Do nanoparticles present ecotoxicological risks for the health of the aquatic environment?

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Abstract

Nanotechnology is a major innovative scientific and economic growth area, which may present a variety of hazards for environmental and human health. The surface properties and very small size of nanoparticles and nanotubes provide surfaces that may bind and transport toxic chemical pollutants, as well as possibly being toxic in their own right by generating reactive radicals. There is a wealth of evidence for the harmful effects of nanoscale combustion-derived particulates (ultrafines), which when inhaled can cause a number of pulmonary pathologies in mammals and humans. However, release of manufactured nanoparticles into the aquatic environment is largely an unknown. This review addresses the possible hazards associated with nanomaterials and harmful effects that may result from exposure of aquatic animals to nanoparticles. Possible nanoparticle association with naturally occurring colloids and particles is considered together with how this could affect their bioavailability and uptake into cells and organisms. Uptake by endocytotic routes are identified as probable major mechanisms of entry into cells; potentially leading to various types of toxic cell injury. The higher level consequences for damage to animal health, ecological risk and possible food chain risks for humans are also considered based on known behaviours and toxicities for inhaled and ingested nanoparticles in the terrestrial environment. It is concluded that a precautionary approach is required with individual evaluation of new nanomaterials for risk to the health of the environment. Although current toxicity testing protocols should be generally applicable to identify harmful effects associated with nanoparticles, research into new methods is required to address the special properties of nanomaterials.
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1. Introduction

1.1. Nanoparticles and biological systems

Nanotechnology is a highly promising and exciting cross-cutting molecular technology that spans many areas of science and technological application. However, due to the relative novelty of this technology very little has been done to assess the risks to biological systems; and concerns about the use of the products of nanotechnology are being increasingly expressed in public and in the media (Colvin, 2003, 2004; Dowling, 2004; Howard, 2004; Royal Society and Royal Academy of Engineering, 2004; Warheit, 2004). The recent report by the Royal Society and the Royal Academy of Engineering on nanoscience and nanotechnology is now focusing the attention of environmental managers and policy makers on the possible toxicological and pathological risks to human health and to the environment that may be presented by novel products resulting from nanotechnologies or from the interface between biotechnology and nanotechnology, as in the development of nanotechnology-based drug delivery systems (Dowling, 2004; Howard, 2004; Moore, 2002; Royal Society and the Royal Academy of Engineering, 2004; Warheit, 2004).

Manufactured nanoparticles represent an intermediate supra-molecular state of matter between bulk and molecular material (Hoet et al., 2004). Apart from particle size (one or more dimensions of the order of 100 nm or less) providing a very large surface to volume ratio, their biocompatibility surface properties depend on the charges carried by the particle and its chemical reactivity. Polycationic macromolecules show a strong interaction with cell membranes *in vitro*; and the interaction of nanoparticles with the surface lining layers of biological tissues is determined by their surface chemistry and reactivity (Hoet et al., 2004).

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Surface modification of C60-fullerenes ("buckyballs") to reduce their lipophilicity results in reduced toxicity (Sayes et al., 2004). However, nanofibres and nanotubes may present special problems with respect to their retention in cells and tissues, since it is believed that there is a threshold for the length of a fibre that is critical for induction of adverse biological effects (Hoet et al., 2004).

The fact that the size of the particles itself can be a factor in direct toxicity and pathology is extremely important, and biodegradability may be a further significant factor in governing harmful biological effects (Brown et al., 2001; Hoet et al., 2004; Howard, 2004). Lack of knowledge about the transport and fluxes of these types of particles in the natural environment presents a further problem, which is exacerbated by the fact that biological systems did not evolve in the presence of nanoparticles of the types now being manufactured (Hoet et al., 2004; Howard, 2004). Nanoparticles, other than sea salt, volcanic dust and natural combustion products (e.g., forest fires), have only really been around in significant amounts (e.g., industrial and automobile combustion products) since the industrial revolution (Colvin, 2004; Howard, 2004).

Our knowledge of the harmful effects of nanoparticles is very limited and is almost non-existent in aquatic animals. Uptake of nanoparticles into biological systems may also be facilitated by the caveolar and endocytotic systems in cells with currently very limited knowledge of the pathological consequences if any (Panyam et al., 2003; Pelkmans and Helenius, 2002; Reiman et al., 2004). In some instances it may be possible to predict the reactivity of manufactured nanoparticle surfaces, although given the diversity of particle types verification of such predictions for any new nanoparticle would be advisable (Hoet et al., 2004).

1.2. What is nanotechnology?

Nanoscience endeavours to understand materials at the nanoscale level (i.e., 0.1–100 nm in diameter), and nanotechnology seeks to synthesise, modify and manipulate matter at this level (Royal Society and the Royal Academy of Engineering, 2004). The US National Nanotechnology Initiative (www.nano.gov/nni2.htm) says: "Nanotechnology is concerned with materials and systems whose structures and components exhibit novel and significantly improved physical, chemical and biological properties, phenomena and processes due to their nanoscale size. The goal is to exploit these properties by gaining control of structures and devices at atomic, molecular and supramolecular levels and to learn to efficiently manufacture and use these devices". Because of the nanoscale nature of nanoscience and nanotechnology, they already bridge many fields including medicine, pharmaceuticals, manufacturing technologies, electronics and telecommunications (Gross, 1999; Kim et al., 2005; Perkel, 2004; Royal Society and the Royal Academy of Engineering, 2004).

