-dependent NF-kappaB activation *in vitro*. Am J Respir Cell Mol Biol 2000, **23**:182–187.
26. Schwartz J, Dockery DW. Increased mortality in Philadelphia associated with daily air pollution concentrations. Amer Rev Respir Dis 1992;**145**:600–604.
27. Clancy L, Goodman P, Sinclair H, Dockery DW. Effect of air-pollution control on death rates in Dublin, Ireland: an intervention study. Lancet. 2002;**360**:1210–1214.
28. Dockery DW, Pope CA, Xu XP, Spengler JD, Ware JH, Fay ME, Ferris BG, Speizer FE. An association between air-pollution and mortality in 6 United States cities. N Engl J Medicine. 1993;**329**:1753–1759.
29. Peters A, Dockery DW, Muller JE, Mittleman MA. Increased particulate air pollution and the triggering of myocardial infarction. Circulation. 2001;**103**:2810–2815.
30. Peters A, von Klot S, Heier M, Trentinaglia I, Hormann A, Wichmann HE, Lowel H. Exposure to traffic and the onset of myocardial infarction. N Engl J Med. 2004;**351**:1721–1730.
31. Nemmar A, Hoet PH, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts MF, Vanbilloen H, Mortelmans L, Nemery B. Passage of inhaled particles into the blood circulation in humans. Circulation. 2002;**105**:411–414.
32. Nemmar A, Vanbilloen H, Hoylaerts MF, Hoet PH, Verbruggen A, Nemery B. Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. Am J Respir Crit Care Med. 2001;**164**:1665–1668.
33. Kreyling WG, Semmler M, Erbe F, Mayer P, Takenaka S, Schulz H, Oberdorster G, Ziesenis A. Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. J Toxicol Environ Health A. 2002;**65**:1513–1530.
34. Routledge HC, Ayres JG, Townend JN. Why cardiologists should be interested in air pollution. Heart. 2003;**89**:1383–1388.
35. Blomberg A, Törnqvist H, Desmyter L, Deneys V, Hermans C: Exposure to diesel exhaust nanoparticles does not induce blood hypercoagulability in an at-risk population. J Thrombosis Haemostasis 2005;**3**:2103–2105.

