NANOTECHNOLOGIES IN THE 21ST CENTURY

Nanomaterials - Health and Environmental Concerns

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Can nanotechnologies assist in solving 21st century environmental challenges?

A critical review of opportunities and risks

The context of this series of papers

Nanotechnologies are the science and business of manipulating matter at the atomic scale. Materials produced with the aid of nanotechnologies are starting to be used in many areas of everyday life (cosmetics, clothing fabrics, sports equipment, paints, packaging, food, etc). As the applications expand, many proponents are positioning nanotechnologies as part of a greener, more sustainable future. Is there a basis to these claims, or will nanotechnologies only lead to more toxic materials, more production and consumption, and a decrease of control over how to create and live our lives?

In this context, it is essential for environmental NGOs to gain knowledge on different aspects of the emerging nanotechnology development and governance debates, especially in relation to critically discussing the promotion of nanotechnologies for use in green technologies (i.e. for renewable energy production and water filtration). Environmental NGOs also need to clarify and become aware of the importance of their involvement in the governance of nanotechnologies and their products and become actively involved in public dialogue about the future development and direction of their use. It is crucial that as nanotechnologies expand into the “green” sector, environmental NGOs formulate political demands and become involved in public debates concerning the sustainable and responsible development of nanotechnologies.

This series of papers is meant to serve as a capacity building tool empowering environmental NGOs to work actively in the field of sustainable governance and use of nanotechnologies and nanomaterials. This objective will be met through the production of four separate publications between April and July 2009. The outline of the issues addressed in each publication is as follows:

1. Challenges and opportunities to green nanotechnologies
2. Environment, health and safety research and emerging concerns about the sustainability of nanotechnologies and nanomaterials
3. Regulatory status and initiatives in Europe and rest of the world on nano materials
4. NGO guidelines on sustainability assessment of nanotechnology and nanomaterials

The published reports are available at:
http://eeb.org/publication/general.html

Uses, Applications and Risks

“Union policy on the environment shall aim at a high level of protection taking into account the diversity of situations in the various regions of the Union. It shall be based on the precautionary principle and on the principles that preventive action should be taken, that environmental damage should as a priority be rectified at source and that the polluter should pay”. Article 191 devoted to the environment in the consolidated version of the EU Treaty.

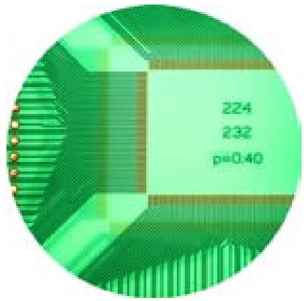
Nanomaterials are continuing to be hailed by governments and industry as the next big technological fix. It is claimed they will offer solutions for many of humanity’s most pressing problems, while being part of a greener, more sustainable future.

The purpose of the second in this series of publications on nanotechnology is to review the current uncertainties associated with the environmental and health effects the use of nanomaterials may present. To answer these questions, this document presents the most commonly used nanomaterials and discusses the potential risks associated with their use in consumer products.

Nanomaterials are already in many consumer products

A key aspect of nanotechnologies is the creation of new materials. These materials (known as nanomaterials) are engineered at the nanoscale (i.e. the scale of atomic particles) to create new and different properties when compared to the “bulk” material. A typical example is carbon, which in its graphite form is used extensively in pencils. When engineered into a carbon nanotube it becomes 100 times stronger than steel, while only being one-sixth its weight.

Nanomaterials are already being used in many everyday objects including window coatings, sunscreens and other cosmetics, textiles, paint, cutting boards, socks, etc. Nanomaterials may also be found in human hip joints, Organic Light Emitting Diodes (OLEDs) for displays and ear implants, to name a few. The most commonly used nanomaterials in consumer products are nanosilver (in the form of ultra tiny silver particles), carbon nanotubes (fibres of carbon rolled into super-strong tubes), nanosized metal oxides (titanium dioxide, zinc oxide), silica and gold (see table 1, on page 7). Other engineered nanomaterials used in consumer, medical and industrial products include additional forms of nanocarbon, cerium oxide, nickel, aluminium oxide and the nanoclays copper oxide, iron oxide and quantum dots [1] .



As of June 2009, the first publicly available on-line inventory of nanotechnology-based consumer products* Project on Emerging Nanotechnologies Consumer Product Inventory listed 807 products containing nanomaterials available worldwide [2]. Sixty percent of the nano products listed fall into the category of health and personal grooming, including such items as cosmetics, sunscreens, clothing, personal care and filtration.

** The inventory of nanomaterials is run by the Project on Emerging Nanotechnologies established as a partnership between the Woodrow Wilson International Centre for Scholars and the Pew Charitable Trusts. The Project is dedicated to helping ensure that as nanotechnologies advance, possible risks are minimised, public and consumer engagement remains strong and the potential benefits of these new technologies are realised.*

The use of nanomaterials is becoming more common

Industry analysts are beginning to speak of nanomaterials as a maturing industry, which generated a global market value for products incorporating nanomaterials of US$83 billion in 2007 and which is predicted to rise to US$263 billion by 2012. Until now the production of nanomaterials has been dominated by the chemical industry, but by 2012 nano healthcare and pharmaceutical applications and products will become more prevalent and important [3]. Unfortunately very little information about the production volume of particular nanomaterials is available, despite this being critical for the enforcement of current legislation on chemicals use at least in the EU**. For instance, industry analysts estimate the global annual production of carbon nanotubes and fibre was 65 tons in 2005, while the annual production volumes of other nanomaterials such as nano-metals and materials with nanostructured surfaces is at present completely unknown [4].

*** REACH is the new EU regulation to control the trade and use of chemicals since June 2007. Manufacturers and importers of chemicals are required to register them with the European Chemicals agency to show that they can be used safely if their volume is above 1 tonne per year.*

The risks remain unaddressed

Nanotoxicologists are starting to agree that the potential risks to health and the environment from the manufacture and use of nanoparticles are real. The issues of concern can be summarised as follows:

- A continuing lack of public information about the toxicity, ecotoxicity and exposure to nano materials.
- An increasing potential for exposure of people and the environment as more and more products containing nanomaterials become commercially available.
- A continuing lack of knowledge about the risks from each particular nanomaterial and how to avoid them.
- Persisting inadequacy of current regulatory regimes to ensure the safe development and use of nanomaterials in commercial products together with unwillingness of producers to convey all available information on possible risks [1].