It is also envisioned that in the future, nanotechnology and biotechnology will coalesce to produce nanoscale systems and devices that use biological principles, since many of the components of cells are already constructed on the nanoscale level,

such as ribosomes, membrane transporters, receptors and cell signalling systems (Moore, 2002; Perkel, 2004).

1.3. Biological interactions of nanoscale particles

A major concern among environmental toxicologists and pathologists is that manufactured nanoparticles may present living systems with a uniquely novel challenge, since such materials were not generally encountered by living organisms during the course of biological evolution (Dowling, 2004; Colvin, 2003, 2004; Howard, 2004; Moore, 2002; Warheit, 2004). Consequently, there will have been little or no selection pressure for defensive or protective systems to counter any adverse properties that such particles may present beyond those already presented by naturally occurring combustion products, volcanic ash, toxic metals and organic xenobiotics.

There is a very large body of evidence that small particles produced by combustion processes known as "ultrafines", or nanoparticles by an older name, can be dangerous to human health (Brook, 2002; Donaldson et al., 2000; Lam et al., 2004; Oberdörster, 2000; Oberdörster et al., 2004; Schwartz, 1994, 2004; Shi et al., 2001; Warheit et al., 2003). Nanoparticles have a proportionately very large surface area and this surface can have a high affinity for metals (e.g., iron) and organic chemical combustion products such as polycyclic aromatic hydrocarbons; PAHs (Cheng et al., 2004). The US Environmental Protection Agency has attributed 60,000 deaths per year to the inhalation of atmospheric nanoparticles; and there is evidence for direct transfer into the brain (Oberdörster et al., 2004; Raloff, 2003).

In medical therapeutics, many drug candidates fail to reach their targets at appropriate concentrations, which can severely limit their effectiveness (Brigger et al., 2002; Oppenheim, 1981). However, when drugs are encapsulated into nanoscale particles and treated to prevent clumping, the result is often a stable and water-soluble material, due to the very large surface to volume ratio (Brigger et al., 2002; Panyam and Labhasetwar, 2003).

New drugs based on nanoparticle-mediated delivery systems are being developed for preventive treatment of the oxidative damage occurring in neurodegenerative diseases like Alzheimer's, Wilson's and Parkinson's (Cui et al., 2005; Dobson, 2001). Other nanoparticle-based medicines are also being investigated for use in cancer therapies and diagnosis where the nanoscale properties facilitate entry and intracellular targeting to specific sites (Brigger et al., 2002). Such particles can have a polymeric matrix (e.g., biotinylated pullulan acetate — BPA), which may or may not be biodegradable, or else consist of a polymeric membrane with either a lipid or aqueous core in which the drug is dissolved (Na et al., 2003).

1.4. Routes of entry into living systems

Uptake of nanoparticles by inhalation or ingestion are likely to be the major routes in terrestrial organisms (Brigger et al., 2002; Dowling, 2004; Colvin, 2003, 2004; Howard, 2004; Moore, 2002; Warheit, 2004). However, in aquatic animals there may be other routes of entry such as direct passage across gill and other

external surface epithelia. Recent studies with fish have indicated that C60-fullerene may be internalised by these routes, although this was a very limited investigation (Oberdörster, 2004).

At the cellular level, most internalisation of nanoparticles will occur via endocytosis. Endocytotic pathways into cells can either lead to the endosomal and lysosomal compartments (conventional endocytosis) or else via cell-surface lipid raft associated domains known as caveolae which avoids the degradative fate of material entering the endosomal/lysosomal system (Na et al., 2003; Panyam and Labhasetwar, 2003; Panyam et al., 2003; Pelkmans and Helenius, 2002; Fig. 1). This latter pathway is a route exploited by many viral pathogens; and in medical nanotechnology,

many of the nanoparticles are designed to enter target cells by these routes (Na et al., 2003; Panyam and Labhasetwar, 2003; Panyam et al., 2003).

