A tale of opportunities, uncertainties, and risks

Nanoscience and nanotechnologies are expected to change industrial production and economics over the decades to come. This new field is also exciting since it sweeps away the traditional barriers between disciplines such as chemistry, physics, and biology. Nanotechnology requires different thinking in management, collaboration, value chain propositions, education, and calls for research grants. Apart from the benefits and challenges, nanotechnologies also produce uncertainties and risks. For some, the degree of potential hazard associated with nanoparticles is so disquieting that in January 2008 the UK Soil Association adopted a nano-free policy for products certified as organic.

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Nanomaterials are being introduced into the market on the basis of claimed benefits and their chemical identity is pegged to already existing legislation, regardless of some unique characteristics. Data on the distribution of some nanomaterials and their toxicity are now emerging and need careful interpretation before generalizing for all nanomaterials.

Regulation is unable to keep pace with development and treats nano-related products as a slippery customer. Consumers are uncertain how they feel about nanotechnology, whether product claims by some proponents represent objective reality, or if a product claiming to be associated with nanotechnology is legitimately a 'nano' product. Unfortunately, neither experts nor the media have added much clarity to the tumult. Experts find their research often under- or misreported and, in too many cases, the media seems more concerned with writing captivating headlines than acting as the empowering estate behind a public sphere[1]. We discuss three examples of nanomaterials that are being explored for wider application. These examples are used to illustrate the opportunities, uncertainties, and risks for nanomaterials.

Image above: detail of a macrophage containing nanoparticles of TiO_2.

Opportunities

The market for nano-based products is increasing rapidly. It includes medical products (e.g. heart valves, drug-delivery systems, and imaging techniques), electronic components, scratch-free paint, sports equipment, wrinkle- and stain-resistant fabrics, sunscreens, and other cosmetics. Analysts estimate that the European market for such products is currently ~€2.5 billion but could rise to hundreds of billions by 2015 and €1 trillion thereafter[2]. Lux Research reports that corporations spent $3.8 billion globally on nanotechnology research and development in 2004. Approximately $900 million in venture capital has gone to nanotechnology companies since 1999, with

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$386 million invested in 2003. Furthermore, Lux predicts that, by 2014, nanotechnology will be associated with 15% of all manufactured goods, worth roughly $2.6 trillion. Products incorporating emerging nanotechnologies will constitute $920 billion in value added, accounting for 2% of global gross domestic product. Manufacturing incorporating nanotechnology will be responsible for 10 million jobs worldwide, comprising 11% of total manufacturing jobs[3].

Another reason why we expect so much from nanotechnology is that we sense it will enable a cleaner environment. Firstly, it can be expected that fabrication will shift from top down to bottom up. Secondly, existing technologies and materials will be improved in such a way that efficiencies increase and properties can be controlled. Thirdly, there are claims that some nanoparticles might be effective in remediating some of our worst toxic wastes.

Uncertainties and risks

Titanium dioxide (TiO_2), amorphous silica (SiO_2), and iron oxides are bulk nano products on the market already and are present in many consumer products, including food additives, pigments, paints, and cosmetics. It is useful to focus our attention on the newer generation of engineered nanoparticles (ENPs), to discriminate between the good, the bad, and the ugly, and design regulation and testing for these materials, lest we find them on the market without proper vetting. Methods that can be used to screen out bad and ugly ENPs in early phase development are among industry's most pressing needs. The same is true for regulation: although regulation seems to be adequate based on the anticipated application of these substances, there are reasonable doubts that current approaches and tests in toxicology will be sufficient to screen all nanomaterials. In the past few years, several expert reports[4], reviews[5–7], and technology assessments[8] have pointed to the potential impact of nanomaterials on society and the need to resolve current uncertainties.

