NANOMATERIALS IN HUMANS: Exposure of environment and humans

Exposure of nanomaterials to workers, consumers, and the environment seems inevitable with the increasing production volumes and the increasing number of commercially available products containing nanomaterials or based on nanotechnology exposure is a key element in risk assessment of nanomaterials since it is a precondition for the potential toxicological and ecotoxicological effects to take place. If there is no exposure – there is no risk. Nanoparticles are already being used in various products and the exposure can happen through multiple routes.

Human routes of exposure are:

- Dermal (for instance through the use of cosmetics containing nanoparticles);
- Inhalation (of nanoparticles for instance in the workpiece);
- Ingestion (for instance food products containing nanoparticles);
- And injection (for instance medicine based on nanotechnology).

Hazard Identification

In order to complete a hazard identification of nanomaterials, the following is ideally required.

- ecotoxicological studies
- Data about toxic effects
- Information on physical – chemical properties
  - Solubility
  - Sorption
- Biodegrability
- Accumulation

And all likely depending on the specific size and detailed composition of the nanoparticles.

In addition to the physical – chemical properties normally considered in relation to chemical substances, the physical chemical properties of nanomateials is dependent on a number of additional factors such as size structure, shape, and surface area. Opinions on, which of these factors are important differ among scientists, and the identification of key properties is a key gap of our current knowledge.

There is little doubt that the physical – chemical properties normally required when doing a hazard identification of chemical substances are not representative for nanomaterials, however there is at current no alternative methods. In the following key issues in regards to determining the destiny and distribution of nanoparticles in the environment will be discussed, however the focus will primarily be on fullerenes such as C60.

Interaction in the environment

Nanoparticles can be used to enhance the bioavailability of other chemical substances so that they are easily degradable or harmful substances can be transported to vulnerable ecosystems.

Besides the toxicity of the nanoparticeles itself, it is furthermore unclear whether nanoparticles increases the bioavailability or toxicity of other xenobiotics in the environment or other substances in the human body. Nanoparticles such as C60 have many potential uses in for inistance in medicine because of their ability to transport drugs to parts of the body which are normally hard to reach. However, this property is exactly what also may be the source to adverse toxic effects. Furthermore research is being done into the application of nanoparticles or spreading of contaminants already in the environment. This is being pursued in order to increase the bioavailability for degradation of microorganism, however it may also lead to increase uptake and increased toxicity of contaminants in plants and animals, but to the best of our knowledge, no scientific information is available that supports this.

Conclusion

It is still too early to determine whether nanomaterials or nanoparticles are harmful or not however the effects observed lately have made many public and governmental institutions aware of the lack of knowledge concerning the properties of nanoparticles the urgent need for a systematic evaluation of the potential adverse effect of Nanotechnology. Furthermore, some guidance is needed as to which precautionary measures are warranted in order to encourage the development of “green nanotechnologies” and other future innovative technologies, while

at the same time minimizing the potential for negative surprises in the form of adverse effects on human health and/or the environment, it is important to understand that there are many different nanomaterials and that the risk they pose will differ substantially depending on their properties. At the moment it is not possible to identify which properties or combination of properties make some nanomaterials harmful and which make them harmless, and property it will depend on the nanomaterial is question. This makes it is extremely difficult to do risk assessments and life-cycle assessment of nanomaterials because, in theory, you would have to do a risk assessment for each of the specific variation of nanomaterials – a daunting task!

Nanotoxicology

Nanotoxicology is the study of the toxicity of nanomaterials. Because of quanum size effects and large surface area to volume ratio, nanomaterials have unique properties compared with their larger counterparts.

Nanotoxicology is a branch of bionanoscience which deals with the study and application of toxicity of nanomaterials.

Nanomaterials, even when made of inert elements like gold, become highly active at nanometer dimensions.

Nanotoxicological studies are intended to determine whether and to what extent these properties may pose a threat to the environment and to human beings. For instance, diesel nanoparticles have been found to damage the cardiovascular system in a mouse model.

Nanotoxicology is a sub-speciality of particle toxic9ology. It addresses the toxicology of nanoparticles (particles <100 nm diameter) which appear to have toxicity effects that are unusual and not seen with larger particles. Nanoparticles can be divided into combustion-derived nanoparticles (like diesel soot), manufactured nanoparticles like carbon nanotubes and naturally occurring nanoparticles from volcanic eruptions, atmospheric chemistry etc.