1.5. Harmful effects and possible consequences of nanoparticles on biological systems

The very large surface area of ultra-small particles can result in the direct generation of harmful oxyradicals (ROS): these can cause cell injury by attacking DNA, proteins and membranes (Brown et al., 2001). Additionally, the natural propensity for many nanoparticles to bind transition metals and organic
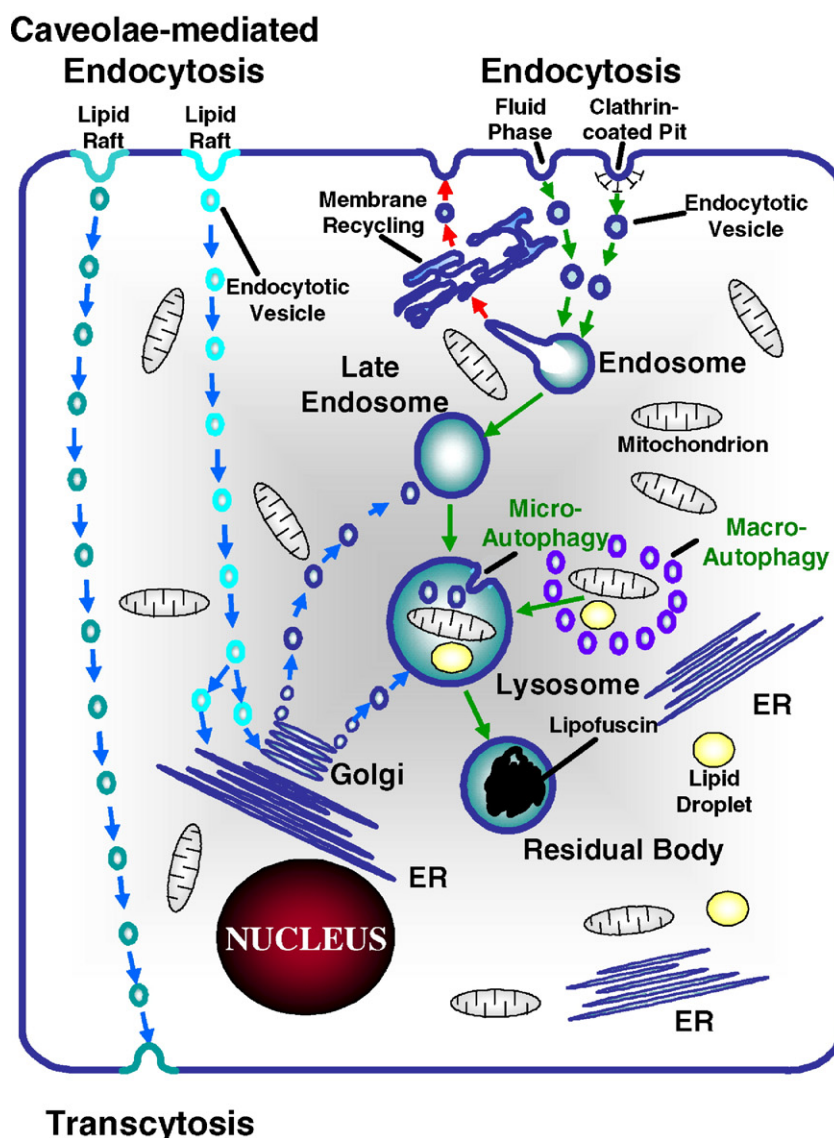

Fig. 1. Pathways for endocytosis in the cell which could be exploited by manufactured nanoparticles. Endocytosis via clathrin-coated pits (receptor mediated) or uncoated pits (fluid phase) transfers materials to the lysosomal degradative compartment, while caveolar endocytosis can result in translocation to the endoplasmic reticulum (ER), Golgi or through the cell by trancytosis (Shin and Abraham, 2001; van der Goot and Gruenberg, 2002).

chemical pollutants is believed to enhance the toxicity of some nanoparticles (Cheng et al., 2004; Gilliland et al., 2004). Furthermore, the ability of the particles to penetrate the body and cells (e.g., via fluid-phase endocytosis and caveolae; Fig. 1) provides potential routes for the delivery of nanoparticle-associated toxic pollutants to sites where they would not normally go (Berry et al., 2004; Lacava et al., 2003; Na et al., 2003; Panyam and Labhasetwar, 2003; Panyam et al., 2003; Pelkmans and Helenius, 2002).

1.6. Environmental release into aquatic ecosystems

Much of what we know about the behaviour of nanoparticles in biological systems has been derived from biomedical investigations of direct injection, ingestion or atmospheric inhalation; and their subsequent nanopathology (Howard, 2004; Royal Society and Royal Academy of Engineering, 2004). Consequently, environmental release of nanoparticles into aquatic systems poses the following questions:

(i) What will be their hydrodynamic behaviour — will ultrasmall particles behave like larger natural particles?
(ii) How will nanoparticles associate with larger sediment and natural colloidal particulates?
(iii) Will nanoparticles bind lipophilic organic and metal pollutants?
(iv) What are the routes of nanoparticle uptake into biota?
(v) Will nanoparticle associated chemical pollutants show enhanced toxicity?
(vi) Will particle size and surface properties be significant factors in determining toxicity and pathogenesis of nanoparticles in aquatic organisms?
(vii) What will be the implications of nanoparticle exposure for organism health and ecosystem integrity?
(viii) Will modelling fluxes and predicted impacts of nanoparticles help to provide an explanatory framework for their environmental behaviour and possible impacts?

This paper will attempt to address these questions in the following sections on the basis of current understanding.