Originally, concern was driven by findings on ultrafine particles (defined as primary particles and aggregates <100 nm), such as those occurring in ambient air pollution. These ultrafine particles mostly consist of combustion-derived nanoparticles (CDNPs), such as diesel exhaust particles, and have been shown to affect cardiovascular rhythm, peripheral blood flow, and blood coagulation, accelerating atherosclerosis in patients with existing cardiovascular disease[9,10]. While there was considerable skepticism in the nanoscience world about the meaning of these findings for 'real' (meaning engineered) nanomaterials, recent studies are showing that we should take the parallels very seriously. *In vitro* and *in vivo* studies with carbon nanotubes (CNTs) demonstrate similar actions of CNTs on blood coagulation as ambient airborne particulate matter (PM), and can exert effects such as granuloma formation and inflammation in the lung after inhalation. The pulmonary effects can largely be explained by the normal paradigms of particle toxicology[11] including surface dose, aspect ratio of fibrous particulates, and dose rate. The effects on cardiovascular response also seem to be in line with recent findings for ultrafine particles. One of the first studies to test ambient particles and ENPs in blood coagulation showed similar effects for CNTs and ambient PM[12].

A relatively new series of studies have been initiated based on the different body distributions of nanoparticles compared with fine (<2.5 μm) particles[13,14]. For instance, the fact that ENPs are able to cross the blood-brain barrier (BBB), reach the olfactory bulb in the brain, and may pass the placental barrier[4] has caused concern about the effects of particles on these organs. It also raises concerns about the potential effects of translocated ENPs on neurodegenerative diseases such as Alzheimer's and Parkinson's[15]. To further compound the uncertainty, relatively little information is available on the environmental distribution and effects of nanomaterials.

Some types of ENPs (e.g. TiO_2, fullerenes) exhibit photocatalytic effects and generate reactive oxygen species. Because of this, TiO_2 nanoparticles have been investigated for the degradation of organic pollutants, water decontamination, air purification, and as photocatalytic coatings. However, these very same properties, desirable in a product or application, may become hazardous if they are active in the wrong place. For example, in the aquatic environment, ENPs will undergo reactions with cations, anions, and natural organic matter (NOM). These reactions may lead to surface modifications, aggregation of ENPs, or aggregation with particles, flocs, and microorganisms.

Real life examples and life cycle assessment

In the following, we detail a few examples showing the complexity of the life cycle and risk-benefit analysis. These are by no means worst or best cases scenarios, but show different sides of the same story.

Nanosilver

Nanosilver coatings are known for their antimicrobial action and are increasingly being used in a range of products such as wound dressings, urinary catheters, self-cleaning clothes, toothpastes, and washing machines[16]. Nanosilver is anticipated to play a major role in many different coatings from food packaging to wall/roof coverings.

Although the principle is based on the slow formation and release of Ag ions, there is little information on the influence of wear on these products and the release of Ag nanoparticles or ions from coatings into the surrounding media. Although the toxicology of Ag ions is well described, it is mostly based on acute spills and high concentrations. From an environmental and health perspective, nanosilver could be dangerous since general exposure to Ag may damage aquatic environments, which are highly sensitive. This concern has led some waste treatment organizations in the US to protest at its use in washing machines. Both TriTAC (a technical advisory group for Publicly-Owned Treatment Works in California) and the National

Association of Clean Water Agencies (NACWA) submitted letters of concern to the US Environmental Protection Agency in 2006.

There is much less data on the effects of chronic exposure to low levels of Ag ions and nothing on Ag nanoparticles, which could act as a carrier system for local release. A strong argument for the introduction of Ag is that bacteria cannot develop resistance to it as they tend to do against antibiotics. Not so, according to some who believe that Ag resistance can be accomplished relatively fast – comparable to susceptibility effects to antibiotics. They claim that Ag resistance is most easily developed in bacteria with already documented resistance mechanisms to antibiotics, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and others. Ag resistance associated with antibiotic resistance has been observed in isolated bacteria from birds[17] and in salmonella[18]. There are Ag-resistant bacteria in our mouths[19], which may possibly be related to the Ag in amalgam fillings. Ag-resistant bacteria have also been found in nature, food, intestinal bacteria from different geographic locations, and hospitals[20]. A systematic review of clinical trials with Ag-coated urinary catheters reveals that few studies have addressed secondary bloodstream infection, mortality, costs, or microbial resistance[21], although some studies[22] have revealed no Ag-resistant pathogens within a two-year trial period.

