

# Nanotherapeutics Magic Bullets- a Boon or Bane to Human Health

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## Abstract

Nanotechnology is a collective definition referring to every technology and science which operates on a nanoscale. Nanoparticles have different properties than larger particles and these properties can be utilized in a wide spectre of areas such as in medicine, information technologies, energy production and storage, materials, manufacturing, and environmental applications. Although nano-derived applications have great potential, there are some concerns about the potential nanoparticles causing adverse effects on human health and the environment. The different properties that make nanoparticles so promising are at the same time properties that are likely to have impact on ecosystems and organisms. This review maps out the current knowledge base of hazards and risks of nanoparticles to human health and the environment. The main findings of this study are that nanoparticles may cause more toxic effects than bigger particles and can translocate within the environment and the body. However, nanoparticles are likely to cause different impacts on human health, occupation health and the environment, depending on the size, shape and chemical composition of the nanoparticle. There is therefore great uncertainty about what the actual risks of nanoparticle to human health and the environment are. Both industry and regulatory bodies are aware of the potential risks of nanoparticles. The producers do not believe that nanoparticles represent a risk to the environment, but feel that nanoparticles can be more problematic in occupational settings. Producers therefore apply some precautionary measures to protect their workers, but limited for the protection of public health or environment. The issues of nanoparticles have a low priority among the regulatory bodies at the moment. The regulators are waiting for more scientific evidence and are therefore not taking any direct precautionary measures.

## Introduction

While nanotechnology and the production of nanoparticles are growing exponentially, research into the toxicological impact and possible hazard of nanoparticles to human health and the environment is still in its infancy. This review aims to give a comprehensive summary of what is known today about nanoparticle toxicology, the mechanisms at the cellular level, entry routes into the body, and possible impacts to public health [1]. Nanoparticles are small-scale substances (<100 nm) with unique properties and, thus, complex exposure and health risk implications [2]. Nanoparticles have been used for centuries. Nano-sized materials are naturally present in forest fires and volcanoes, also generated unintentionally from anthropogenic sources as a by-product of combustion and deliberately as manufactured nanomaterials. The colored glass that we see in many old cathedrals from the middle age was made of gold nanosized clusters that created different color depending on the size of the nanoparticles. The most prominent example of engineered nanoparticulate material is carbon black which has been around us for decades in applications like printing inks, toners, coatings, plastics, paper, tires and building products. Nanoparticles have different properties than larger particles and these properties can be utilized in a wide spectre of areas such as in medicine, information technologies, energy production and storage, materials, manufacturing and environmental applications. Although nano-derived applications have great potential, there are some concerns about the potential nanoparticles causing adverse effects on human health and the environment. The different properties that make nanoparticles so promising are at the same time properties that are likely to have impact on ecosystems and organisms. Therefore, nanotoxicology has emerged only recently, years after the first boom of nanotechnology, when various nanomaterial's had already been introduced into a number of industrial processes and products [3]. Nanotechnology is considered by some to be the next industrial revolution and is believed to cause enormous impacts on the society, economy and life in general in the future [4]. The fields in which the nanotechnology is and will be used reaches over a wide spectre of areas as in medicine, information technologies, biotechnologies, energy production and storage, material

technologies, manufacturing, instrumentation, environmental applications and security. There are few industries that will escape the influence of nanotechnology [5] and consequently will affect our daily life in the future. However, it is important to understand that nanotechnology as such is not an industry, but an enabling technology that, combined with other technologies, has the potential to impact most other industries in various ways.

There are also some potential negative environmental and health aspects that may follow the nanotechnology. The urgency to react to a growing unregulated use of new nanomaterial's, leading inevitably to some degree of environmental and human exposure, was apparent. Since then, investigations into the toxicological potential of nanomaterials have been constantly trying to catch up with the rapid growth of nanotechnology [6]. Engineered nanomaterial's can penetrate the skin, lungs and intestinal tract with unknown effects to human health as nanoparticles can travel around in the body and reach for example the brain [7]. The new engineered nanoparticles have novel properties not previously known and it is likely because of their novel properties will cause impacts on ecosystems and organisms. As an example, a study led by Eva Oberdorster found that a type of buckyball - a carbon nanoparticle that shows promise for electronic and pharmaceutical uses - can cause brain damage in fish [8]. Nanoparticles can cause other effects if they react with other substances or even carry other substances into organisms, soil or groundwater.

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Received April 23, 2012; Accepted June 04, 2012; Published June 10, 2012

Citation: Gauri B (2012) Nanotherapeutics Magic Bullets- a Boon or Bane to Human Health. J Nanomed Nanotechnol 3:142. doi:10.4172/2157-7439.1000142

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There are currently no regulations that cover nanomaterials and there is no demand to do risk assessment of nanoparticles as such, if the parent, bulk compound is already assessed. When risk assessments are conducted on for example chemicals, one of the biggest uncertainties lie in the exposure assessment. Additionally, as nanoparticles can have such widely different applications and forms of hazards and risks, the limited literature available is published in very different journals which make it difficult to get an overview of the relevant literature. This is time consuming and also an expressed problem for producers, researchers and authorities.

This article therefore attempts to give an overview of the most recent knowledge about the hazards and the potential risks of nanoparticles. The objective of this article is to provide a basic understanding of the risks to human health and the environment involved in technologies that brings materials down to the size of nanometers and how we currently are facing these risks.

### Types of nanoparticles for clinical applications

There are several types of these new nanomaterial's like the quantum dots, fullerenes and nanotubes which have a vast potential in the medical arena as drug and gene delivery vehicles, fluorescent labels and contrast agents.

**Liquid crystals:** Liquid crystal pharmaceuticals are composed of organic liquid crystal materials that mimic naturally occurring biomolecules like proteins or lipids. They are considered a very safe method for drug delivery and can target specific areas of the body where tissues are inflamed, or where tumors are found. Liquid crystal materials generally have several common characteristics. Among these are rod-like molecular structures, rigidity of the long axis, and strong dipoles and/or easily polarizable substituents [9].