2. Nanoparticles as potential aquatic pollutants

2.1. Nanoparticles entering waterways

Industrial products and wastes tend to end up in waterways (e.g., drainage ditches, rivers, lakes, estuaries and coastal waters) despite safeguards (Daughton, 2004; Moore, 2002; Moore et al., 2004). Consequently as the nanotechnology industries start to come on line with larger scale production, it is inevitable that nanoscale products and by-products will enter the aquatic environment (Daughton, 2004; Howard, 2004; Moore, 2002; Moore et al., 2004; Royal Society and Royal Academy of Engineering, 2004). This makes it an imperative that we have effective risk assessment procedures in place as soon as possible to deal with potential hazards. In developing a risk strategy for manufactured nanoparticles, much can probably be learned from our past ex-

perience with conventional industrial materials and pollutant chemicals such as lipophilic organic xenobiotics (e.g., PAHs, heterocyclics and organohalogens). For instance, fullerenes are lipophilic while many inorganic and polymeric nanoparticles will be hydrophilic (Oppenheim, 1981; Brigger et al., 2002; Sayes et al., 2004).

2.2. Uptake and bioavailability

How will contaminant nanoparticles behave in aquatic systems? We may well be able to use the known behaviours of natural nanoscale or microscale particles, such as colloids (humics), viruses and bacteria; and how these adsorb to or associate with larger biotic and non-biotic particles in the suspended and deposited sediment (Daughton, 2004; Moore et al., 2004; Orlanducci et al., 2004; Readman et al., 1984; Smedes, 1994; Thomas et al., 2000). Suspended sediment particles are known to be important in sequestering and transporting contaminant chemicals over significant distances; and the hydrodynamic and morphological characteristics of bodies of water and coastal zones will largely determine the distribution of bound nanoparticles (Smedes, 1994).

In the marine and estuarine context, we also have to consider what part the sea-surface microlayer, with its lipid, carbohydrate and proteinaceous components, will play in influencing the behaviour of nanoparticles (Wurl and Obbard, 2004). Here the uppermost lipid moiety is probably going to be the major factor providing a medium for lipophilic nanoparticles, such as fullerenes or carbon nanofibres, to partition into. Larger colloidal aggregates of lipophilic nanoparticles may also be coated by microlayer lipid. This behaviour will undoubtedly influence their behaviour and bioavailability in relation to the sub-surface ecosystem, which although largely microbial (bacteria and protists) also includes the pelagic eggs and larvae of many invertebrate and fish species (Wurl and Obbard, 2004).

Uptake of nanoparticles into the aquatic biota is a major concern. Potential routes include direct ingestion or entry across epithelial boundaries such as gills, olfactory organs or body wall. At the cellular level, prokaryotes like bacteria may well be largely protected against the uptake of many types of nanomaterials, since they do not have mechanisms for the bulk transport of supramolecular and colloidal particles across the cell wall. However, with eukaryotes (i.e., protists and metazoans) the situation is very different, since they have highly developed processes for the cellular internalisation of nanoscale (100 nm or less) and microscale (100 nm–c. 100,000 nm) particles, namely endocytosis and phagocytosis respectively (Na et al., 2003; Panyam and Labhasetwar, 2003; Panyam et al., 2003; Pelkmans and Helenius, 2002; Reiman et al., 2004; Synnes et al., 1999; Fig. 1). These processes are integral to key physiological functions such as intracellular digestion and cellular immunity. In invertebrate animals, the cellular immune system, gut epithelium and hepatopancreas (digestive or midgut gland), where present, is likely to be targeted (Moore, 1990). The hepatopancreas is involved in uptake and digestion of food and storage of nutrient reserves; and is frequently specialised for intracellular lysosomal digestion of food via internalisation by endocytosis (Bayne and Moore, 1998; Moore, 1990, 2002; Moore et al., 2004; Owen, 1970). In fish, the

liver is a probable target following endocytotic transport across the intestinal epithelium into the hepatic portal blood system followed by endocytosis into hepatocytes (Smedsrud et al., 1984). Oberdörster (2004) has claimed that colloidal C60 fullerenes are taken up into the brains of largemouth bass and has hypothesised that this transport is via the olfactory nerve.

2.3. Hazard identification — potential harmful effects

Studies with cultured mammalian cells have shown that fullerenes can cause oxidative damage and that their cytotoxicity is related to their lipophilicity (Colvin, 2003, 2004; Sayes et al., 2004). Sayes et al. (2004) have shown that modification of the surface of fullerenes to reduce their lipophilicity, by introducing aliphatic and hydroxyl groups, reduces the cytotoxicity.

Uncoated colloidal fullerenes may cause oxidative damage in the brains of largemouth bass (Oberdörster, 2004). However, this was a very limited investigation and it cannot be ruled out that the effects observed were due to the toxic action of residual solvent used to disperse the fullerenes (Oberdörster, 2004). In the same study there was evidence for a decrease in lipid peroxidation in the liver and gills that may be linked to an increased anti-oxidant defence capacity. These limited findings indicate that interpreta-tion of the effects of fullerenes is problematic and underlines the need for more rigorous experimental exposures.