Table 1: Commonly used nanomaterials in consumer products

Nanomaterial	Number of products available worldwide	Typical example
Nanosilver	235	Socks, hairbrushes, plasters, food packaging, food supplements
Carbon, incl. fullerenes	71	Sporting goods, filtration and storage devices, OLEDs, computer RAM, cosmetics, computer hardware
Titanium dioxide	38	Paints, sunscreens, antibacterial coatings, cleaning products
Zinc Oxide	29	Sunscreens
Silica (silicon dioxide)	31	Paints, cleaning products, cosmetics, food supplements, sporting goods
Gold	16	Cosmetics, personal care products, fuel catalysts, food supplements

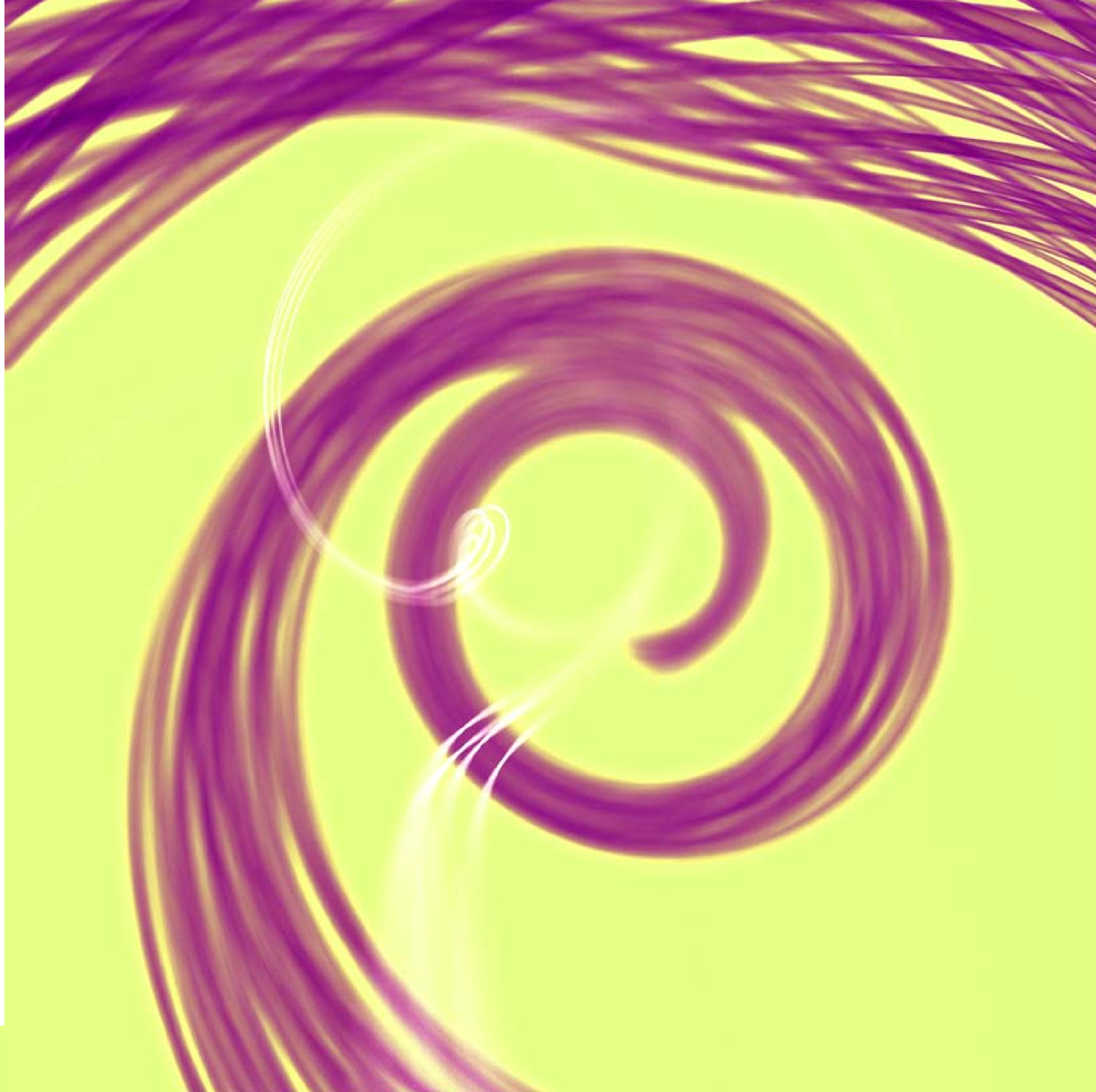
Source: adapted from the Project on Emerging Nanotechnologies Consumer Product Inventory list, August 2008 [2]

A recent review [1] of research being conducted or completed into the environmental, health and safety aspects of nanomaterials has raised concerns about at least three of the above materials: carbon nanotubes, nanosilver and nano titanium dioxide. There is evidence that carbon nanotubes may have an adverse effect on human health and that silver nanoparticles and titanium dioxide nanoparticles are detrimental to the environment. In these three cases the review suggests further research is needed and that in the meantime that the “precautionary principle” should be invoked. Of course, this is one of the fundamental principles of EU legislation: if there is no data showing a product is safe, it shall not be allowed on the market.
The evidence is not as clear cut in the case of nano zinc oxide. However there are worrying indications that this material may also be unsafe. We will review carbon nanotubes and nanosilver as well as zinc oxide and nano titanium dioxide in more detail below. These four are the most commonly produced nanomaterials and therefore currently have the largest potential to be harmful, as they are used in substantial quantities in consumer products available on the market today.
There are numerous other metals, metal oxides, carbon structures and other materials that are beginning to be used in nanoform. Unfortunately not much is known about the potentially harmful effects they may have on human health or the environment so they are not addressed in this document.

What are nanomaterials and why worry about them?

Nanotechnology has conventionally been defined as the intentional engineering of matter at a scale of approximately 1 to 100 nanometres. However, the scientific and political communities have increasingly come to agree and understand that a definition in terms of dimensions is not sufficient and that a number of other factors determine not only the novel properties of a material, but also their potential to cause harm. The physical/chemical properties determining the behaviour of nanomaterials include shape, surface charge, mass, surface area, aspect ratio (the ratio of width to height of an object), crystal structure, stability, and distributions in size and degree of dispersion of the particles [5].

Nanomaterials can be roughly divided into metals (e.g. iron, gold, silver, platinum), metal oxides (zinc oxide, titanium dioxide, aluminium oxide), carbon-based nanomaterials (fullerenes, carbon nanotubes) and hybrid structures such as quantum dots, core-shell structures, functionalised materials that combine nanomaterials into elaborate engineered structures for the purpose of exhibiting particular properties [6]. These engineered structures may add a further layer of issues in assessing their potential toxicity, apart from those introduced by the nanomaterial used itself.



For instance, quantum dots are essentially semiconductor nanoparticles. Their key property is their luminescence. Their unique properties result from a combination of their core structure/composition, size and coating. How toxic quantum dots are hence depends on multiple factors including their physicochemical properties as well as the environmental conditions they encounter [7].

For toxicological purposes, nanomaterials can be further categorised according to where the nanomaterial is located or what form it takes in the product, e.g. being airborne, surface bound, suspended in liquids or solids or being bound in a nano structured surface [8].