36. Oberdorster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, Cox C. Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol. 2004;**16**:437–45.
37. Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, Potter R, Maynard A, Ito Y, Finkelstein J, Oberdorster G. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect. 2006;**114**:1172–8.
38. Yu IJ, Park JD, Park ES, Song KS, Han KT, Han JH, Chung YH, Choi BS, Chung KH, Cho MH: Manganese distribution in brains of Sprague-Dawley rats after 60 days of stainless steel welding fume exposure. Neurotoxicology 2003;**24**:777–785.
39. Borm PJA, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K, Schins R, Stone V, Kreyling W, Lademann J, Krutmann J, Warheit D, Oberdorster E. The potential risks of nanomaterials: a review carried out for ECETOC. Part Fibre Toxicol. 2006;**3**:11–23.
40. Lademann J, Weigmann H, Rickmeyer C, Barthelmes H, Schaefer H, Mueller G, Sterry W. Penetration of titanium dioxide microparticles in a sunscreen formulation into the horny layer and the follicular orifice. Skin Pharmacol Appl Skin Physiol. 1999;**12**:247–256.
41. Gamer AO, Leibold E, van Ravenzwaay B. The *in vitro* absorption of microfine zinc oxide and titanium dioxide through porcine skin. Toxicol *In vitro*. 2006:301–307.
42. Alvarez-Roman R, Naik A, Kalia YN, Guy RH, Fessi H. Skin penetration and distribution of polymeric nanoparticles. J Control Release. 2004;**99**:53–62.
43. Schaefer H, Lademann J. The role of follicular penetration. A differential view. Skin Pharmacol Appl Skin Physiol. 2001;**14**:23–27.
44. Pflucker F, Wendel V, Hohenberg H, Gartner E, Will T, Pfeiffer S, et al. The human stratum corneum layer: an effective barrier against dermal uptake of different forms of topically applied micronised titanium dioxide. Skin Pharmacol Appl Skin Physiol. 2001;**14**:92–97.
45. Lomer MC, Thompson RP, Powell JJ. Fine and ultrafine particles of the diet: influence on the mucosal immune response and association with Crohn's disease. Proc Nutr Soc. 2002;**61**:123–130.
46. McCullough JS, Hodges GM, Dickson GR,Yarwood A,Carr KE. A morphological and microanalytical investigation into the uptake of particulate iron across the gastrointestinal tract of rats J Submicrosc Cytol Pathol 1995;**27**:119–24.
47. Florence AT, Hillery AM, Hussain N, Jani PU. Factors affecting the oral uptake and translocation of polystyrene nanoparticles: histological and analytical evidence J Drug Target 1995;**3**:65–70.
48. Kreyling WG, Semmler M, Erbe F, Mayer P, Takenaka S, Schulz H, et al. Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. J Toxicol Environ Health A. 2002;**65**:1513–1530.
49. Muldoon LL, Pagel MA, Kroll RA, Roman-Goldstein S, Jones RS, Neuwelt EA. A physiological barrier distal to the anatomic blood-brain barrier in a model of transvascular delivery. Am J Neuroradiol. 1999;**20**:217–222.
50. LeFevre ME, Green FH, Joel DD, Laqueur W. Frequency of black pigment in livers and spleens of coal workers: correlation with pulmonary pathology and occupational information. Hum Pathol. 1982;**13**:1121–1126.
51. Khandoga A, Stampfl A, Takenaka S, Schulz H, Radykewicz R, Kreyling W, Krombach F. Ultrafine particles exert prothrombotic but not inflammatory effects on the hepatic microcirculation in healthy mice in vivo. Circulation. 2004;**109**:1320–1325.
52. Saeed S, Hing K, Damien E, Revell PA. Hydroxyapatite activates T lymphocytes and causes inflammation in the liver and spleen of rabbits following intra-osseous implantation. World Congress of Biomaterials. Sydney, 2004.
53. Revell PA, Gatti AM, Gambarelli A, Monari E, Hercus B, Saeed S, MacInnes T. Detection of CoCr particles in the spleen of guinea pigs six weeks after their intra-osseous implantation. Proc 7th World Biomaterials Congress, Sydney, Australia, 753; 2004.
54. Revell PA, Al-Saffar N, Kobayashi A. Biological reaction to debris in relation to joint prostheses. Proc Instn Mech Engrs 1997;**211H**:187–197.