Nano-size particles have been demonstrated to enter the digestive gland cells of blue mussels and cockles by endocytosis (Moore et al., 1997; Owen, 1970). In mussels, these particles were composed of a sucrose polyester, a zero-calorie food additive, which was taken up endocytotically by isolated hepatopancreatic digestive cells and entered the lysosomal degradative compartment (Fig. 2). The lysosomes in these cells are also a major site of oxyradical generation (Fig. 2; Winston et al., 1991). Uptake of sucrose polyester into the hepatopancreas of whole mussels from seawater was also demonstrated and was found to increase the uptake (160%) and cellular toxicity (122%) of the PAH anthracene (Fig. 2; Moore et al., 1997).

Exploitation of caveolar/endocytotic routes of entry to the cell may allow pollutant nanoparticles to embed themselves within the functional machinery of the cell in ways that are toxicologically quite different from conventional toxic chemicals (Fig. 1). Nanoparticles situated in the endoplasmic reticulum, Golgi and endo-lysosomal system could conceivably act as foci of oxidative damage that could not readily be expelled from the cell; while generation of radicals could lead to organelle dysfunction.
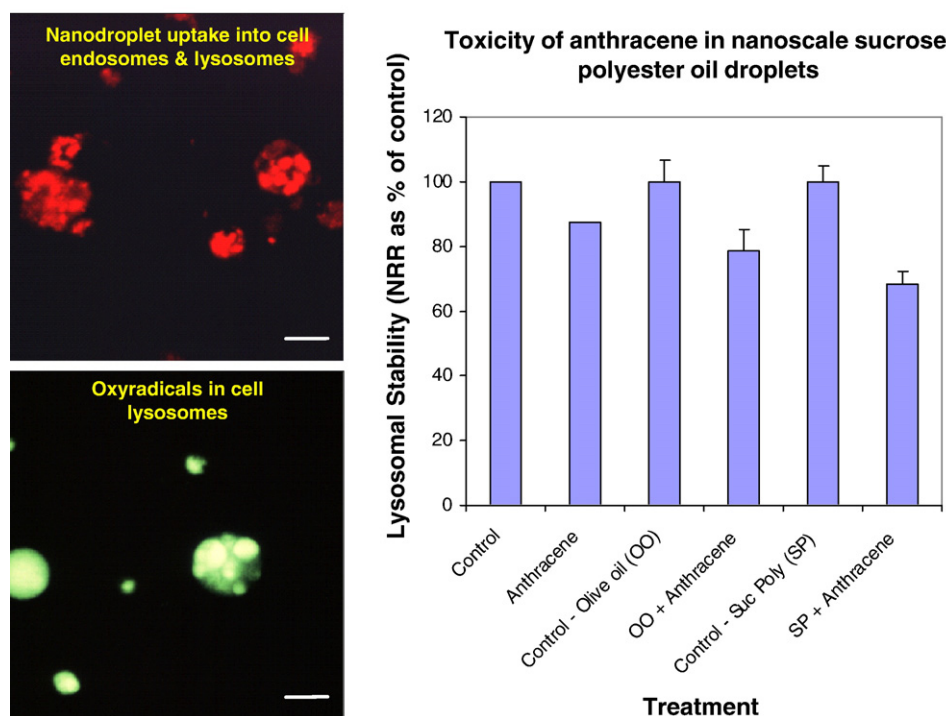

Fig. 2. Confocal images of endosomal and lysosomal accumulation (red fluorescence) following endocytosis of Nile red labelled sucrose polyester nanodroplets (nanoparticles) by mussel isolated hepatopancreatic digestive cells; and normal oxyradical generation (green fluorescence) in lysosomes of isolated digestive cells (dihydrorhodamine 123 method). Uptake of sucrose polyester nanodroplets by digestive cells in whole animals enhances the toxicity, of the polycyclic aromatic hydrocarbon anthracene, measured as reduction of lysosomal membrane stability (3 day exposure at 10 °C; 2 mussels/l of seawater; initial daily concentration of both oils 10 µl l^{-1}; initial daily anthracene concentration 200 µg l^{-1}; mean±95% CL, n=10; Moore et al., 1997; Winston et al., 1991). Control values on the graph have been standardised for clarity as olive oil and sucrose polyester had no significant effect on lysosomal stability. Scale bar on fluorescence micrographs≡10 µm.

2.4. Naturally occurring biologically derived nanoscale particles

Inorganic nanoparticles have been identified in human liver and kidney tissues (Gatti and Rivasi, 2002). These authors coined the term "nanopathology" and believe that these particles, which are of exogenous (ceramic?) origin, could have a causal link with cryptogenic granulomas in the tissues examined. In aquatic and terrestrial invertebrates, metalliferous nano- and microscale granules are frequently found in cells of digestive and excretory tissues (Viarengo and Nott, 1993). Nott and Nicolaidou (1990) have developed models for the formation of these granules, some of which are related to the lysosomal system, and their role in the sequestration, detoxication and biomineralisation of toxic metals such as iron, copper, mercury, lead, silver, chromium and nickel (Nott and Nicolaidou, 1990; Viarengo and Nott, 1993).