Categorising nanomaterials in such a way clearly shows not only that the same material may occur in a diversity of locations and forms, but also important ramifications regarding how toxic a material may be at different points in the product's life cycle. For instance, surface bound materials are expected to be less toxic (at least in their intended use) than airborne particles which can be readily inhaled. However, some nanomaterials in consumer products will also change their toxicity potential during use.

Nanoparticles in paint (such as titanium dioxide) are usually suspended in the liquid, but become solid when the paint has dried. Little exposure to the consumer is expected once the paint has dried, but as wear and tear inevitably affects the paint, the nanomaterial will wash off into the environment and new and additional exposure risks for humans and the environment may occur.

Applying the above categorisation scheme to the products in the nanomaterial consumer inventory showed that 45% of the consumer products listed will likely lead to consumer exposure. Zinc oxide, gold and titanium dioxide nanomaterials were the most likely to result in this exposure. Additionally there were a large number of consumer products for which no information about the type of nanomaterial used was available, which raises concerns about the potential exposure and hazard these products may pose to humans and the environment [8].

How do we know a nanomaterial is toxic or ecotoxic?

Unfortunately current nanomaterial exposure assessment is complex and limited by insufficient knowledge. Much work still needs to be done to identify hazards and coordinated research efforts and research strategies need to be devised and implemented to obtain a comprehensive exposure assessment for each particular nanomaterial. It is known, however, that some nanoparticles can penetrate at the sub-cellular level, where they can intervene with and disrupt the molecular processes within the cell. Ecotoxic effects on the environment have also been demonstrated. It is still largely unknown which properties determine and/or influence the toxicity of nanoparticles, but physical and chemical properties such as particle size, size distribution, number concentration, agglomeration state (i.e. how much the particles have gathered into a single unit), shape, crystal structure, chemical composition, surface area, surface chemistry, surface charge, porosity and method of synthesis are all properties that need to be considered [4].

Very few studies have addressed mammalian toxicity and ecotoxicity of nanoparticles, but when researchers examined 428 studies they noted that the vast majority of the studies demonstrated some degree of adverse effects on tested animals or cells.

Exposure routes for humans

A full assessment of the effects of exposure to nanomaterials requires knowledge about manufacturing conditions, volume of production, industrial applications, uses, consumer products and behaviour and environmental fate and distribution. Currently this kind of information is not available for any nanomaterial, making it virtually impossible to assess exposure levels.

Exposure to nanomaterials can occur at different life-cycle stages of the materials and/or products. External exposure routes for humans comprise inhalation (e.g. as aerosols or during manufacture), and absorption through the skin, eye and gastrointestinal tract. Once in the body internal exposure mechanisms include further absorption, distribution and metabolism. Exposure of the unborn child via the placenta is also a possibility [9, 10]. An additional issue is that while the nanomaterial itself may be relatively safe, it may act as a Trojan horse, i.e. a perhaps more toxic material may attach itself to the nanomaterial and gain entry to the body, organs or cells within the body [11].

Environmental exposure routes

There are a number of pathways for nanomaterials into the environment. They may be emitted during manufacture or accidentally released, but ultimately their end use determines how they will enter the environment. Sunscreens containing nanomaterials provide a suitable illustration. The sunscreen might wash off during washing and swimming and enter the sewage system or surface water bodies. Any unused portion of the sunscreen will be disposed of in household waste and then either incinerated or end up in landfill. Nanoparticles used in fuel as a catalyst are likely to end up in the air [12].Table 2 gives an overview of the most likely entry point into the environment for commonly used nanomaterials depending on product category and material used.

Little is known currently about the end of life fate of nanomaterials. Direct disposal of nanomaterial may occur as landfill and incineration or during wastewater treatment. For instance, up to 95% of nanoparticles used in cosmetics, paints and coatings may ultimately end up in wastewater, either as result of runoff during application or 'wear and tear' abrasion during the product's lifetime [13]. Materials such as carbon nanotubes, used in plastics, sporting equipment and electronics, are likely to remain intact until their disposal in landfills or incinerators where they may leach into the environment or be released into the air. Recycling may be a long-term goal, but currently little is known about whether this option is feasible.

Once in the environment nanomaterials may behave differently from their intended use i.e. they may aggregate, attach themselves to other soil contaminants, or be absorbed into solid particles, bioaccumulated or biomagnified. While ecotoxicological effects have been reported for a number of aquatic and soil organisms, much remains unknown.

Table 2: Major nanomaterials input routes into the environment of for different types of applications (adapted from Boxhall et al. [12])

Product Type	Typical Nanomaterial used	Air	Surface Water	Sewage	Soil
Cosmetics and Personal care	Titanium dioxide, zinc oxide, fullerenes, gold,	✓	✓	✓	
Catalysts and lubricants	Cerium oxide, platinum, molybdenum trioxide		✓	✓	
Paints and coatings	Titanium dioxide, gold, quantum dots	✓	✓	✓	
Environmental and water remediation	Iron, polyurethane, carbon nanotubes		✓	✓	✓
Agrochemicals	Silica as carrier	✓	✓		✓
Food packaging	Gold, nanoclays, titanium dioxide, silver		✓		✓
Pharmaceuticals and medicine	Nanomedicines and carriers			✓	✓

Note: empty fields denote less likely input route

Carbon nanotubes

Carbon nanotubes (CNTs) are tubes of rolled graphene sheets made of one atom thick carbons arranged in a honeycomb crystal lattice. CNTs are typically long and thin and may either consist of a single wall (SWCNT) or be multi-walled (MWCNT). Their unusual length-to-diameter ratio (1:1,000,000) gives them unusual physiochemical properties including high tensile strength and hardness (harder than diamonds). They are also flexible, lightweight, heat-resistant and have high electrical conductivity. Their unusual hollow one nanometre structure makes them useful for biological applications, such as drug delivery and medical imaging [14]. Untreated CNTs are not water-soluble and are non-biodegradable, biopersistent and lipophilic (i.e. an ability to enter fatty cell membranes). Depending on how they are manufactured and treated their behaviours in terms of water solubility, transport behaviour and specific toxicity may change.

There are 20 different structural types of single-walled carbon nanotubes alone, with an average length from 5 to 300 nm. Four different processes exist for their manufacture, five for their purification, and ten surface coatings are typically applied – hence there are up to 50,000 potential combinations of single-walled CNTs – and each version may have different chemical, physical, and biological properties that determine their overall hazard [4]. Similarly there are many different variations of MWCNTs.
MWCNTs have recently become the topic of much concern, as the long-held suspicion by scientists that some not only resemble asbestos fibres (long and thin), but may also cause similar health risks (see ”potential harm”) has been confirmed.

Common products
Bicycle components, tennis rackets, golf clubs, display technology, solar cells, computer hardware.

How much is produced in Europe annually
Exact figures for CNT production are not readily available, but the biggest producer in Europe Bayer AG has recently opened a manufacturing plant in Germany with capacity to produce 30 tonnes per year. One of Bayer’s biggest competitors, the French-based company Arkema, has the capacity to produce between 10 and 30 tonnes of CNTs per year [15]. It is estimated that in 2007/2008 about 350 tons of CNTs were produced worldwide [13].