Typical nanoparticles that have been studied are titanium dioxide, alumina, zinc oxide, carbon black, and carbon nanotubes, and "nano-nC60". Nanoparticles have much larger surface area to unit mass ratios which in some cases may lead to greater pro-inflammatory effects (in, for example lung tissue). In addition, some nanoparticles seem to be able to translocate from their site of deposition to distant sites such as the blood and the brain. This has resulted in a sea-change in how particle toxicology is viewed – instead of being confined to the lungs, nanoparticle toxicologists study the brain, blood, liver, skin and gut.

Toxicity of QDs

There are different opinions about the toxicity of QDs; therefore, we investigated their toxicity in amoeba as primary eukaryotes, in plant, and in animal.

a) In amoeba

It has been determined that QD labeling had no detectable effect on cell growth and had no deleterious effects on cellular signaling and motility during development of the Dictyostelium discoideum cells.

b) in plant

The ratio of reduced glutathione levels (GSH) relative to the oxidized glutathione (GSSG) in plants suggests that QDs caused oxidative stress on the plant at this condition.

c) In animal

Yan et al. investigated the potential vascular endothelial toxicity of mercaptosuccinic acid (2-sulfanylbutanedioic acid) – capped QDs in vitro.

Their results suggested that QDs could not only impair mitochondria but also exert endothelial toxicity through activation of mitochondrial death pathway and induction of endothelial apoptosis.

More recently, Chen et al. have studied the cytotoxicity of CdTe/CDs (core-shell) structured and also CdTe/CDs/ZnS (core-shell-shell) structured aqueous synthesized QDs, and their results suggest that the cytotoxicity of CdTe QDs not only comes from the release of Cd^{2+} ions but also intracellular distribution of QDs in cells and the associated nanoscale effects.

Metal nano particles

Nanomaterials are consistently being released into the environmental via spillages, wear washing and disposal at a rate proportional to their level of use. We don't know where the go or what happens to them after they are released: they may accumulate on land or water and enter the food chain via plants and aquatic life, where they could exist harmlessly or react. They might enter the human body: routes include inhalation, ingestion or even possibly through our skin.

It has been suggested that once inside the body, the smallness of nanomaterials allows them to easily could allow them to penetrate living cells, where they can amass, disrupt cell activity or corrupt genes. They can also bypass the visual transportation channels, cross the blood-brain barrier.

i) Lungs

There has been much interest in the health effects of airborne particles, specifically PM10 (thoracic fraction).

PM2.5 (respirable fraction), PM1, and ultrafine particles (PM0.1), which are = 10, 2.5, 1, and 0.1 μm (100 nm), respectively. One to 5 nm air suspended ENMs that enter the lungs are not predicted to reach the alveoli; instead a high percentage is likely to deposit in the mucus – lined upper airways (tracheobronchial region) due to their strong diffusion properties. On the other hand -45% of 10 nm, - 50% of 20 nm, and – 25% of 100 nm ENMs deposit in the alveoli. Deposition is greater during exercises. Chronic obstructive pulmonary and decreases alveolar particle deposition.

ii) Nasal cavity

Uptake from the nasal cavity into the olfactory nerve, followed by retrograde axonal transport to the olfactory bulb and beyond, was shown in studies of the polio virus (30 nm) and colloidal silver coated gold (50 nm). Uptake of -35 nm 13C particles along the olfactory pathway to the olfactory bulb, and to a lesser extent into the cerebrum and cerebellum, was shown 1 to 7 days later. Exposure to ~30 nm agglomerates of Mn by inhalation resulted in upto a 3.5 fold increase of Mn in the olfactory bulb, and lower (but significant) increases in 4 rat brain regions. The increase of Mn in brain regions other than the olfactory bulb may have resulted from translocation to the brain by route(s) other than via the olfactory nerve, such as through cerebrospinal fluid or across the blood-brain barrier. The nasal cavity is the only site where the nervous system is exposed directly to the environment. This is an often overlooked potential route of small amounts of ENMs into the brain.

iii) Dermal exposure

Skin is composed of 4 primary layers, the outermost epidermis (which contains the stratum corneum, stratum granulosum and stratum spinosum), dermis, and hypodermis. The hair follicle is an invagination of the stratum comeum, lined by a horny layer (acroinfundibulum). Dermal uptake routes are intercellular, intracellular, and follicular penetration. Uptake is primarily by diffusion. Materials that diffuse through the lipid-rich intercellular space of the stratum comeum typically have a low molecular weight (< 500 Da) and are lipophilic.