Other naturally generated nanoparticles include age- or stress pigment granules (lipofuscin) which are produced in cells by oxidative attack on lipoproteins (Brunk and Terman, 2002; Moore, 1990; Winston et al., 1991, 1996). Lipofuscin is formed in mitochondria and lysosomes: the mitochondrial lipofuscin is transferred to the lysosomal compartment by macroautophagy as part of organelle turnover (Brunk and Terman, 2002; Moore et al., 2006). Lipofuscin was previously believed to be an inert product of cellular ageing which had little effect other than to occupy cell volume. Recently, Brunk and Terman (2002) have proposed that lipofuscin is not in fact inert, as was previously thought to be the case, since it binds iron, and probably other transition metals such as copper and nickel, which result in the generation of reactive oxygen species. They further proposed that lipofuscin may also bind lysosomal hydrolases, hence blocking their active sites; and consequently inhibiting lysosomal degradation of proteins and complex carbohydrates as part of normal heterophagic and autophagic recycling (Terman and Brunk, 2004; Fig. 1).

If we consider metalliferous granules and lipofuscin granules as naturally occurring and biologically derived nanoparticles, then perhaps we can use their toxico-pathological properties as baselines for comparison of their properties with those of some types of manufactured nanoparticles. Furthermore, based on Brunk and Terman's hypothesis for reactive lipofuscin (Terman and Brunk, 2004), the question arises as to whether intralysosomal nanoparticles with specific surface properties capable of generating reactive nitrogen and oxygen species (RNOS) could contribute to cellular ageing? We also need to consider the possibility of nanoparticle overload of the endo-lysosomal system, which in itself could lead to dysfunction in lysosomal degradative capacity and interfere with programmed autophagic cell death and breakdown of ingested pathogens by cells of the immune system (Cuervo, 2004).

2.5. Risks to aquatic biota and ecosystems

As we can glean from the above, manufactured nanoparticles are still largely an unknown quantity in terms of how they will behave in any environment, let alone the aquatic medium.

However, we may be able to make some reasonable predictions about potential hazards based on what we know of their size, surface charges and chemical reactivity; but as they are essentially supramolecular entities they may also have novel emergent properties in respect of their biological interactions that are not readily predictable from knowledge of the surface characteristics (Colvin, 2003; Hoet et al., 2004; Howard, 2004; Warheit, 2004).

Given the complexity of aquatic systems just at the physical level of suspended sediment particles, natural colloids like humics and the microlayer; predicting the physical behaviours of nanoparticles is likely to be much more difficult than predicting those of conventional chemical pollutants, which is still often a major challenge (Howard, 1997; Readman et al., 1984; Smedes, 1994; Thomas et al., 2000; Warne and Hawker, 1995; Zhou et al., 1998, 1999).

Uptake by organisms again presents us with another set of issues including phyletic and species differences superimposed onto the role of particular groups of organisms within the functional ecology of particular assemblages and ecosystems (Rice, 2003).

Similar constraints on our understanding also apply to the putative toxicities of RNOS, hence conventional cellular toxicology will largely apply in pinpointing hazards; and we may be able to make valid assumptions leading to reasonable predictions of cell and tissue injury (Howard, 2004; Livingstone, 2001; Livingstone et al., 2000). However, if nanoscale dimensions confer novel supramolecular characteristics resulting in direct interactions with cellular components such as cytoskeleton, mitochondria, specific receptors or membrane transporters, then the problem assumes a higher level of complexity. And this is likely to prove less tractable in the short term, for the purpose of evaluating probable risk factors, unless we are able to identify a set of generic rules governing the probable nanotoxicological characteristics for the ever increasing variety of manufactured nanoparticles (Colvin, 2004; Howard, 2004; Moore et al., 2004; Warheit, 2004). However, it seems reasonable to infer that oxidative damage will be a probable outcome from exposure to some types of surface reactive nanoparticles; and here we can draw on the existing body of knowledge for this type of stress in aquatic species (Livingstone, 2001).

Extrapolating to the higher levels of impact on ecosystem health status only compounds the problem of prediction, until we have sufficient baseline data on toxicity and pathology induced by nanoparticles. Consequently, until we can effectively discount specific or generalised hazards associated with various types of nanoparticle we should invoke a precautionary approach (Colvin, 2003; Howard, 2004; Royal Society and Royal Academy of Engineering, 2004). This will require testing of existing and new nanomaterials to determine individual level impacts on animal health status.

2.6. Risks to human health via water and aquatic systems

Accidental spillages or permitted release of industrial effluents in waterways and aquatic systems may result in direct exposure to nanoparticles of humans via skin contact, inhalation of water aerosols and direct ingestion of contaminated drinking

water or particles adsorbed on vegetables or other foodstuffs (Daughton, 2004; Howard, 2004).