Potential harm
CNT cytotoxicity is influenced by length, size distribution, metal impurities, dispersion, aggregation status, coatings and probably a number of other as yet unknown factors. The coating of a nanoparticle can be of special importance, as uncoated particles may be more or less toxic, have a different charge or have different mobility.

CNT cytotoxicity has been observed for a number of different organs and organ-specific cell lines, including human epidermal cells, human dermal fibroblasts, human embryo kidney cells and human bronchial cells, causing cellular toxicity (see box) [16, 17, 18] and DNA damage [19]. Pulmonary disease and death was observed in mice [20], as well as dose-dependent inflammatory reactions [21].

Cytotoxicity, or cellular toxicity, is the quality of being toxic to cells. The toxic effects can lead to cells necrosis, whereby cells lose membrane integrity and die rapidly or stop their active growing and dividing.

As stated already, there is increasing evidence that some CNTs behave like asbestos fibres. When mice were exposed to 50mg of four different MWCNTs of various chemical composition, length, shape and diameters for up to 7 days, it was found that the exposure *“produced length dependent inflammation … that were qualitatively and quantitatively similar to the foreign body inflammatory response caused by long asbestos*” [22]. This was the first experiment that clearly showed that long thin carbon nanotubes may in theory behave like asbestos and cause lesions in the lung that are precursors for carcinogenic formations/ cancer. Preliminary research results released by NIOSH (National Institute for Occupational Health and Safety, USA) in March 2009 confirmed these findings [23].

Another recent study showed that mice that already suffer from asthma and inhale MWCNTs have a greater tendency to contract airway fibrosis, suggesting that ”individuals with pre-existing allergic inflammation may be susceptible to the same effects” [24]. See table 3 for a summary of the key results.

Environmental toxicity

SWCNTs have also shown ecotoxic potential. For example, juvenile rainbow trout showed a dose-dependent rise in ventilation rate, gill disease and mucus secretion [25]. Other ecological indicator species such as zebrafish showed delayed hatching, while estuarine crustaceans showed increased mortality [26].
Plants have also recently been shown to react adversely to MWCNTs and fullerenes. Rice plants exposed to carbon fullerenes transmitted nanomaterials to the next generation. Exposure to both carbon fullerenes and carbon nanotubes also delayed the onset of rice flowering by at least one month and reduced the seed set. Given that over half the world's population relies on rice as a staple crop, the environmental and food safety implications of the observed results could potentially be devastating [27].

Table 3: Summary of some key results for carbon nanotubes

Type of Carbon Nanotube	Cell type or Species	Effects
SWCNT	Human embryo kidney exposed to 0.78-200 ¤g/mL for up to 5 days	Dose-and time dependent inhibition of cell proliferation, and a decrease in cell adhesive ability
SWCNT (unrefined, 30% iron)	Human skin cells exposed to 0.6-0.24 mg/mL after 2 to 18 hours in	Oxidative stress, involved in many diseases, such as atherosclerosis, Parkinson's disease, Heart Failure, Alzheimer's disease
SWCNT (various)	Human skin cells	Cytotoxicity dependent on the density of surface functionalisation
SWCNT	Mice exposed to concentrations between 0 and 0.5 mg in during 7 and 90 days	Dose-dependent granulomas and interstitial inflammation
MWCNT	Intratracheal exposure of mice to a single dose of 1, 2.5, or 5 mg/kg	Pulmonary effects, then death
MWCNT various diameters, lengths, shape and chemical composition	Mice lungs injected with 50 mg MWCNT for 24 hours or 7 days	Significant increase in inflammation
MWCNT	Asthmatic and non asthmatic mice were exposed to a MWCNT aerosol (100 mg/m3) for 6 hours	Asthmatic mice responded with airway fibrosis

Effect and impact

Some carbon nanofibres have physical-chemical and biopersistent characteristics similar to those of hazardous asbestos fibres. They can induce similar inflammatory reactions.
It is unclear whether carbon nanotubes are released when bound in a matrix, but it is inevitable at some point of their lifecycle [28].

Verdict

Extreme caution should be used when handling CNTs, especially during manufacture but potentially during all stages of use (manufacturing, use and end-of-life phases.
There is sufficient evidence to suggest that CNTs may be harmful to human health and therefore their use in consumer goods should be banned until they have been proven scientifically safe.

Silver nanoparticles

Silver biocides are used in a range of products including water treatment, textiles, washing machines, dyes/paints and varnishes, polymers, medical applications, sinks and sanitary ceramics as well as various 'consumer' applications such as disinfectants, cosmetics, cleaning agents, baby bottles, etc.

An increasing amount of silver in consumer and industrial products is now in nano form. Nanoscale silver or "nanosilver" has become one of the most commonly used nanomaterials in consumer products, predominately as a bactericide. A study analysing the risk to freshwater ecosystems of silver nanoparticles incorporated into textiles and plastic predicted that in the future 15% of the total silver released into water in the European Union would come from biocidal plastics and textiles [29]. Most of the nanosilver is predicted to end up in sewage sludge and at least some of it may be spread onto agricultural fields.

A BIOCIDE IS A CHEMICAL SUBSTANCE CAPABLE OF KILLING LIVING ORGANISMS. BIOCIDES ARE COMMONLY USED IN MEDICINE, AGRICULTURE, FORESTRY, AND IN INDUSTRY WHERE THEY PREVENT THE DEVELOPMENT OF UNDESIRED BACTERIA.

Common products
Food contact materials (such as cups, bowls and cutting boards), cosmetics and personal care products, clothing, childrens' toys, infant products, 'health' supplements, paints and surface coatings, medical devices, wound plasters.

How much is produced in Europe annually
The European market for silver-containing biocidal products is projected to reach between 110 and 230 metric tonnes of silver annually by 2010 and a significant portion of this will be nanosilver [29].

Potential harm

The toxicity of silver nanoparticles may exceed the toxicity of the most toxic silver compound in its standard form [30, 31] and may have greater antimicrobial properties because they are able to kill bacteria for longer periods of time than normal silver [32]. Indeed, silver has been shown to be comparatively more toxic than other heavy metals when in nanoparticle form [33]. In addition, different sized and shaped silver nanoparticles have different toxicities. Nanoparticles of silver less than 10 nanometres can penetrate the cell wall [34]. The most common nanosilver toxicity studies focus on bacteria and to a lesser extent on complex animal species such as fish, rats, mice and quails. A study on bioluminescent bacteria showed that nanosilver particles can disrupt cell membranes resulting in cell toxicity and cell deformation [35]. A number of researchers have shown that nanosilver particles can destroy the ability of bacterial DNA to replicate or can damage DNA [36].