55. Case CP, Langkamer VG, James C, Palmer MR, Kemp AJ, Heap PF and Solomon L. Widespread dissemination of metal debris from implants. J Bone Jt Surg 1994;**76B**:701–712.
56. Revell PA, Tissue reactions to joint prostheses and the products of wear and corrosion. Chapter 3: Current Topics in Pathology Vol 71. Springer-Verlag, Berlin, Heidelberg, New York, 1982, pp 73–102.
57. Revell PA, Pathology of Bone. Springer-Verlag, Berlin, Heidelberg, New York and Tokyo, pp 217–223, 1986.
58. Yamac T, The extraction and characterisation of wear particles from tissues around failed orthopaedic implants of different designs PhD Thesis, University of London, 1999.
59. Doorn PF, Campbell PA, Worrall J, Benya PD, McKellop HA, Amstutz HC. Metal wear particle characterization from metal on metal total hip replacements. J Biomed Mater Res 1998;**42**:103–111.
60. Galvin AL, Tipper JL, McEwen HMJ, Williams S, Stone MH, Ingham E, Fisher J. Nanometre Sized Polyethylene Wear Debris In Hip And Knee Prostheses. Proc 7th World Biomaterials Congress, Sydney,Australia, 345; 2004.
61. Al-Saffar N, Revell PA. Pathology of the bone-implant interfaces. J Long-Term Effects Medical Implants 1999;**9**:319–347.
62. Curtis P.E. Signalling in macrophages following exposure to retrieved wear particles. PhD Thesis, University of London, 2002.
63. Altaf H PhD thesis. The inflammatory response to particlulate wear debris in the context of total hip replacement. University of London, 2006.
64. Altaf H, MacFarlane T and Revell PA. The inflammatory potential of microparticles vs nanoparticles *in vitro*. 20th European Society of Biomaterials Conference. Nantes, France. 29th September 2006.
65. Koike E, Kobayashi T. Organic extract of diesel exhaust particles stimulates expression of Ia and costimulatory molecules associated with antigen presentation in rat peripheral blood monocytes but not in alveolar macrophages Toxicol Appl Pharmacol 2005;**209**:277–285.
66. Altaf H, Saeed S, Bhatt R, Revell PA. The Effects of Biomaterials on the Expression of Costimulatory Molecules Present on Antigen Presenting Cells. 18th European Conference on Biomaterials Stuttgart, Germany T 147, 2003.
67. Green TR, Fisher J, Matthews JB, Stone MH, Ingham E. Effect of size and dose on bone resorption activity of macrophages by *in vitro* clinically relevant ultra high molecular weight polyethylene particles J Biomed Mater Res 2000;**53**:490–7.
68. Stearns RC, Paulauskis JD, Godleski JJ. Endocytosis of ultrafine particles by A549 cells. Am J Respir Cell Mol Biol. 2001;**24**:108–15.
69. Geiser M, Rothen-Rutishauser B, Kapp N, Schürch S, Kreyling W, Schulz H, Semmler M, Im Hof V, Heyder J, Gehr P. Ultrafine Particles Cross Cellular Membranes by Nonphagocytic Mechanisms in Lungs and in Cultured Cells. Environ Health Perspect. 2005;**113**:1555–1560.
70. Catelas I, Vali H, Petit A, Zukor DJ, Antoniou J, Huk OL. How Do Chromium Ions Interact With Macrophages *In vitro*? Trans 29th Annual Meeting Society for Biomaterials 310; 2004.
71. Allen, M. Butter, R. Chandra, L. Lettington, A. Rushton, N. Toxicity of particulate silicon carbide for macrophages, fibroblasts and osteoblast-like cells *in vitro* Biomed Mater Eng 1995;**5**:151–9.
72. Aspenberg, P. Anttila, A. Konttinen, Y.T. Lappalainen, R. Goodman, S.B. Nordsletten, L . Santavirta, S. Benign response to particles of diamond and SiC: bone chamber studies of new joint replacement coating materials in rabbits Biomaterials 1996;**17**:807–812.
73. Peters K, Unger RE, Gatti AM, Monari E, Kirkpatrick CJ. Effects Of Nano-Scaled Particles On Endothelial Cell Functions *In vitro*, Proc 7th World Biomaterials Congress, Sydney Australia. 1881, 2004.
74. Schins RP: Mechanisms of genotoxicity of particles and fibers. Inhal Toxicol 2002;**14**:57–78.
75. Nikula KJ, Snipes MB, Barr EB, Griffith WC, Henderson RF, Mauderly JL: Comparative pulmonary toxicities and carcinogenicities of chronically inhaled diesel exhaust and carbon-black in f344 rats. Fund Appl Toxicol 1995;**25**:80–94.

76. Maeng SH, Chung HW, Yu IJ, Kim HY, Lim CH, Kim KJ, Kim SJ, Ootsuyama Y, Kasai H: Changes of 8-OH-dG levels in DNA and its base excision repair activity in rat lungs after inhalation exposure to hexavalent chromium. Mutat Res 2003;**539**:109–116.

77. Godschalk RW, Moonen EJ, Schilderman PA, Broekmans WM, Kleinjans JC, Van Schooten FJ: Exposure-route-dependent DNA adduct formation by polycyclic aromatic hydrocarbons. Carcinogenesis 2000;**21**:87–92.

78. Hansen T, Boutrand, JP2, Eloy R, Clermont G, Alves A, Gatti AM, Kirkpatrick CJ. Biological Tolerance Of Different Nanoparticles In A Rat Model—Clinical And Histological Findings 7 th World Biomaterials Congress, Sydney, Australia. 752; 2004.

79. Kirkpatrick CJ, Alves A, Kohler H, Kriegsmann J, Bittinger F, Otto M, Williams DF, Eloy R. Biomaterial-induced sarcoma: a novel model to study preneoplastic change. Am J Pathol 2000; **156**:1455–1467.