**Quantum dots:** Quantum dots are often referred to as artificial atoms. They are tiny particles, or "nanoparticles", of a semiconductor material, traditionally chalcogenides (selenides or sulfides) of metals like cadmium or zinc (CdSe or ZnS, for example), which range from 2 to 10 nanometers in diameter (about the width of 50 atoms) [10]. Quantum dots work under the laws of quantum mechanics that cannot be explained by the classical mechanics and electromagnetic theory. Quantum dots are often fabricated in semiconductor material. The size and shape can be precisely controlled and a quantum dot may have certain number of electrons. Quantum dots show among other, potential within the information technology. Quantum dots (QDs) are semiconductor nanocrystals that emit fluorescence on excitation with a light source. They have excellent optical properties, including high brightness, resistance to photo bleaching and tunable wavelength. Recent developments in surface modification of QDs enable their potential application in cancer imaging. QDs with near-infrared emission could be applied to sentinel lymph-node mapping to aid biopsy and surgery. Conjugation of Quantum Dots with biomolecules, including peptides and antibodies, could be used to target tumors in vivo. In-vitro studies indicated that quantum dots may be toxic [11,12] of which some toxicity could be attributed to surface coating [11]( Figure 1).

**Fullerenes:** Fullerenes are carbon molecules formed as large hollow closed-caged clusters and have several special properties that were not found in any other compound before. Fullerenes are being explored as potential new antimicrobial agents in view of their potency for induction of reactive oxygen species after photo excitation. However, this may have an impact on microbial communities if they are released into the environment via effluents. Therefore, various studies with

fullerenes have been published with regard to the ecotoxicity of these important building blocks in nanomaterials [13,14].

The first fullerene discovered was the buckyball in 1985 [15]. Buckyballs (Figure 2) are soccer-ball-shaped molecules that were first observed in a space 25 years ago. They are named for their resemblance to architect Buckminster Fuller's geodesic domes, which have interlocking circles on the surface of a partial sphere. Buckyballs were thought to float around in space, but had escaped detection until now. Buckyballs are made of 60 carbon atoms arranged in three-dimensional, spherical structures. Their alternating patterns of hexagons and pentagons match a typical black-and-white soccer ball. The C<sub>60</sub> fullerene can reduce the hepatic enzyme activity of glutathione (glutathione-S-transferase, glutathione peroxidase et glutathion reductase) *in vitro* in humans (liver coming from an autopsy), mice and rats [16].

**Nanotubes:** The nanotubes are typically a few nanometers in diameter, but can be several micrometers long. These nanotubes show

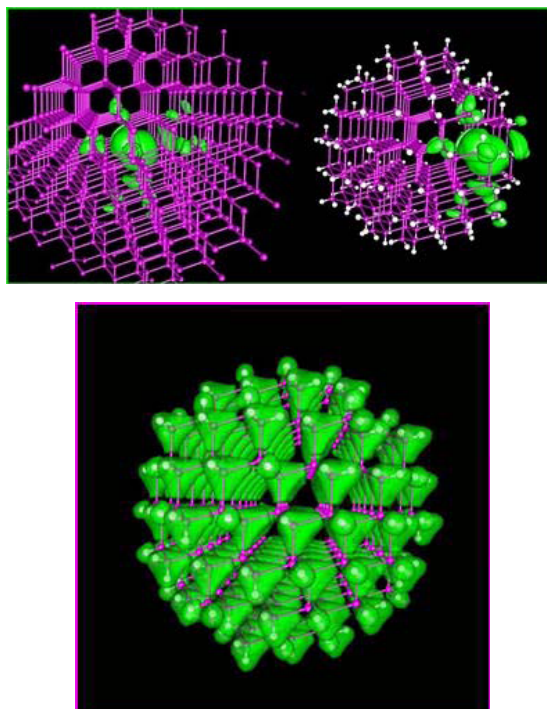


Figure 1: Systematic representation of functionalized Quantum Dots.

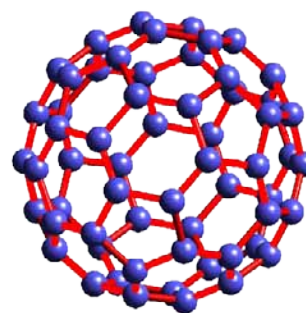


Figure 2: Systematic Representation of Structure of C-60 Buckyball [17].

highly promising potentials as they often are described as having one hundred times the tensile strength of steel, thermal conductivity better than all but the purest copper, and electrical conductivity similar to or better than copper, but with the ability to carry much higher currents. There are very different nanotubes with different properties and shape: long, short, single-walled, multi-walled, open, close, with different types of spiral structure etc. A vast developed nanotube is Carbon Nanotube. Carbon nanotubes are long carbon-based tubes that can be either single- or multiwalled and have the potential to act as biopersistent fibers. Nanotubes have aspect ratios >100, with lengths of several mm and diameters of 0.7 to 1.5 nm for single-walled carbon nanotubes (SWCNT) and 2 to 50 nm for Multiwalled Carbon Nanotubes (MWCNT) [18,19]. Recent studies with carbon derived nanomaterials showed that platelet aggregation was induced by both single and multi-wall carbon nanotubes, but not by the C60-fullerenes that are used as building blocks for these carbon nanotubes [20]. MWCNT also elicit pro-inflammatory effects in keratinocytes [21].

Nanotubes are very good at conducting communicative impulses, whether in the body or through technological devices. With the creation of special coatings for the nanotubes, the science may very well give sight to the blind, sound to the hearing impaired, and motion to the paralyzed. Medically speaking we could soon find a new field of specialty known as nanosurgery. In these procedures, cancer cells or other diseased cells could be eradicated from the body and then replaced by engineered nanotubes that are ready to redevelop the diseased cells with healthy impulses (Figure 3).

**Dendrimers:** Because of their specific nature dendrimers are specifically suited for drug delivery purposes. Although their small size (up to 10 nm) limits extensive drug incorporation into the dendrimers, their dendritic nature and branching allows for drug loading onto the outside surfaces of the polymeric structure [22]. Functionalization of the surface with specific antibodies may further enhance potential targeting. Apart from application in drug-delivery, dendrimers are being investigated for many other uses including bacterial cell killing, as gene transfer agents and trans-membrane transport. Little published data is available on the toxicity of this class of particles. A recent review on this topic [23] concluded that it will only ever be possible to designate a dendrimers as “safe” when related to a specific application. The so far limited clinical experience with dendrimers makes it impossible to designate any particular chemistry intrinsically “safe” or “toxic”.