Cerium oxide nanoparticles

Cerium oxide (CeO_2) nanoparticles are being added to diesel as a catalyst to reduce toxic exhaust emission gases and particulate emission from diesel vehicles. Envirox™, a fuel-borne CeO_2 catalyst that reduces fuel consumption and particulate emissions, has been developed by Oxonica, who claims it enables fuel savings of up to 10% and reduces particulate emissions. Envirox has been successfully trialed in buses in Hong Kong and is now being introduced by one of the UK's largest bus operators, Stagecoach. It is unclear to what extent the emission of CeO_2 will influence the current ambient exposure to nanoparticles and its potential hazards.

Data from Oxonica describe a number of classic endpoint studies (dermal irritation, cytotoxicity, bacterial mutagenicity, and *Daphnia magna* immobilization) with two forms of CeO_2 (9 nm and 320 nm)[23]. Neither of these oxides show *in vitro* cytotoxicity, mutagenicity, effects on *Daphnia*, nor inhibition of sewage sludge respiration. Although the tests are not specifically validated for nanomaterials, they show that neither normal nor nanosized CeO_2 used in diesel fuel has an effect. Of course, in the application of CeO_2, the most relevant tests will be inhalation of relevant doses. With respect to this, in tests, exposure of lung tissue to neat CeO_2 aerosols and diesel particulates with and without CeO_2 show no toxic effects. An environmental impact and life cycle assessment taking into account fuel efficiency savings was positive as well. Medical applications of CeO_2 may also be pursued based on its radical scavenging properties, and recent reports suggest a protective effect of autocatalytic ceria in different cell types[24].

Titanium dioxide and zinc oxide nanoparticles

TiO_2 and ZnO nanoparticles are increasingly being used in many different products, especially sunscreens where they are receiving attention from environmental groups[25]. Uptake of nanoparticles from these products through the skin would cause a considerable burden in the body. A discussion is therefore crucial as to whether nanoparticles remain on the skin or can reach systemic circulation and target organs. While there is little evidence that nanoparticles in sunscreens cross the stratum corneum into the dermis and from there migrate elsewhere, concerns remain about entry via damaged skin and there is some evidence of oxidative stress and damage to naked DNA *in vitro* involving inorganic sunscreen ingredients[4,26]. In addition, recent work suggests that, once in the bloodstream, these particles might be taxing on the liver, placenta, and brain[27].

TiO_2 nanoparticles are used in many other applications. For example, they are employed in coatings for saltwater vessels to control antifouling and reduce corrosion. Since they need to be reapplied, wear or sloughing does take place. The effect of TiO_2 nanoparticles on ocean organisms remains unknown. TiO_2 is also a component of some water-treatment technologies. It degrades volatile organic compounds and kills bacteria (like *Escherichia coli*) in the presence of light. Currently, a TiO_2 granular media – Adsorbia™GTO™ – is marketed by Dow to remove As from water. Whether these particles end up elsewhere, from handling or leeching into drinking water, remains to be resolved.