In addition to being an effective bactericide, silver nanoparticles are also toxic to mammalian cell cultures, such as rat liver cells [37], cultured neuroendocrine cell lines, which are a model for human brain cells [38], and rat cells used as a model for human toxicity after inhalation [39]. The significant toxicity of silver nanoparticles to mammalian stem cells indicates the potential of these particles to interfere with the male reproductive system. There is also some evidence that nanosilver can be detrimental to human health when ingested or used in medical devices [40].

Environmental toxicity

There is increasing evidence that silver nanoparticles at low concentrations will harm aquatic invertebrates and fish. Silver nanoparticles administered in vivo to zebra fish embryo increased deformation rates and ultimately led to death. Individual silver nanoparticles were found inside embryos at each developmental stage [41]. This is one of the few available in vivo studies to observe passive diffusion of nanoparticles and points to the severe consequences that the release of large amounts of silver nanoparticles may have, if the nanoparticles remain unchanged when reaching aquatic environments.

A recent study on zebra fish found that nanosilver particles can also induce altered physiology, including the degeneration of body parts and an increase in mortality and hatching delay [42]. Nanosilver particles also impair the growth and reproductive ability of certain nematodes [43]. Early studies suggest that microorganisms and plants may be able to modify and concentrate nanoparticles that can then bioaccumulate (or even biomagnify) along the food chain [44].

BIOPERSISTENCE IS THE ABILITY OF A MATERIAL TO REMAIN IN AN ORGAN IN SPITE OF THE ORGAN'S PHYSIOLOGICAL CLEARANCE MECHANISMS.

BIOACCUMULATION IS A GENERAL TERM FOR THE ACCUMULATION OF A HARMFUL SUBSTANCE IN AN ORGANISM OR PART OF AN ORGANISM.

BIOMAGNIFICATION IS A SEQUENCE OF PROCESSES IN AN ECOSYSTEM BY WHICH HIGHER CONCENTRATIONS OF A SUBSTANCE ARE ATTAINED IN AN ORGANISM THAN IN ITS FOOD, I.E. THE HIGHER AN ORGANISM STANDS IN THE FOOD CHAIN THE MORE LIKELY IT IS TO ACCUMULATE HARMFUL SUBSTANCES IN ITS SYSTEM.

Table 4: Summary of some key results for nanosilver

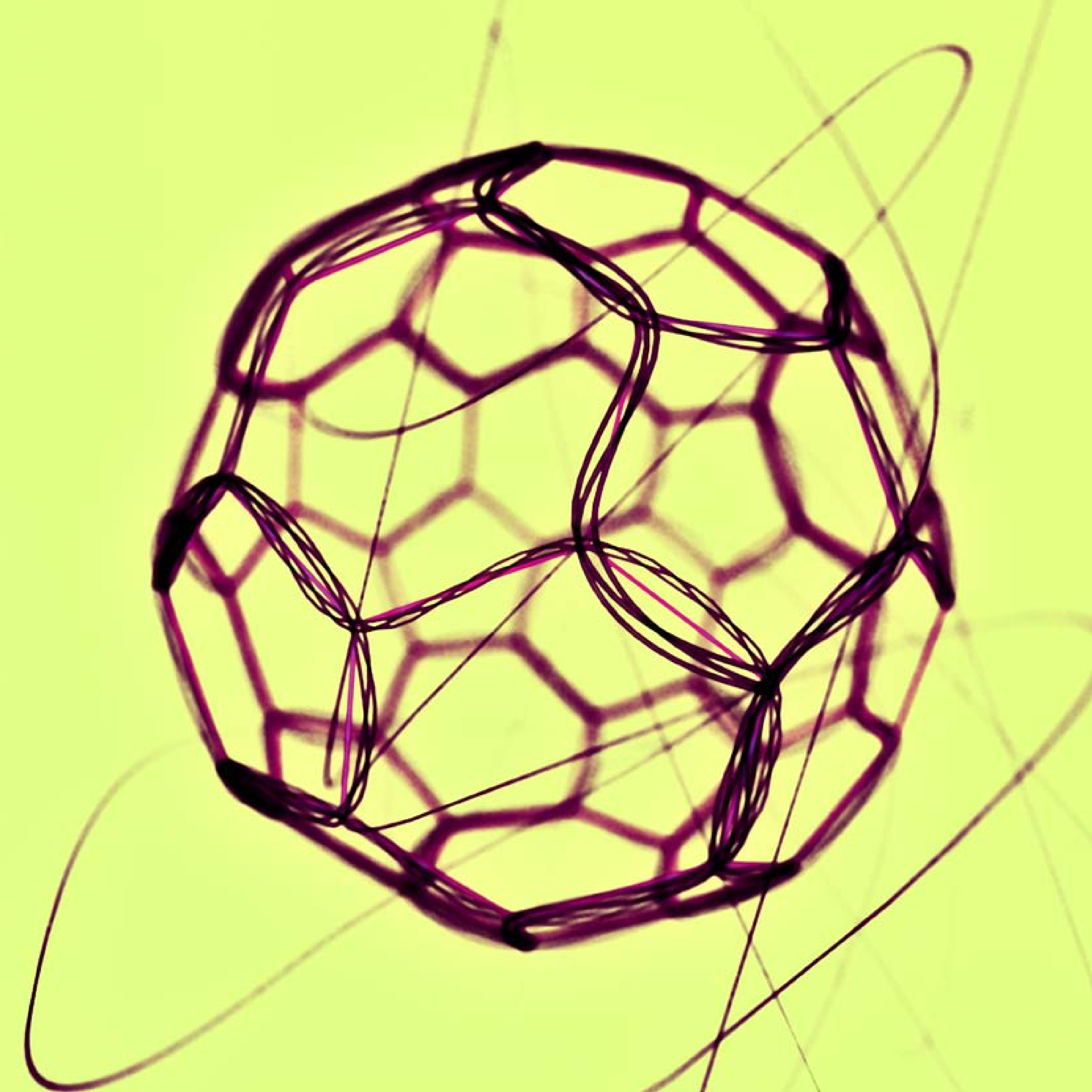
Cell or Species	Effects
Rat liver cells exposed to nanosilver particles	Mitochondrial function, an indicator of energy available to the cells, is decreased Cell death and reduced cell metabolism
Neuroendocrine cell lines (an in vitro model for brain cells)	Mitochondrial activity reduced Cells decreased in size and became irregular in shape
Mammalian stem cells exposed to a number of metal oxides	Silver nanoparticles were more toxic than other metal oxides Significantly reduced mitochondrial function and interference with cell metabolism Potential to interfere with the male reproductive system.
Rat cells (a model for toxicity to humans after inhalation) for 24 hours to hydro-carbon coated silver particles	Size-dependent toxicity was confirmed

Effect and impact

- Nanosilver is one of the most commonly used nanomaterials in a wide range of consumer products. There is convincing evidence that some nanosilver will wash into wastewater.
- Soil, aquatic and terrestrial organisms may be harmed by the release of nanosilver into the environment.
- It is recommended that the release of untreated silver nanoparticle waste into the environment be controlled/restricted.