**Gold nanoparticles/nanoshells:** Metallic colloidal gold nanoparticles are widely used, can be synthesized in different forms (rods, dots), are commercially available in various size ranges and can be detected at low concentrations. Cells can take up gold nanoparticles without cytotoxic effects [24,25]. For biomedical applications, they are used as potential carriers for drug delivery, imaging molecules and even genes [26], and for the development of novel cancer therapy products [27-31]. For gold nanorods the cytotoxicity could be attributed to the presence of the stabilizer CTAB of which even residual presence after washing resulted in considerable cytotoxicity. PEG-modified gold nanorods with removal the excess CTAB did not show cytotoxicity [32]. In an acute oral toxicity study no signs of gross toxicity or adverse effects were noted when a nanogold suspension (nanoparticle diameter ca. 50 nm) was evaluated, the single dose for acute oral LD50 being greater than 5000 mg/kg body weight [33]. Gold solutions are also used to prepare nanoshells composed of gold and copper, or gold and silver to function as contrast agents in Magnetic Resonance Imaging (RMI) [34], and gold-silica for photothermal ablation of tumor cells [35]. *In vitro* the non targeted nanoshells did not show cytotoxicity for the tumor cells, whereas after binding to the tumor cells, cell death could

be obtained after laser activation [36,37]. Also in vivo positive results were obtained with photothermal ablation therapy in a mouse model for colon carcinoma after intravenous administration of PEG coated gold nanoshells of approximately 130 nm [30] (Figure 4).

### Potential applications of nanoparticles

Applications involving nanoparticles that exist or show promises are presented here.

1. Nanotechnology is already being used in commercial applications for bulk products, such as sunscreens with increased transparency and cosmetics containing nanoparticles with the ability to target deeper into the body. The cosmetic companies have been active in using nanotechnology to improve their existing products and e.g. L’Oreal holds a very high number of nanotechnology patents [38].

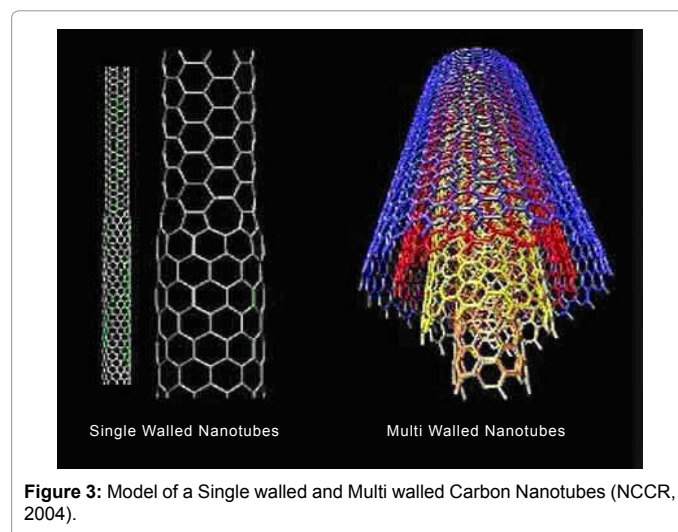


Figure 3: Model of a Single walled and Multi walled Carbon Nanotubes (NCCR, 2004).

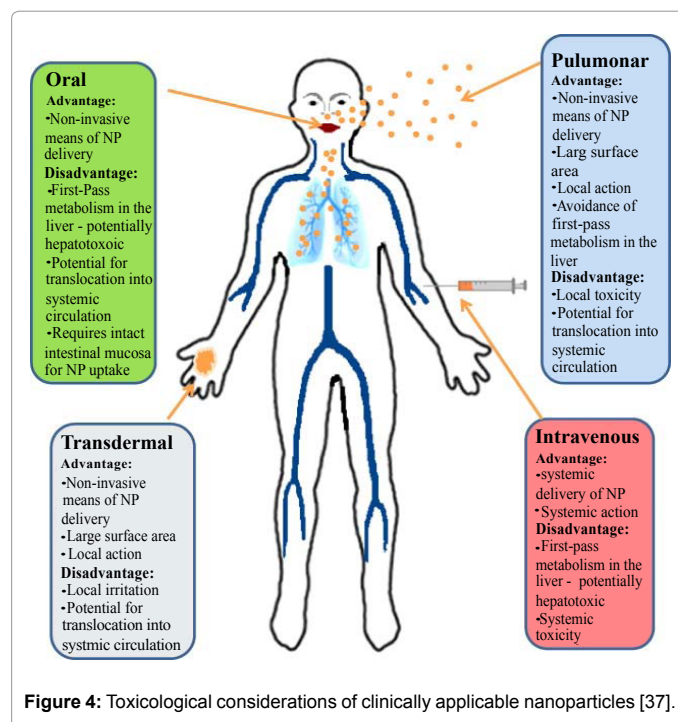


Figure 4: Toxicological considerations of clinically applicable nanoparticles [37].

2. These have especially potentials in aerospace industry, packaging and in the car industry where they already have been introduced in the GM Motors Safari and Chevrolet Astro vans [38]. The nanoparticles change the material's properties as e.g. metal gets harder, ceramics get softer and mixtures like alloys may get harder up to a point where they get softer again. By introducing clay nanoparticles it is possible to make the materials stronger, lighter, more durable and often transparent.
3. Other short-term uses includes solar energy collection (photovoltaics), medical diagnostic tools and sensors, flexible display technologies and e-paper, glues, paints and lubricants, various optical components, and new forms of computer memories and electronic circuit boards [39].
4. Smart textiles developed with the help of nanotechnology and in the long run textiles are expected to be able to change their physical properties according to the surrounding conditions, or even monitor vital signals [40]. The introduction of nanoparticles in textiles can make it possible to produce very light and durable textiles with resistance against water, stains and wrinkling.
5. Medical applications are one of the fields with the biggest expectations regarding human welfare. With the development of new materials and a combination of nanotechnology and biotechnology it could be possible to make artificial organs and implants through cell growth which could repair damaged nerve cells, replace damaged skin, tissue or bone [38]. Furthermore in the synergy of information technology and medicine there are expectations to e.g. diagnosis instruments for personal health monitoring providing ultra-fine precision and quick response time to the diagnosis tests.
6. Another application field in medicine is drug-delivery where research is especially intensive on the possibility of manipulating nanoparticles to deliver drugs because nanoparticles can have a better solubility and absorption potential than bigger particles. The nanoparticles can carry the drug and perhaps release it in fine-tuned doses over a long time period to a targeted area, reducing the side-effects of the traditional drugs.
7. Nano is also behind the creation of lighter weight and much stronger materials for use in lots of sports. Nanotech will eventually make better-performing yacht racing masts, hockey sticks, vaulting poles, softball bats, golf clubs and tennis rackets [41]. The technology will help make lighter racing bikes and Indy cars. Eventually, nanotech could produce some truly exotic sports products. Adidas, a shoe manufacturing company worked with Olympics 400 m runner Jeremy Wariner for more than two years to create the revolutionary Adidas Lone star spike (Figure 5), which is named after Wariner's home state of Texas. The Lone star is the world's first asymmetrical 400 m spike, featuring the first ever full length carbon nanotubes reinforced plate and the exclusive adidas progressive compression spike. The tapered grooves of the progressive, compression spike provide the best penetration/compression ratio, thus optimizing the spring like elastic property of the track surface. The French tennis racket manufacturer Babolat introduced a racket with carbon nanotubes which lead to an increased torsion and flex resistance. They were more rigid than current carbon rackets and pack more power. The Inmat company made tennis balls which have twice the lifetime than a conventional ball. Antibacterial fabrics based on silver

nanoparticles are used extensively for sports socks as well as ones for day to day wear just to keep the odor at bay. Jackets and towels are also using nano silver to kill bacteria.