More indirect exposure could arise from ingestion of organisms such as fish and shellfish (i.e., molluscs and crustaceans) as part of the human diet. Surface sediment- and filter-feeding molluscs are prime candidates for uptake of manufactured nanoparticles from environmental releases, if it transpires that some of these nanomaterials will associate with natural particulates; since the molluscs are already known to accumulate suspended particle- and sediment-associated conventional pollutants (Galloway et al., 2002; Livingstone, 2001).

2.7. Biomarkers in key biota as early warning indicators of risk to humans and ecosystems

Biomarkers of exposure and effect may provide tools for assessing the uptake, bioavailability and harmful effects of nanoscale materials in the aquatic environment, as they already do for more conventional chemical pollutants (Depledge et al., 1993; Galloway et al., 2002, 2004; Livingstone et al., 2000; Lowe et al., 1995a,b; Moore et al., 2004; Wells et al., 2001). However, as with more conventional chemical contaminants, we are probably unlikely to find specific biomarkers for nanoparticles and will have to rely on biomarker tests for generic effects such as oxidative damage, depletion or enhancement of antioxidant defences, mitochondrial and lysosomal dysfuntion, and cell and tissue pathology (Livingstone, 1993, 2001; Livingstone et al., 1990, 2000). The phase I and II biotransformation systems are perhaps unlikely to be directly affected by lipophilic nanoparticles. However, this type of particle may well act as a carrier for lipophilic xenobiotics such as PAHs and organohalogens, in which case the biotransfomation system may come into play if the xenobiotics are released from the confines of the particle (Cheng et al., 2004; Gilliland et al., 2004; Moore et al., 1997). Similarly, facilitated xenobiotic induced cell injury is unlikely to occur if the chemical is trapped within the particle or within a hollow micelle formed by the nanoparticles. However, the facilitated uptake of anthracene by nanoscale sucrose polyester particles did result in damage to the lysosomal system in the hepatopancreatic cells of mussels; indicating that although the sucrose polyester was not biodegradable, even in lysosomes, the PAH must have been released in order to cause cell injury (Fig. 2; Moore et al., 1997).

We also need to be able to identify "common targets" in the biota. These may well include evolutionarily highly conserved systems such as vesicular transport (i.e., caveolae, endocytosis, lysosomal degradation), mitochondria, biotransformation systems and xenobiotic/drug transporters (Depledge et al., 1993; Kurelec, 1993; Livingstone, 1991; Livingstone et al., 2000; Lowe et al., 1995a,b; Moore et al., 2006; Shin and Abraham, 2001; Svendseb and Weeks, 1995; van der Goot and Gruenberg, 2002). For example, lysosomal functional integrity is one such generic common target in all eukaryotic organisms, that is also a good diagnostic and prognostic biomarker for individual health status (Allen and Moore, 2004; Bayne and Moore, 1998; Galloway et al., 2002, 2004; Köhler et al., 1992, 2002; Lawrence et al., 2003; Lekube et al., 2000; Moore, 2002; Moore et al., 2006; Winston

et al., 2002). This biomarker can also be used to predict liver damage and tumour progression in fish liver, as well as enhanced protein turnover (i.e., lysosomal autophagy), as a result of radical attack on proteins, immunocompetence and energetic status (i.e., scope for growth) as a predictive indicator of fitness of individuals within a population (Allen and Moore, 2004; Kirchin et al., 1992; Moore et al., 2004, 2006).

Similarly we may be able to use lipid peroxidation and protein carbonyl or adduct formation as general indicators of oxidative damage (Livingstone et al., 2000; Kirchin et al., 1992). Biomarkers that are specific to nanoparticles are perhaps more likely to emerge at higher levels of cell and tissue organisation. These may manifest as overload of the lysosomal system in cells of the immune system, fish liver and invertebrate hepatopancreas or midgut gland; or amyloid plaque formation in brain tissues, as observed in some neurodegenerative disorders such as BSE, CJD and Alzheimer's (Cuervo, 2004).

By extending the concept of common targets for potential pollutant nanoparticles in multicellular organisms, it is perhaps not unreasonable to suggest that evidence of harmful environmental impact in the aquatic biota could act as an early warning for possible risk to human health.

2.8. Modelling nanotoxicity

Computational modelling of whole biological systems from cells to organs is gaining momentum in cell biology and disease studies. This development is essential for the derivation of explanatory frameworks that will enable a predictive capacity for estimating outcomes or risk associated with particular disease processes and therapeutic or stressful treatments (Bock and Goode, 2002; Chicurel, 1999; Hunter et al., 2002; Moore and Noble, 2004; Moore and Willows, 1998; Noble et al., 1999; Noble, 2002a,b,c). Recent advances in measuring key molecular and cellular reactions in disease have provided a wealth of detail on the processes involved, and this, coupled with the dramatic increase in computing power, has allowed the development of numerical and computational models of cellular function and perturbation (Hunter et al., 2002). Complex structured models can provide vital tools in the investigation of fundamental induced disease processes; and also provide a predictive capability for risk management (Allen and Moore, 2004; Moore and Noble, 2004). In an ecotoxicological context, the envisaged longer-term end-product is the creation of "Virtual Animals" as tools for integration and prediction in environmental health risk management (Allen and Moore, 2004; Moore, 2002; Noble, 2002a).