Verdict

- Because there is sufficient evidence to suggest that nanosilver may be harmful to various organisms and ecosystems, their release into the environment should no longer be allowed;
- Products containing nanosilver should be assessed for safety throughout their whole lifecycle, requiring assessment beyond the use phase and including end-of-life impacts.



Titanium dioxide (TiO2) in its bulk form is the most widely used white pigment because of its brightness and high reflectivity. It comes in three crystalline forms. Depending on its crystalline structure, TiO2 can have significantly different physical and chemical properties.
It was commonly used in sunscreens, but gave the skin a white or milky appearance. In nanoform TiO2 loses its opaqueness, a property now widely preferred for "clear" sunscreens. Certain forms of nano titanium dioxide are also highly photocatalytic, which means that they are capable of absorbing light and using it to change their rate of chemical reaction.
An increase in damage to newly installed metal roofs in Australia was traced back to this photocatalytic property of nanosized titanium dioxide, which had come into contact with the roofs via the sunscreen that the roof installers had applied to their hands [45].

Common products

Cosmetics, sunscreens, food packaging, paints, wall coatings, dirt-repellent coatings for windows, car coatings and as a catalyst for the decomposition of organic contaminants present in water and waste water.

How much is produced/used in Europe annually

TiO2 nanoparticles are used in amounts greater than 1000kg/company/year [1].

Potential harm

While the bulk material is generally considered safe, some studies have suggested that chronic inhalation of TiO2 nanoparticles is harmful. Exposure to nanosized TiO2 has been associated with a variety of pulmonary effects in rats, including inflammation, pulmonary damage, fibrosis, and lung tumours [1]. In vitro experiments show that nano TiO2 can damage DNA, disrupt the function of cells and interfere with the defence activities of immune cells [46. 47, 48]. It can also provoke inflammation by absorbing fragments of bacteria and 'smuggling' them across the gastro-intestinal tract [11], which can be a factor in the rising incidence of auto-immune diseases like irritable bowel syndrome and Crohn's disease [49].

In 2009 a study performed on mice reported that nanosized TiO2 particles can be passed from mother to child. The nanoparticles were found in the offspring's brain and testes, causing, for instance, decreased sperm count [50].

Crystalline structure, shape and exposure route as well as surface area and coating of the particles all influence TiO2 toxicity. Ultraviolet light also appears to increase the cytotoxicity of titanium dioxide. Several studies observed increased cell death after UV light exposure, especially if the TiO2 was the crystalline form that is more reactive to light) [4].These findings are important in determining which form of nano TiO2 should be used in sunscreen. See table 5 for a summary of key results.

Environmental toxicity

Due to their small size, large surface area and strong electrostatic attraction, TiO2 nanoparticles can absorb other metallic particles. For example, cadmium, which is extremely toxic and can bioaccumulate in organisms and ecosystems, has been shown to be strongly absorbed within nano TiO2 and then transported into and accumulated in carp. This process raises the issue of nano TiO2 acting as a magnifier for cadmium pollution [51]. Nanosized TiO2 has also been shown to increase the mortality [52] and to have antibacterial properties towards different bacteria some of which beneficial to humans (such as in wastewater treatment installations).

Cell Line or Species	Effects
Mice brain	• Interference with mitochondrial energy production
Female mice	• Liver and kidney damage • TiO2 accumulated in liver, spleen, kidneys and lung tissues
Human immunity cells	• Changes in defensive functions
Pregnant mice	• Nanoparticles found in the offsprings' brains and testes

Release of nanosized tiatinium dioxide into water could have detrimental effects on overall ecosystem health, especially given that the concentration is large enough to have some detrimental effect on organisms and that it readily accumulates in drinking water [1].

Effect and impact

Nanosized T02 is very commonly used in a wide range of consumer and industrial products. It is inevitable that much of it will end up in the environment.
T02 nanoparticles may be present in the environment at concentrations that may be detrimental to organisms.
There are increasing indications that nano titantium dioxide can be harmful to humans, though further research needs to be conducted to confirm this.

Verdict

Given the uncertainties surrounding the potential toxicity of titanium dioxide, its further application in consumer goods should be barred until there is scientific evidence of no hazard.

Zinc Oxide (ZnO)

Zinc oxide is considered relatively safe in its bulk form. In nano size, zinc oxide is widely used as a pigment for paints and cosmetics, as well as a semiconductor. It can also exhibit antibacterial properties.

Common products

Cosmetics, sunscreens, food packaging, food additives, paints, wall coatings.

How much is produced/used per year in Europe?

Zinc oxide is used in quantities greater than 1000kg/company/year [1].

Potential harm

Nanosized zinc oxide is toxic to human and rat cells even at very low concentrations [54]. Test mice showed severe symptoms of lethargy, vomiting and diarrhoea. A study in mice showed severe responses, ranging from death to heavy kidney damage, anaemia and liver damage [55]. Another experiment recorded damage in mice liver, heart, spleen and pancreas [56].

Recently the potential of zinc oxide to harm genetic makeup when applied to the skin has been demonstrated [57]. The study observed cytotoxicity, oxidative stress and DNA damage. See table 6 for a summary of key results.

Environmental toxicity

Some studies report the toxicity of nanosized zinc oxide in bacteria and vertebrates [58, 59]. It can also inhibit seed germination and root growth in plants [60]. Zinc oxide is also toxic to some roundworms and can inhibit their growth and reproductive capabilities [61].

Like other metal oxides, nano zinc oxide tends to aggregate and settle so most of the material can be found in sediments. A study presented evidence that nano zinc oxide aggregates cause toxicity to zebrafish embryos and larvae, including malformation in the cardiovascular system, blocked hatching and mortality in embryos. The researchers urged a reassessment of existing regulations for soluble nano-sized metal ions [62].

Table 6: Summary of some key results for Zinc Oxide

Cell Line or Species	Effects
Adult mice	Lethargy, vomiting and diarrhoea. Kidney damage and aneamia.
Adult mice	Liver, heart and spleen damage. 20nm particles damaged liver, spleen and pancreas
Human skin cell	Demonstrated DNA damaging potential Induced oxidative stress in cells

Effect and impact

Nanosized zinc oxide is widely used in many products. There are increasing indications that it may be harmful to humans and the environment under certain circumstances.

Verdict

Further research needs to be conducted because not enough is known on the human health and environment impacts of nano-sized zinc oxide. In the interim it should be banned from application in consumer goods until proven safe for human health and the environment.

The challenge of nanomaterials use

While the use of nanomaterials promises consumer goods with new and perhaps useful properties, studies are starting to identify the potential health risks of these materials.