8. There are also future environmental applications developed with the help of nanotechnology. For example carbon nanotubes show promises as a storage medium for hydrogen giving new possibilities for renewable energy. Other applications researched are nanoparticles as bioremediation. Biological organisms that are used to clean up soil pollution face the problem that in the soil most pollutants are not bioavailable, but locked up within pores in the soil structure. By using nanoparticles it may be possible to deliberately mobilize pollutants in a controlled manner so that they become bioavailable and ensuring that clean-up organisms are not killed by a rapid release.

The development of doing things smaller, lighter and faster than before has already been going on for many years. This enhanced precision could enable existing products and processes to be more effective, hence require less raw materials and energy. This is especially true in the field of IT, electronic and energy industry.

#### Hazards and risks of nanotechnology and nanoparticles

There are many concerns regarding the possible impacts on human health and the environment that can arise when the smaller constituents of materials are brought down to the nanoscale. Although these impacts may not be any different from those that can be caused by chemicals. The very young field of nanotoxicity has already linked some nanoparticles to:

- Damage to DNA
- Disruption of cellular function and production of reactive oxygen species
- Asbestos-like pathogenicity
- Neurologic problems (such as seizures)
- Organ damage, including significant lesions on the liver and kidneys



Figure 5: The "Lone star spike" shoes used by Jeremy Wariner.

- Destruction of beneficial bacteria in wastewater treatment systems
- Stunted root growth in corn, soybeans, carrots, cucumber and cabbage
- Gill damage, respiratory problems and oxidative stress in fish.

### Inhalation of nanoparticles

The most elementary property that nanoparticles have is the size. Small sized particles have an increase both in number and in relative surface area compared to particles of a bigger size, but with the same mass. If comparing bigger particles with e.g. a mass concentration 10  $\mu\text{g}/\text{m}^3$  with nanoparticles of the same mass concentration, there seems to be a correlation between a decrease in particle size and an increase in toxicity. This is a well established hypothesis when it comes to lung toxicity, where it is shown that ultrafine particles made of low-soluble, low-toxicity materials causes a stronger defence reaction in the lung tissue of rats than finer respirable particles made from the same material [42-44]. If we take the example of nickel, it is shown that indicators of lung injury were greater with ultrafine nickel (20 nm) than standard nickel (5  $\mu\text{m}$ ) [44] suggesting that ultrafine particles have a much more toxic effect than finer particles of the same material. However, the mechanisms behind are poorly understood. There are theories that are trying to explain the mechanisms behind the increased toxicity of ultrafine particles. The most well established theory is that it has to do with the increased surface area and/or combination with the increasing number of particles [45]. The increase in particle surface area is believed to be linked to lung cancer, lung fibrosis and inflammation in the lung. There are a considerable epidemiological studies suggesting that an increase in ambient particle concentration is related to increase in mortality and diseases in the exposed population. The strongest associations are seen for respiratory and cardiac deaths, particularly among the elderly and particulate air pollution is also associated with asthma exacerbations, increased respiratory symptoms, decreased lung function and increased medication use [46].

As already mentioned, there are a lot of concerns of nanoparticles, but nobody knows yet if they can be biodegradable. Particles that disappear within 1 to 2 weeks will probably not cause many problems, according to Paul Borm of the Center for Expertise in Life Sciences, Hogeschool Zuyd, the problems start when you use insoluble particles like carbon black, gold and so on [47]. According to Borm and Kreyling two third of inhaled fibres that are less than 20  $\mu\text{m}$  are not cleared out of the lungs, which means that the fibers will bio-accumulate in the lungs unless they are biodegradable or cleared out by other mechanisms than macrophages clearing that transport particles from the lower parts of the lung [48]. To make nanoparticles biodegradable will therefore prevent many problems.

### Dermal Penetration of nanomaterials

Skin can be exposed to solid nanoscale particles through either intentional or nonintentional means. Intentional dermal exposure to nanoscale materials may include the application of lotions or creams [2]. There are many consumer products containing nanoparticles already in the market that are applied to the skin. There are nanoparticles in cosmetics, suntan lotion and baby products that regulate and improve the moisture, odor or color. Up till today, there is no clear evidence whether nanoparticles can be absorbed through the skin, but the studies that are done are highly debated by scientists as the examination methods are of various characters. For example some studies are not based on living samples of skin which makes them only

of limited relevance [4]. It is also unknown how susceptible damaged skin is to nanoparticles. On the other hand there is some evidence that dermal exposure to nanoparticles may lead to direct penetration into top layer of the skin and possibly beyond into the blood stream [49]. Considering the wide variety of products already on the market, this needs to be found out urgently.

**Absorption through the intestinal tract:** Nanoparticles that are swallowed will sooner or later end up in the intestinal tract. A very important consideration is what happens with food containing nanoparticles. Will they remain in the intestinal tract or will they move on into the body? The two tasks of the intestine is to take up nutrition while protecting the body from unwanted substances in the food. It is not known whether nanoparticles are regarded as an 'unwanted substance' and excreted or will be absorbed. According to a report by the Swiss Re, can particles of under some 300 nm reach the bloodstream, while particles that are smaller than 100 nm are also absorbed in various tissues and organs [4]. As a general rule, the smaller the particles are, the more of them are absorbed and the deeper into the body they can go.

**Nanoparticle Translocation in the body:** Normally, foreign substances that enter the bloodstream are absorbed by special cells called phagocytes, which remove the foreign substance from the bloodstream. However, everything smaller than about 200 nm, is no longer specifically absorbed by these phagocytes, but by cells that are not actually "designed" for this function [4]. This can mean that nanoparticles, once entered the body can travel freely in the blood and through the body. Additionally small size means increased mobility. Nanoparticles diffuse more easily than solid particles and behave more like gas molecules in the air and like large molecules in solutions, being less subject to sedimentation than bigger particles. This may have implications also for the movement of nanoparticles in tissue. Whether nanoparticles enter and transfer within the body to different organs can have a significant importance for the impacts of nanoparticles on human health and in the environment.