Such simulation models will have to incorporate all of the major cellular physiological processes, including the various endocytotic and caveolar pathways into the cell, which specify the route of uptake, aspects of intracellular behaviour and eventual fate; and also those that are essential for maintaining cellular defence and tissue and organ integrity (Allen and McVeigh, 2004; Livingstone et al., 2000; Lowe et al., 1995a; McVeigh et al., 2004; Moore and Allen, 2002; Moore et al., 2004; Stegeman and Lech, 1991). The increasing deployment of nanoparticles in biomedicine for probing processes and in drug delivery has not only

opened up a potential cornucopia of possibilities for drug design and therapeutics, but has also provided new insights into basic biological processes (Brigger et al., 2002; Dowling, 2004; Gould, 2004; Hoet et al., 2004; Howard, 2004; Panyam and Labhasetwar, 2003). This knowledge will aid our understanding of the biological interaction of nanoparticles, which in turn will facilitate the development of *in silico* simulation as a tool to predict the potential environmental toxicity of manufactured nanoparticles. Model design and development will require a capacity for incorporation of data on particle surface properties, size and biodegradability if simulation is to provide an effective tool for hazard identification and risk management (Brigger et al., 2002; Gatti and Rivasi, 2002; Hoet et al., 2004; Howard, 2004; Panyam and Labhasetwar, 2003).

3. Conclusions

Nanotechnology holds out the promise of immense improvements in economic growth, health and manufacturing technologies; and even environmental remediation. Both the nanotechnology industries and governments are now seriously considering the possibility of unforeseen risks for human health and environmental degradation as a result of this novel technology (Colvin, 2004; Hoet et al., 2004; Howard, 2004; Perkel, 2004; Royal Society and Royal Academy of Engineering, 2004; Wilsdon and Willis, 2004). The environmental science community also needs to embrace this positive approach, and devise appropriate testing protocols and predictive tools for addressing the crucial issue of risk of harmful impacts. Only by doing so, will environmental managers and policy makers have the necessary information on which to base their decisions for environmental regulations regarding the safe use and disposal of industrial nanoparticles (see US Environmental Protection Agency webpage; http://es.epa.gov/ncer/nano/factsheet). By following such an approach they will be better placed to avoid the problems that have occurred with the products of plant biotechnology, where a combination of poor public understanding of science coupled with the actions of some environmental activists, and a section of the media, who used the relative lack of effective risk assessments to label genetically modified plants as "Frankenstein Crops".

The development of an effective working relationship between industry, governments and an independent environmental science community will facilitate the development of a coherent approach to the identification of environmental hazards and the design of nano-risk protocols (Royal Society and Royal Academy of Engineering, 2004).

For regulatory purposes, a precautionary approach has been recommended by the Royal Society and Royal Academy of Engineering (2004). This will probably require that each type of new nanomaterial should be treated individually for toxicity and risks to the health of the environment, as it is not feasible to generalise about the toxicity of nanoparticles. Even though existing nanomaterials are very diverse in their composition and surface properties, current toxicity testing protocols should still be generally applicable to identify harmful effects associated with nanoparticles (Hoet et al., 2004). Proposed new regulatory frameworks for chemical risk assessment procedures such as the European Union's REACH (*R*egistration, *E*valuation and *A*uthorisation of *Ch*emicals) may be suitable for adaptation to include nanomaterials (http://europa.eu.int/comm/environment/chemicals/reach.htm).

Since there is so little data available for aquatic environments, research is required to test the behaviour and particulate binding properties of manufactured nanoparticles in both freshwater and seawater: salinity may alter surface characteristics of nanomaterials. The relative importance of the endocytotic and caveolar routes of uptake identified above, also needs to be assessed in representative aquatic species, since this will be a crucial factor governing intracellular behaviour, distribution, fate and toxicity of internalised nanomaterials.

A major challenge for ecotoxicologists will be the derivation of toxicity thresholds for nanomaterials; and determining whether or not currently available biomarkers of harmful effect will also be effective for environmental nanotoxicity and nanopathology. If new methods are required to assess the toxicity of nanomaterials, then these tests will also need to be linked if possible with functional ecosystem indices (Depledge et al., 1993; Galloway et al., 2002, 2004; Rice, 2003; Winston et al., 2002). Such linkage would be desirable in order to bridge the gap between individual organism "health-status" and ecosystem-level functional properties; and how they affect and are quantifiably connected to health of the environment (Allen and Moore, 2004; Colvin, 2003; Depledge et al., 1993; Galloway et al., 2002, 2004; Lowe et al., 1995a; Rice, 2003; Winston et al., 2002).

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