Materials that penetrate to the stratum corneum into the stratum granulosum can induce the resident keratinocytes to release proinflammatory cytokines. Materials that penetrate to the stratum spinosum, which contains langerhands cells (dendritic cells of the immune system), can initiate an immunological response.

This is mediated by the Langerhans cells, which can become antigen-presenting cells and can interact with T-cells. Once materials reach the stratum granulosum or stratum spinosum there is little barrier to absorption into the circulatory and lymphatic systems. Whereas dry powder ENMs pose a greater risk for inhalation exposure than those in liquids, liquid dispersed ENMs present a greater risk for dermal exposure.

Consumer materials most relevant to dermal exposure include quntum dots, titania, and zinc oxide in sunscreens, and silver as an anti-microbial agent in clothing and other products. Prolonged dermal application of microfine titania sunscreen suggested penetration into the epidermis and dermis. However, subsequent studies did not verify penetration of titania from sunscreens into epidermis or dermis of human, porcine or psoriatic skin or find evidence of skin penetration of zinc oxide from sunscreen or positively or negatively – changed iron containing ENMs. Nanoparticles with dye penetrated deeper into hair follicles of massaged porcine skin in vitro and persisted thirty nm carbodylated quantum dots applied to the skin of mice were localized in the folds and defects in the stratum corneum and hair follicles. A small amount penetrated as deep as the dermis. Ultraviolet radiation increased penetration, raising concern that these results might generalize and enter the central nervous system. The high surface area and surface activity of nanomaterials means they may have amplified effects tests on human cells grown in laboratories have shown immune reactions and inflammations in lung tissue. However, it is important to remember that many of these dangers are still speculative, man made nanoparticles are relatively simple, and existing materials are not considered to have the complexity of systems like viruses that themselves struggle to enter and corrupt cells, and are of a similar size to nanomaterials. There is only one way to find out whether these risks are realistic or speculative at normal nanomaterial concentrations – testing. But this is rather difficult.

Problems with Testing

Toxicity is not only idiosyncratic to the chemical composition and structure of each nanomaterial, but is also dependent upon other factors in particular, shape and solubility. Shape may affect interactions with binding sites on enzymes, and sphercity or regularity may affect surface reactivity and mobility.

Surface charge fundamentally changes reactivity negatively charged membranes interact with positively charged particles, but not negative ones. Even size matters. After all, a 100 nm diameter particles is one million times bigger (in volume) than a 1 nm diameter particles – and even across the nanosclae, transitions may occur between fundamental properties.

Whether the tests are carried out n plants, cells, or organisms can also affect the conclusions of testing, as can the materials used during nanomaterial synthesis to direct the nano size and shape since most of these are toxic themselves and might contaminate products. These factors all depend on the experimental conditions under which the nanomaterial was synthesized and tested, making comparison between research groups almost impossible.

For the toxicity of nanomaterials to be meaningful, they need to be compared with the toxicity of the bulk material, but not all materials can exist when the same chemical structure for nano and bulk types. Even if they do, we can't be sure nanomaterials retain their dimensions under testing they might form chains, aggregate lumps, or break down into smaller nanoparticles. Without accounting for these variations, we can't be sure how the "nano" factor affects toxicity, and whether nanomaterials pose us any risk. This is wahy no regulatory guidelines exist, and why nanomaterials are so freely used. But precisely because they are being freely used, we really need to know the answers.