Inhaled ultrafine particles are depending on the particle size, deposited in the nose region, and upper and lower level of the respiratory system. A recent study concluded that the central nerve system and the brain can be targeted by airborne solid ultrafine particles and that the most likely mechanism is from deposits in the nose region [50]. The study furthermore concluded that the nose region could provide a portal of entry into the central nerve system for solid ultrafine particles, circumventing the tight blood-brain barrier, but the potential effects on the central nerve system needs to be determined by further studies. The blood-brain barrier represents an insurmountable obstacle for a large number of drugs, including antibiotics, antineoplastic agents, and a variety of central nervous system-active drugs, especially neuropeptides and scientists have successfully transported drugs through this barrier of drug delivery to the brain by using nanoparticles [51]. If nanoparticles designed for drug-delivery can target the brain it is also likely that other nanoparticles can do the same. There are a number of studies done that support the hypothesis that ultrafine particles are able to translocate from the lung into the systemic circulation and reach organs like the liver in animals [52-56]. The large surface of nanoparticles also means that it can be able to bind, absorb and carry compounds such as drugs, probes and proteins [48], but also other substances like metals or toxic substances. This increasing reactivity with other substances can have consequences both for human health and the environment.

**Novel properties, different toxicity:** The novel properties are exactly what are making novel nanoparticles of such interest for

the industry and society. However the properties which make the nanoparticles interesting are also believed to change the toxicity of the material. Single-walled carbon nanotubes (SWCNT) have a very broad commercial application potential due to their superior mechanical and electrical properties, but are very light and can become airborne. In a study by [57], SWCNTs were induced into lungs of mice. The results showed that if carbon nanotubes reach the lungs, they are much more toxic than carbon black and can be more toxic than quartz, which is considered a serious occupational health hazard in chronic inhalation exposures. Another study showed that SWCNTs induced in rats caused inflammation and does not appear to follow the normal paradigm of toxic dusts such as quartz and silica suggesting a potentially new mechanism of pulmonary toxicity and injury by SWCNTs [45]. The ability of SWCNTs to cause lung damage may be consistent with their unique physicochemical properties which impart to them enhanced structural properties, but may also make them more persistent in biological and ecological systems [58]. In current practice SWCNTs are classified as a new form of graphite on material safety data sheets provided by manufacturers of these nanoparticles. As the two studies showed, the exposure limits for graphite-based material may not be adequate for setting a safe exposure level for SWCNTs and it is a question whether SWCNTs should have different exposure limits than bigger graphite material.

**Chemical composition and coating:** In addition, another consideration is the toxicity of the material the nanoparticles consists of, [59] showed in a rat study that the different materials such as nickel, cobalt and titanium dioxide give dramatically different inflammatory responses in the lung. Nickel was more toxic than cobalt with titanium dioxide giving the least toxic effect. It further showed that the substances had different ability to create free radical activity. This difference in free-radical-generation activity therefore could underlie the difference in inflammation that these three ultrafine particle types induced. Free radicals are atoms that possess an “unsatisfactory” number of electrons and the atoms either snatch or force electrons on the neighboring atoms. In this way the original free radicals optimize their own structure, but transform other atoms into free radicals, triggering a chain reaction. This process is completely natural in healthy cells, but when this process is reinforced by external factors they can harm cell walls and these radicals can initiate a chain reaction causing effects in the entire body [4].

Warheit et al. [60] have studied different commercial formulations of the surface coatings on titanium dioxide particles and found that one surface coating demonstrated more toxicity than the other coatings. The absence, presence or composition of surface coatings is therefore complicating the toxicity of nanoparticles. However, it is unknown how long safety coatings stay on the particles. The likelihood of coating breakdown has been shown in cell culture systems, where quantum dots were initially rendered non-toxic with coatings, but if the quantum dots were either exposed to air or ultraviolet radiation for as little as 30 min, they became very destructive to living cells [61].

**Environment:** In the environment, natural enzymes can change the surface properties of nanoparticles so the nanoparticles become colloids. In colloidal solutions nanoparticles remain mobile because they do not form conglomeration and are not deposited. According to a report by Swiss Re, one of the leading global reinsurers, nanoparticles with colloidal characteristics can carry toxic material, such as water-repelling pollutants and heavy metals [4], over long distances and could potentially pollute aquifers. As they tend to be more reactive, due to the size, they may react with other substances in the environment and

lead to new and possible toxic compounds. If the roots of plants were to absorb nanoparticles, the nanoparticles could enter the food-chains. The Swiss Re report posed the question whether the nanoparticles could then also leak into the drinking water and be directly taken up by humans [4]. However, the high reactivity also means that they react much faster with the surroundings and may be neutralized before causing harm. On the other hand, pollutants binding to a nanotube might neutralize pollutants and reduce the harm they normally would cause [62]. In the long term it is a possibility for a wide exposure of the entire ecosystem to engineered nanomaterials through the water and soil. One of the prominent environmental nano-researchers, Vicky Colvin, anticipates that if engineered nanomaterial applications develop as projected, the increasing concentrations of nanomaterials in groundwater and soil may present the most significant exposure avenues for assessing environmental risk [63]. She further imposed that even though it is difficult to create water-soluble nanostructures, some nanomaterials can form a stable colloidal species in water from both a powder and an organic solution and hence enters groundwater.

One type of nanomaterials, the fullerenes are lipophilic and localize into lipid-rich regions such as cell membranes in living organisms, and being redox active and they therefore have the potential to be toxic. A study by Eva Oberdorster showed that fullerenes can induce oxidative stress in the brain of fish [64]. This is the first study done with new nanomaterials showing that nanomaterials can damage aquatic organisms. Further studies that evaluate the potential toxicity of manufactured nanomaterials, especially related with respect to translocation in the brain are needed.

**Special Risks:** The potential risk about nanoparticle is that they dissolve in the bloodstream, penetrate deeply into the lung, can cross the placenta in pregnant women and reach the foetus and can cross the blood-brain barrier. There is a particular problem that nanoparticles are not confined only to the lungs, but can reach anywhere in the body. The most potent risk is the toxicity associated with the large surface area and that if there are some toxic properties like metals on that surface, the toxicity will be multiplied up (Table 1).

### Precautionary measures

1. We should be very careful in situations where there might be exposure to nanoparticles and that we should make recommendations for good practice for companies handling engineered or synthetic particles. Companies should know how to use and produce them safely and know which nanoparticles are more hazardous and which are less hazardous.
2. It is important that authorities work together with the producers and scientists, to find out what kind of precautionary measures should be taken, based on information from epidemiology and toxicity studies and the material scientists. Authorities should initiate proper attempts to assess what the exposure is and do proper toxicology to get a feeling for what risks those different kinds of nanoparticles represent.
3. We need to test more types of nanomaterials and look at the whole range of nanoparticles that the nanotechnology industries are using. One example of the new nanomaterials is the nanotubes, because they might behave like fibres. These nanotubes may behave like asbestos because they are long and thin. The nanotubes are cleaned in acid which means that the lungs will not manage to dissolve the nanotubes.
4. There is apparently a need to change the testing protocols. More fundamental and pragmatic testing is needed.