One of the greatest challenges in the coming years will be how to successfully assess the potential of nanomaterials to cause harm before many more products containing such materials are available on the market. It is already becoming obvious that in vitro testing may not be sufficient to predict hazards accurately, but to date only a few in vivo studies have been performed and are notoriously difficult to replicate.

For most nanomaterials it has not been established whether safe thresholds exist or what these could be. **Little or no detailed information is available on nanomaterials safety assessment either in research phase or in production, distribution and use in consumer products and hence no full exposure assessment can yet be performed.** Nanomaterials are difficult to assess and monitor as often new testing methods are needed to gauge their impacts on human health and the environment. This does not necessarily mean that only new tests are needed, rather it may also require that existing tests be assessed to ensure they are relevant and effective at the nano level. Environmental and biological exposure pathways for many nanomaterials are still largely unknown as they have not been observed.

Public exposure is virtually impossible to assess for now as there is a lack of information about commercial products, including which contain nanomaterials, their precise nano content and therefore the public's potential level of exposure..

People cannot even decide to avoid products containing nanomaterials, as manufacturers or importers are not required generally to communicate this information. However, recent decisions at EU level (Cosmetics regulation, Novel Foods regulation) are starting to require such information provision, through ingredients lists. EU chemicals regulation requires safety data information to be provided as part of registration processes, although this does not lead specifically to public communication.

Given the present lack of information on nanomaterials' impacts, the most rational approach is the application of the precautionary principle to any nanomaterial-containing consumer product - this would mean the banning of consumer products containing nanomaterials from commercial circulation until appropriate data was available to be able to make a safety assessment; products already available on the market should be removed until similar safety data is available.

The next report in this series will examine the state of play of broad governance issues including regulatory initiatives and responses, voluntary codes and practices, as well as the progress of international efforts in coordinating nanotechnology governance and public engagement initiatives in Europe. It will review NGO responses and initiatives regarding nanotechnology regulation with particular reference to the precautionary principle and pre-market registration demands in Europe.