Metal oxide nanomaterials

The knowledge on potential harmful effects of metallic nanomaterials lags behind their increased use in consumer products and therefore, the safety data on various nanomaterials applicable for risk assessment are urgently needed. In this study, 11 metal oxide nanoparticles (MeOx NPs) prepared using flame pyrolysis method were analysed for their toxicity nanoscale sunscreens. PEG-coated ~37 nm quantum dots accumulated in the lymphatic duct system after intra-dermal injection in mice. Cadmium, determined by ICP-MS, from cadmium-containing quantum dots was seen in liver, spleen, and heart, however, it is uncertain if this was from cadmium or translocation of the quantum dots because methods were not used to show the presence of quantum dots. The above results suggest topically applied ENMs that penetrate to the dermis might enter the lymphatic system, and the ENMs or dissolved components distribute systematically. To address these concerns ENMs intended for dermal application such as titania, are often surface coated, eg. With silica, alumina, or manganese. One goal of the surface treatment is to minimize toxicity by trapping the free radicals of reactive oxygen species (ROS).

An in vitro study showed that mechanical stretching of human skin increased penetration of 500 and 100 nm fluorescent dextran particles through the stratum corneum, with some distribution into the epidermis and dermis. Similarly, mechanical flexing increased penetration of a 3-5 nm phenylalanine based C60 amino acid ENM through porcine skin in vitro. The contribution of skin flexing and immune system response was further addressed wit three titania formulations applied to minipigs. There was some ENM penetration into epidermis and abdominal and neck dermis, but no elevation of titanium in lymph nodes or liver. Topical exposure of mice to SWCNTs resulted in oxidative stress in the skin and skin thickening, demonstrating the potential for toxicity not revealed by in vitro studies of ENM skin penetration. There are no reports of long-term studies with topical ENM exposure.

In the absence of organic solvents, the above suggests that topically applied ENMs do not penetrate normal skin. Not surprisingly, organic solvents (chloroform > coclohexane > toluene) increased penetration of fullerene into skin that had the stratum corneum removed by tape stripping. As the fullernes were not detected in systemic circulation, there was no evidence of systemic absorption.

Oral exposure

Little is known about the bioavailability of ENMs from the buccal cavity or the sub-lingual site, or possible adverse effects from oral ingestion.

Particle absorption from the intestine results from diffusion though the mucus layer, initial contact with enterocytes or M (microfold or membranous specialized phatocytic enterocyte) cells, cellular trafficking, and post-translocation events . Colloidal bismuth subcitrate particles (4.5 nm at neutral pH) rapidly penetrated the mucosa of dyspeptic humans, resulting in bismuth in the blood. Particles appeared to penetrate only in regions of gastric epithelial disruption. Greater uptake of 50 to 60 nm polysterene particles was seen through Peyer's patches and enterocytes in the villous region of the GI tract than in non-lymphoid tissue, although the latter has a much larger intestinal surface area. Peyer's patches are one element of gut-associated lymphoid tissue, which consist of M cells and epithelial cells with a reduced number of goblet cells, resulting in lower mucin production. It was estimated that ~7% of 50 nm and 4% of 100 – nm polysterene ENMs were absorbed.

Fifty nm polystyrene ENMs fed to rats for 10 days by gavage showed 34% absorption, of which about 75% was in the liver, spleen, blood and bone marrow, no ENMs were seen in heart or lung. After oral administration of 50 nm fluorescence – labeled polystyrene ENMs, 18% of the dose appeared in the bile within 24 h and 9% was seen in the blood at 24h, none was observed in urine. The mechanism of GI uptake of 4, 10, 28 or 58 nm colloidal (maltodextran) gold ENMs from the drinking water of mice was shown to be penetration through gaps created by enterocytes that had died and were being extruded from the villus. Gold abundance in peripheral organs inversely correlated with particle size.

In summary, there appears to be significant absorption of some ENMs from the GI tract, with absorption inversely related to ENM size. The absorption site seems to be regions of compromised gastric epithelial integrity and low mucin content.

Ocular and mucous membrane exposure

Ocular exposure might occur from ENMs that are airborne, intentionality placed near the eye (e.g. cosmetics), accidently splashed onto the eye, or by transfer from the hands during rubbing of the eyes, which was shown to occur in 37% of 124 adults every hour. This route of exposure could result in ENM uptake through the cornea into the eye or drainage from the eye socket into the nasal cavity through the nasolacrimal duct. Other than a study that found uptake of a polymer ENM into conjuctival and corneal cells, this route has been largely ignored in research studies of ENM exposure.