Target	Nanoparticle	Major outcomes	References
Brain	Silver NP	Time- and dose-dependent increase in pro-inflammatory cytokine release and correlating increases in permeability and cytotoxicity of cells.	[65]
	Silver NP	Dose-dependent accumulation of NP was observed in the brain and other organs suggesting systemic distribution after oral administration. ALP and cholesterol increased significantly in high-dose group (1000 mg/kg/day) indicating hepatotoxicity.	[66]
	Silver NP	Neurobehavioural abnormalities were observed in adult zebrafish with increased DA and 5HT turnover in previously exposed embryos secondary to altered synaptic functioning.	[67]
	U (SPION)	Direct inoculation of all 3 SPION agents resulted in the uptake into the CNS parenchyma. No pathological changes were detected.	[68]
Liver	Gold NP	Naked NP: severe adverse effects with resultant death with particles ranging from 8 to 37 nm in diameter. Microscopically, Kupffer cell activation in the liver and lung parenchymal destruction was observed. Surface modified NP: elicited increased host immune response and improved cytocompatibility.	[69]
	Gold NP	NPs were found to accumulate in liver and spleen. Significant upregulation of inflammatory cytokines (IL-1, 6, 10 and TNF- $\alpha$ ) with subsequent apoptosis of hepatocytes at highest concentrations (4.26 mg/kg). No significant changes in the liver at lower doses.	[70]
	Silver NP	Oxidative stress-mediated toxicity due to free Ag <sup>+</sup> liberation. Induction of pro-apoptotic signals in liver tissues.	[71]
	Silver NP	NP enter cells which results in the production of mediators of oxidative-stress. However, protective mechanisms could be observed which increase GSH production to avoid oxidative damage.	[72]
	Silica NP	Significant hepatotoxicity (degenerative necrosis of hepatocytes) was observed with smaller NP (<100 nm) whereas no pathological changes were seen with larger particles (300 or 1000 nm), even at relatively higher concentrations of NP (100 mg/kg).	[73]
Dermal	Silver NP	Reversible hepatotoxicity and argyria-like discoloration of treated area of skin, elevated plasma and urine silver concentrations and increased liver enzymes.	[74]
	Titanium Dioxide NP	Cytotoxicity was observed affecting cellular functions such as cell proliferation, differentiation and mobility resulting in apoptosis.	[75]
	Silica NP	Size-related toxicity with faster cellular uptake of smaller particles and concomitant higher toxicity.	[76]
	Silica NP	Reduced Cell Viability	[77]
	Gold NP	Maximum cytotoxicity with smaller NP (1.4 nm) characterized by apoptosis and necrosis.	[78]
	Gold NP	Dose-dependent reduction in cell proliferation.	[79]
Lung	PLGA-Chitosan NP	Non-toxic even at highest concentrations.	[80]
	SWCNP	Low acute cytotoxicity was further reduced by dispersion of SWCNTs in serum.	[81]
	MWCNP	Uniform particle uptake by pulmonary macrophages. No inflammatory or fibrotic changes were observed.	[82]
	Silica NP	Dose- and time-dependent decrease in cell viability: up to 50% reduction at highest dosage after 72 h. Oxidative stress indicated as mechanism of cytotoxicity.	[83]
	Silica NP	Dose dependent Increase in Cytotoxicity	[84]
	Silver NP	Dose- and time-dependent increase in blood Ag nanoparticle concentration was observed along with correlating increases in alveolar inflammation and small granulomatous lesions.	[85]

**Table 1:** Summary of nanoparticle toxicity.

- We must think surface chemistry of nanoparticles. We need more epidemiological studies on humans, where the effects of nanoparticles are separated out from the effects of other particles.
- We have to look more at the mechanisms of cell activation and move away from only acute toxicity models, which have abnormally high levels of exposure that may not be relevant.
- A moratorium should be posed on nanotechnology until more scientific evidence is established, proving that the technology and its applications are not only safe to the environment or human health, but also that the social implications are understood.

## Conclusion

The bottom line is not whether nanoparticles are hazardous or represent risks to human health or environment. The bottom line is whether the applications of nanoparticles can substitute applications that are more hazardous and represent a larger risk to human health or environment. If the benefits prove to be as true as the most enthusiastic supporters of nanotechnology believe, the risks may prove to be acceptable. Until that day comes, reducing the uncertainties and the risks should be the main focus. That would, according to the

precautionary principle, mean to handle nanoparticles as in a worst-case scenario and take adequate precautionary measures to cope with the worst-case, because it is better to be safe than sorry for all parties involved.

## References

- Elsaesser A, Howard CV (2012) Toxicology of Nanoparticles. *Adv Drug Deliv Rev* 64: 129-137.
- Tsuji JS, Maynard AD, Howard PC, James JT, Lam C, et al. (2006) Research Strategies for Safety Evaluation of Nanomaterials, Part IV: Risk Assessment of Nanoparticles. *Toxicol Sci* 89: 42-50.
- Donaldson K, Stone V, Tran CL, Kreyling W, Borm PJ (2004) Nanotoxicology. *Occup Environ Med* 61: 727-728.
- Hett A, Gesellschaft SR (2005) Nanotechnology. Small matter, many unknowns. Zurich: Swiss Reinsurance Company.
- DTI (2002) New Dimensions for Manufacturing: A UK Strategy for Nanotechnology. Great Britain Dept of Trade and Industry.
- Stone V, Donaldson K (2006) Nanotoxicology: signs of stress, *Nat Nanotechnol* 1: 23-24.
- Oberdorster G, Sharp Z, Atudorei V, Elder A, Gelein R, et al. (2004) Translocation of inhaled ultrafine particles to the brain. *Inhal Toxicol* 16: 437-445.
- Oberdorster E (2004) Manufactured Nanomaterials (Fullerenes, C60) Induce