References

1. Aitken, R.J., et al., EMERGNANO: A review of completed and near completed environment, health and safety research on nanomaterials and nanotechnology. 2009, IOM. p. 198.
2. Project_on_Emerging_Nanotechnologies. Consumer Product Inventory - Analysis. 2009 [cited 16th April 2009]; Available from: http://www.nanotechproject.org/inventories/consumer/analysis_draft/.
3. Cientifica. Halfway to the trillion dollar market. 2007 [cited; Available from: http://www.cientifica.eu/files/Whitepapers/A%20Reassessment%20of%20the%20Trillion%20WP.pdf.
4. Hansen, S.F., Regulation and risk assessment of nanomaterials – too little, too late?, in Department of Environmental Engineering. 2009, Technical University of Denmark: Lyngby.
5. Hassello, M., et al., Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles. Ecotoxicology, 2008. 17: p. 344–361.
6. Christian, P., et al., Nanoparticles: structure, properties, preparation and behaviour in environmental media. Ecotoxicology, 2008. 17: p. 326–343.
7. Hardman, R., A Toxicologic Review of Quantum Dots: Toxicity Depends on Physicochemical and Environmental Factors. Environmental Health Perspectives, 2006. 114(2): p. 165-172.
8. Hansen, S.F., et al., Categorization framework to aid exposure assessment of nanomaterials in consumer products. Ecotoxicology, 2008. 17: p. 438–447.
9. Takeda, K., et al., Nanoparticles Transferred from Pregnant Mice to Their Offspring Can Damage the Genital and Cranial Nerve Systems. Journal of Health Sciences, 2009. 55(1): p. 95-102.
10. Tsuchiya T, et al., Novel harmful effects of [60]fullerene on mouse embryos in vitro and in vivo. FEBS 1996 Lett 393 (1): 139-45.
11. Ashwood, P., R. Thompson, and J. Powell, Fine particles that adsorb lipopolysaccharide via bridging calcium cations may mimic bacterial pathogenicity towards cells. Exp Biol Med 2007. 232(1): p. 107-117.
12. Boxall, A.B.A., K. Tiede, and Q. Chaudry, Engineered nanomaterials in soils and water: how do they behave and could they pose a risk to human health. Nanomedicine, 2007. 2(6): p. 919-927.
13. Mueller, N.C. and B. Nowack, Exposure Modeling of Engineered Nanoparticles in the Environment. Environmental Science Technolology, 2008. 42: p. 4447-4453.
14. Hussain, M.A., M.A. Kabir, and A.K. Sood, On the cytotoxicity of carbon nanotubes. Current science, 2009. 96(5): p. 664-673.
15. Thayer, A.M., Carbon nanotubes by the metric ton. Chemical & Engineering News, 2007: p. 29-35.
16. Shvedova, A.A., et al., Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. . American Journal of Lung Cellular and Molecular Physiology, 2005. A289: p. 698-708.
17. Sayes, C.M., et al., Functionalization density dependence of single-walled carbon nanotubes cytotoxicity in vitro. Toxicological Letters, 2006. 161(1): p. 135-142.
18. Cui, D.X., et al., Effect of single wall carbon nanotubes on human HEK293 cells. . Toxicological Letters, 2005. 155(1): p. 73-85.
19. Lindberg, H.K., et al., Genotoxicity of nanomaterials: DNA damage and micronuclei induced by carbon nanotubes and graphite nanofibres in human bronchial epithelial cells in vitro. Toxicology Letters 2009. 186(3): p. 166-173.
20. Carrero-Sanchez, J.C., et al., Biocompatibility and toxicological studies of Carbon Nanotubes doped with nitrogen. . Nano Lett 6(8): , 2006. 6(8): p. 1609-1616.
21. Lam, C.W., et al., Pulmonary toxicity of single-wall Carbon Nanotubes in mice 7 and 90 days after intratracheal instillation. Toxicological Science, 2004. 77(126-134).
22. Poland, C.A., et al., Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nature Nanotechnology 2008. 3: p. 423 - 428.
23. NIOSH_Science_Blog, Persistent Pulmonary Fibrosis, Migration to the Pleura, and Other Preliminary New Findings after Subchronic Exposure to Multi-Walled Carbon Nanotubes. 2009.
24. Ryman-Rasmussen, J.P., et al., Inhaled Multiwalled Carbon Nanotubes Potentiate Airway Fibrosis in Murine Allergic Asthma. American Journal of Respiratory Cell and Molecular Biology, 2009. 40(3): p. 349-358.
25. Smith, C.J., B.J. Shaw, and R.D. Handy, Toxicity of Single Walled Carbon Nanotubes on Rainbow Trout, (Oncorhynchus mykiss): Respiratory Toxicity, Organ Pathologies, and Other Physiological Effects. . Aquatic Toxicology, 2007. 82(2): p. 94-109.
26. Cheng, J.P., E. Flahaut, and S.H. Cheng, Effect of carbon nanotubes on developing zebrafish (Danio rerio) embryos. . Environmental Toxicololgy and Chemistry, 2007. 26(4): p. 708-716 and Templeton, R.C., et al., Life-cycle effects of single-walled carbon nanotubes (swnts) on an estuarine meiobenthic copepod. Environmental Science Technolology, 2006. 40(23): p. 7387-7393.
27. Lin, S., et al., Uptake, Translocation, and Transmission of Carbon Nanomaterials in Rice Plants. Small, 2009. 5(10): p. 1128-1132.
28. Meyer, D.E., M.A. Curran, and M.A. Gonzalez, An Examination of Existing Data for the Industrial Manufacture and Use of Nanocomponents and Their Role in the Life Cycle Impact of Nanoproducts. Environmental Science Technolology, 2009. 43(6): p. 1256-1263.
29. Blaser, S.A., et al., Estimation of cumulative aquatic exposure and risk due to silver: Contribution of nano-functionalized plastics and textiles. Science of the total environment 2008. 390: p. 396-409.
30. Pal, S., Y.K. Tak, and S.J. Myong, Does the Antibacterial Activity of Silver Nanoparticles Depend on the Shape of the Nanoparticle? A Study of the Gram-Negative Bacterium Escherichia coli. APPLIED AND ENVIRONMENTAL MICROBIOLOGY, 2007. 73(6): p. 1712–1720.
31. Gram-Negative Bacterium Escherichia coli. APPLIED AND ENVIRONMENTAL MICROBIOLOGY, 2007. 73(6): p. 1712–1720.
32. Elechiguerra, J.L., et al., Interaction of silver nanoparticles with HIV-1. Journal of Nanobiotechnology, 2005. 3(6).
33. Zeng, F., et al., Silver nanoparticles directly formed on natural macroporous matrix and their anti microbial activities. Nanotechnology, 2007. 18.
34. Braydich-Stolle, L., et al., In Vitro Cytotoxicity of Nanoparticles in Mammalian Germline Stem Cells. Toxicological Sciences 88 2005. 88(2): p. 412–419
35. Morones, J.R., et al., The bacterial effect of silver nanoparticles. . Nanotechnology 2005. 16: p. 2346 - 2533.
36. Hwang, E.T., et al., Analysis of the toxic Mode of Action of silver nanoparticles using stress-specific bioluminescent bacteria. Small 2007. 4(6): p. 746-750.
37. Berger, M., Stabilizing antimicrobial nanosilver on a natural porous plant material. Nanowerk 17 Jan 07, 2007.
38. Hussain, S.M., et al., In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicology in Vitro. , 2005(19): p. 975–983.
39. Hussain, S.M., et al., The Interaction of Manganese Nanoparticles with PC-12 Cells Induces Dopamine Depletion. Toxocological Sciences, 2006. 92(2): p. 456-463.
40. Carlson, C., et al., Unique Cellular Interaction of Silver Nanoparticles: Size-Dependent Generation of Reactive Oxygen Species. The Journal of Physical Chemistry B, 2008. 112(43): p. 13608-13619.
41. Landsdown, A.B.G., Critical observations on the neurotoxictiy of silver Critical reviews in toxicology, 2007. 37(3): p. 237-250.
42. Lee, K.J., et al., In Vivo Imaging of Transport and Biocompatibility of Single Silver Nanoparticles in Early Development of Zebrafish Embryos. ACS Nano, 2007. 1(2): p. 133-143.
43. Asharani, P.V., et al., Toxicity of silver nanoparticles in zebrafish models. Nanotechnology, 2008. 19.
44. Wang, H., R.L. Wick, and B. Xing, Toxicity of nanoparticulate and bulk ZnO, Al2O3 and TiO2 to the nematode Caenorhabditis elegans. Environmental Pollution 2009. 157: p. 1171–1177.
45. Tran, C., et al., A scoping study to identify hazard data needs for addressing the risks presented by nanoparticles and nanotubes. . 2005.
46. Barker, P. and A. Branch, The interaction of modern sunscreen formulations with surface coatings. Progress in Organic Coatings, 2008. 62.
47. Long, T., et al., Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): Implications for nanoparticle neurotoxicity. . Environmental Science Technolology, 2006. 40(14): p. 4346-4352.
48. Lucarelli, M., et al., Innate defence functions of macrophages can be biased by nano-sized ceramic and metallic particles. European Cytok Net 2004. 15(4): p. 339-346.
49. Wang, J., et al., Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. . Toxicological Letters, 2007. 168(2): p. 176-185.
50. Schneider, J., Can microparticles contribute to inflammatory bowel disease: Innocuous or inflammatory? Exp Biol Med 2007. 232(1-2).
51. Takeda, K., et al., Nanoparticles Transferred from Pregnant Mice to Their Offspring Can Damage the Genital and Cranial Nerve Systems. Journal of Health Sciences, 2009. 55(1): p. 95-102.
52. Zhang, X., et al., Enhanced bioaccumulation of cadmium in carp in the presence of titanium dioxide nanoparticles. Chemosphere, 2007. 76: p. 160-166.
53. Lovern, S.B. and R. Klaper, Daphnia Magna mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles. . Environ Toxicol Chem 2006. 25(4): p. 1132-1137.
54. Brunner, T.I., et al., Environ. Sci. Technol. In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica and the effect of particle solubility., 2006. 40: p. 4374-4381.
55. Wang, B., et al., Acute toxicity of nano- and micro-scale zinc powder in healthy adult mice. Toxicological Letters, 2006. 161: p. 115–123.
56. Wang, B., et al., Acute toxicological impact of nano- and submicro-scaled zinc oxide powder on healthy adult mice. . Journal of Nanoparticle Research, 2007. 10(2): p. 263-276.
57. Sharmaa, V., et al., DNA damaging potential of zinc oxide nanoparticles in human epidermal cells Toxicological Letters, 2009. 185(3).
58. Brayner, R., et al., Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. . Nano Letters, 2006. 6: p. 866-870.
59. Heinlaan, M., et al., Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria Vibrio fischeri and crustaceans Daphnia magna and Thamnocephalus platyurus. . Chemosphere 71, , 2008. 71: p. 1308–1316.
60. Lin, D. and B. Xing, Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. . Environmental Pollution, 2007. 150: p. 243–250.
61. Wanga, H., R.L. Wicka, and B. Xing, Toxicity of nanoparticulate and bulk ZnO, Al2O3 and TiO2 to the nematode Caenorhabditis elegans. Environmental Pollution, 2008. 157(4): p. 1171-1177.
62. Zhu, X., et al., The impact of ZnO nanoparticle aggregates on the embryonic development of zebrafish (Danio rerio). Nnaotechnology, 2009. 20(19).

NanoCap is a European project that sets up a consortium of 5 environmental civil society groups, 5 trade unions and 5 universities. It focuses on capacity building enabling the project partners to work in the filed of nanotechnologies safe and sustainable development and applications. It aims to deepen the understanding of environmental and occupational health impacts, safety risks and ethical and regulatory aspects of these technologies.

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