While nearly one third of sunscreens allegedly contain ZnO nanoparticles, they have received much less attention. Because ZnO offers ultraviolet (UV) protection and tends to stay on the skin longer, they have been used in sunscreens for many years. In 2005, the Scientific Committee on Consumer and Non-Food Products of the European Commission Health and Consumer Protection Directorate-General (SCCNFP) pointed out that the physico-chemical specifications of ZnO used in many of the submitted studies were incomplete[28]. The SCCNFP's main concern is related to the risk assessment of 200 nm ZnO particles, which may be coated with other compounds and are used as an ingredient in sunscreens. In addition, 200 nm ZnO particles have been demonstrated to be photoclastogenic, possibly photo-aneugenic, and a photo-DNA damaging agent in mammalian cells cultured *in vitro*. Clarification of the relevance of these findings is required by appropriate investigations *in vivo*. There is a lack of reliable data on the percutaneous absorption of microfine ZnO. SCCNFP is of the opinion that more information is required to enable a proper safety evaluation of microfine ZnO for use as a UV filter in cosmetics[28].

Different perceptions and lack of data

What emerges from the examples above is a risk-benefit evaluation with incomplete information on the risk side and, we may discover, the same on the benefit side as well. This is typically the dilemma for many applications of nanoparticles. It appears that we do not have a full understanding or appreciation of the longer-term implications or toxic

effects of free, as opposed to fixed or biodegradable, nanoparticles. There remains an incomplete conceptual understanding of the properties of ENPs that could cause toxicity, or the routes by which they can be taken up and distributed in the body. The traditional model of risk analysis could be insufficient for studying the implications of ENPs given what little we know about dosage and exposure.

This makes nanotechnology a slippery customer for regulators[29] since a huge area of legislation (e.g. environment, food, workforce) is affected. It has been seriously discussed whether Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) – the new chemical inventory system in the European Union (EU) – and the Toxic Substances Control Act (TSCA) in the US are applicable and valid for nanomaterials. However, the flood of consumer 'nano' products tells us that nanotechnology is already on the market without life cycle analysis or sufficient toxicity testing. ENPs are not anticipated to be regulated in the EU in the immediate future because they are not distinguished from the same chemicals as coarse particles or bulk material under REACH. A very similar situation exists with TSCA in the US. The wide variety of routes by which ENPs could be taken up by the body complicates the definition used in risk assessment and regulation, since there is insufficient understanding of the properties that could cause toxicity[30,31]. For risk assessment and future regulation of ENPs, several crucial issues need to be considered:

- Effects may be specific to ENPs and not present for the same or other materials of larger size or agglomerates. In this case, ENP effects may be quantitatively different and regulation may need to be adapted by changing values and/or metrics;
- Effects may be qualitatively different based on size, surface chemistry, or other specific interactions. In this case, existing standards can be used since the critical effect is different for fine analogs. However, this implies that the same material at different sizes may have different standards, based on different effects;
- Effects will be substantiated by studies of all sorts, but given the pace of commercialization, we need to focus on some nanoparticles more than others, to study some characteristics of nanoparticles more than others, and to establish protocols that will allow us to take findings, collate and concatenate them, producing usable information for regulators and society as a whole; and
- Most importantly, extrapolation of available data and bridging of concepts might be difficult. To illustrate this epidemiological studies have revealed several effects on susceptible population groups after inhalation of CDNPs. It is crucial to explore whether these concepts can also be used for ENPs. If yes, this means we should start testing ENPs in susceptible animal models, such as the hypertensive rat.

In conclusion, nanotechnologies pose a classic dilemma for modern society: use its potential and go full speed ahead or perform the necessary risk and technology assessments first. The difference with previous technologies is that nanotechnology may affect all aspects of human life, and an error of this magnitude might be irreversible. One thing is sure: to fully comprehend and realize its potential, a renaissance of science and education is needed, accompanied by open minds in politics, investment funds, and grant-awarding bodies. This includes giving full attention to sustainable development, which may be enabled by new methods and protocols for testing nanomaterials for their potential adverse effects. We would benefit by heeding the following: 'nano' is a society of creative 'yes-sayers' associated, and as such it may well be that this discussion will prelude the end of environmental protectionism[32]. Nanotechnology will allow bottom-up clean production, as well as the clean up of current environmental problems. However, much effort is needed to enable this promise. nt

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