- Oxidative Stress in the Brain of Juvenile Largemouth Bass. *Environ Health Perspect* 112: 1058-1062.
9. Davidson P, Gabriel JCP (2004) Mineral liquid crystals. *Current Opinion in Colloid & Interface Science* 9: 377-383.
  10. Hardman R (2006) A toxicological reviews of quantum dots: toxicity depends on physicochemical and environmental factors. *Environ Health Perspect* 114: 165-172.
  11. Hoshino A, Fujioka K, Oku T, Suga M, Sasaki Yu F, et al. (2004) Physicochemical properties and cellular toxicity of nanocrystal quantum dots depend on their surface modification. *Nano Lett* 4: 2163-2169.
  12. Shiohara A, Hoshino A, Hanaki K, Suzuki K, Yamamoto K (2004) On the cytotoxicity caused by quantum dots. *Microbiol Immunol* 48: 669-675.
  13. Lovern SB, Klaper R (2006) *Daphnia magna* mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles. *Environ Toxicol Chem* 25: 1132-1137.
  14. Zhu S, Oberdörster E, Haasch ML (2006) Toxicity of an engineered nanoparticle (fullerene, C60) in two aquatic species, *Daphnia* and flathead minnow. *Mar Environ Res* 62: S5-S9.
  15. Kroto HW, Heath JR, O'Brian SC, Curl RF, Smalley RE (1985) C<sub>60</sub>: Buckminster fullerene. *Nature* 318: 162-163.
  16. Lyon DY, Alvarez Pedro JJ (2008) Fullerene Water Suspension (nC60) Exerts Antibacterial Effects via ROS-Independent Protein Oxidation. *Environ Sci Technol* 42: 8127-8132.
  17. NCCR (2004) Model of a bucky ball (fulleren) and carbon nanotubes.
  18. Shvedova AA, Castranova V, Kisin ER, Murray A, Gandelsman V, et al. (2003) Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells. *J Toxicol Environ Health Part A* 66: 1909-1926.
  19. Sayes CM, Liang F, Hudson JL, Mendez J, Guo W, et al. (2006) Functionalization density dependence of single-walled carbon nanotubes cytotoxicity *in vitro*. *Toxicol Lett* 161: 135-142.
  20. Radomski A, Jurasz P, Alonso-Escolano D, Drews M, Morandi M, et al. (2005) Nanoparticle-induced platelet aggregation and vascular thrombosis. *Brit J Pharmacol* 146: 882-893.
  21. Monteiro-Riviere NA, Nemanich RJ, Inman AO, Wang YY, Riviere JE (2005) Multi-walled carbon nanotube interactions with human epidermal keratinocytes. *Toxicol Lett* 155: 377-384.
  22. Svenson S and Tomalia DA (2005) Dendrimers in biomedical applications- reflections on the field. *Adv Drug Deliv Rev* 57: 2106-2129.
  23. Duncan R, Izzo L (2005) Dendrimer biocompatibility and toxicity. *Adv Drug Deliv Rev* 57: 2215-2237.
  24. Connor EE, Mwamuka J, Gole A, Murphy CJ, Wyatt MD (2005) Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. *Small* 1: 325-327.
  25. Shenoy D, Fu W, Li J, Crasto C, Jones G, et al. (2006) Surface functionalization of gold nanoparticles using hetero-bifunctional poly (ethylene glycol) spacer for intracellular tracking and delivery. *Int J Nanomedicine* 1: 51-57.
  26. Kawano T, Yamagata M, Takahishi H, Niidome Y, Yamada S, et al. (2006) Stabilizing of plasmid DNA *in vivo* by PEG-modified cationic gold nanoparticles and the gene expression assisted with electrical pulses. *J Control Release* 111: 382-389.
  27. Hirsch LR, Stafford RJ, Bankson JA, Sershen SR, Rivera B, et al. (2003) Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance. *Proc Natl Acad Sci USA* 100: 13549-13554.
  28. Hainfeld JF, Slatkin DN, Smilowitz HM (2004) The use of gold nanoparticles to enhance radiotherapy in mice. *Phys Med Biol* 49: 309-315.
  29. Loo C, Lin A, Hirsch L, Lee MH, Barton J, et al. (2004) Nanoshell-enabled photonics-based imaging and therapy of cancer. *Technol Cancer Res Treat* 3: 33-40.
  30. O'Neal DP, Hirsch LR, Halas NJ, Payne JD, West JL (2004) Photo-thermal tumor ablation in mice using near infrared-absorbing nanoparticles. *Cancer Lett* 209: 171-176.
  31. Radt B, Smith TA, Caruso F (2004) Optically addressable nanostructured capsules. *Adv Mater* 16: 2184-2189.
  32. Niidome T, Yamagata M, Okamoto Y, Akiyama Y, Takahashi H, et al. (2006) PEG-modified gold nanorods with a stealth character for *in vivo* applications. *J Control Release* 114: 343-347.
  33. Lai MK, Chang CY, Lien YW (2006) Application of gold nanoparticles to microencapsulation of thioridazine. *J Control Release* 111: 352-361.
  34. Su CH, Sheu HS, Lin CY, Huang CC, Low YW, et al. (2007) Nanoshell resonance imaging contrast agents. *J Am Chem Soc* 129: 2139-2146.
  35. Stern JM, Stanfield J, Lotan Y, Park S, Hsieh JT, et al. (2007) Efficacy of laser-activated gold nanoshells in ablating prostate cancer cells *in vitro*. *J Endourol* 21: 939-943.
  36. Lowery AR, Gobin AM, Day Es, Halas NJ, West JL (2006) Immunonanoshells for targeted photothermal ablation of tumor cells. *Int J Nanomedicine* 1: 149-154.
  37. Yildirim L, Thanh NTK, Loizidou M, Seifalian AM (2011) Toxicological considerations of clinically applicable nanoparticles. *Nano Today* 6: 585-607.
  38. Wood SJ, Geldart A, Jones R (2003) The social and economic challenges of nanotechnology. *The Economic and Social Research Council* 72-73.
  39. <http://news.bbc.co.uk/2/hi/science/nature/3920685.stm>
  40. Holister P (2002) Nanotech - the tiny revolution. *CMP Cientifica*.
  41. K Maney (2004) Nanotech could put a new spin on sports. *USA Today*.
  42. Donaldson K, Brown D, Clouter A, Duffin R, MacNee W, et al. (2002) The pulmonary toxicology of ultrafine particles. *J Aerosol Med* 15: 213-220.
  43. Oberdorster G (2000) Pulmonary effects of inhaled ultrafine particles. *Int Arch Occup Environ Health* 74: 1-8.
  44. Zhang Q, Kusaka Y, Zhu X, Sato K, Mo Y, et al. (2003) Comparative toxicity of standard nickel and ultrafine nickel in lung after intratracheal instillation. *J Occup Health* 45: 23-30.
  45. Warheit DB. (2004) Nanoparticles: Health impacts? *Materials Today* 7: 32-35.
  46. Utell MJ, Frampton MW (2000) Acute health effects of ambient air pollution: the ultrafine particle hypothesis. *J Aerosol Med* 13: 355-359.
  47. Walgate R (2004) Degrading Nanoparticles. *The Scientist*.
  48. Borm PJA, Kreyling W (2004) Toxicological Hazards of Inhaled Nanoparticles - Potential Implications for Drug Delivery. *J Nanosci Nanotechnol* 4: 521-531.
  49. Aitken R, Creely K, Tran C (2004) Nanoparticles: An occupational hygiene review. *Edinburgh: Institute of Occupational Medicine (IOM)*.
  50. Oberdorster G, Sharp Z, Atudorei V, Elder A, Gelein R, et al. (2004) Translocation of inhaled ultrafine particles to the brain. *Inhal Toxicol* 16: 437-445.
  51. Kreuter J (2001) Nanoparticulate systems for brain delivery of drugs. *Adv Drug Deliv Rev* 47: 65-81.
  52. Kreyling WG, Semmler M, Erbe F, Mayer P, Takenaka S, et al. (2002) Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. *J Toxicol Environ Health A* 65: 1513-1530.
  53. Nemmar A, Vanbilloen H, Hoylaerts MF, Hoet PH, Verbruggen A, et al. (2001) Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. *Am J Respir Crit Care Med* 164: 1665-1668.
  54. Oberdorster G, Sharp Z, Atudorei V, Elder A, Gelein R, et al. (2002) Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. *J Toxicol Environ Health A* 65: 1531-1543.
  55. Takenaka S, Karg E, Kreyling WG, Lentner B, Schulz H, et al. (2004) Fate and toxic effects of inhaled ultrafine cadmium oxide particles in the rat lung. *Inhal Toxicol* 16: 83-92.
  56. Takenaka S, Karg E, Roth C, Schulz H, Ziesenis A, et al. (2001) Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. *Environ Health Perspect* 109: 547-551.
  57. Lam CW, James JT, McCluskey R, Hunter RL (2004) pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. *Toxicol Sci* 77: 126-134.
  58. Dreher KL (2004) health and environmental impact of nanotechnology: toxicological assessment of manufactured nanoparticles. *Toxicol Sci* 77: 3-5.



59. Zhang Q, Kusaka Y, Sato K, Nakakuki K, Kohyama N, et al. (1998) Differences in the extent of inflammation caused by intratracheal exposure to three ultrafine metals: role of free radicals. *J Toxicol Environ Health A* 53: 423-438.
60. Warheit DB, Reed KL, Webb TR (2003) Pulmonary toxicity studies in rats with tri-ethoxyoctylsilane (OTES)-coated, pigment-grade titanium dioxide particles: bridging studies to predict inhalation hazard. *Exp Lung Res* 29: 593-606.
61. Derfus AM, Chan WCW, Bhatia SN (2004) Probing the Cytotoxicity of Semiconductor Quantum Dots. *Nano Lett* 4: 11-18.
62. Kleiner K, Hogan J (2003) How safe is nanotech? *New Scientist* 177: 14-15.
63. Colvin VL (2003) The potential environmental impact of engineered nanomaterials. *Nat Biotechnol* 21: 1166-1170.
64. Oberdorster E (2004) Manufactured Nanomaterials (Fullerenes, C60) Induce Oxidative Stress in the Brain of Juvenile Large mouth Bass. *Environmental Health Perspectives*, 112: 1058-1062.
65. Trickler WJ, Lantz SM, Murdock RC, Schrand AM, Robinson BL, et al. (2010) Silver Nanoparticle Induced Blood-Brain Barrier Inflammation and Increased Permeability in Primary Rat Brain Micro vessel Endothelial Cells. *Toxicol Sci* 118: 160-170.
66. Kim YS, Kim JS, Cho HS, Rha DS, Kim JM, et al. (2008) twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. *Inhal Toxicol* 20: 575-583.
67. Powers CM, Levin ED, Seidler FJ, Slotkin TA (2010) Silver exposure in developing zebrafish produces persistent synaptic and behavioral changes. *Neurotoxicol Teratol* 2: 329-332.
68. Muldoon LL, Sandor M, Pinkston KE, Neuwelt EA (2005) Imaging, distribution, and toxicity of superparamagnetic iron oxide magnetic resonance nanoparticles in the rat brain and intracerebral tumor. *Neurosurgery* 57: 785-796.
69. Chen YS, Hung YC, Liao I, Huang GS (2009) Assessment of the In-Vivo Toxicity of Gold Nanoparticles. *Nanoscale Res Lett* 4: 858-864.
70. Cho WS, Cho M, Jeong J, Choi M, Cho HY, et al. (2009) Size-dependent tissue kinetics of PEG-coated gold nanoparticles. *Toxicol Appl Pharmacol* 236: 16-24.
71. Choi JE, Kim S, Ahn JH, Youn P, Kang JS, et al. (2010) Induction of oxidative stress and apoptosis by silver nanoparticles in the liver of adult zebrafish. *Aquat Toxicol* 100: 151-159.
72. Arora S, Jain J, Rajwade JM, Paknikar KM (2009) Interactions of silver nanoparticles with primary mouse fibroblasts and liver cells. *Toxicol Appl Pharmacol* 236: 310-318.
73. Nishimori H, Kondoh M, Isoda K, Tsunoda S, Tsutsumi Y, et al. (2009) Silica nanoparticles as hepatotoxicants. *Eur J Pharm Biopharm* 72: 496-501.
74. Trop M, Novak M, Rodl S, Hellborn B, Kroell W, et al. (2006) Silver-coated dressing acticoat caused raised liver enzymes and argyria-like symptoms in burn patient. *J Trauma* 60: 648-652.
75. Kiss B, Biro T, Czifra G, Toth BI, Kertesz Z, et al. (2008) Investigation of micronized titanium dioxide penetration in human skin xenografts and its effect on cellular functions of human skin-derived cells. *Exp Dermatol* 17: 659-667.
76. Nabeshi H, Yoshikawa T, Matsuyama K, Nakazato Y, Arimori A, et al. (2010) Size-dependent cytotoxic effects of amorphous silica nanoparticles on Langerhans cells. *Pharmazie* 65: 199-201.
77. Park YH, Kim JN, Jeong SH, Choi JE, Lee SH, et al. (2010) Assessment of dermal toxicity of nanosilica using cultured keratinocytes, a human skin equivalent model and an in vivo model. *Toxicology* 267: 178-181.
78. Pan Y, Neuss S, Leifert A, Fischler M, Wen F, et al. (2007) Size-dependent cytotoxicity of gold nanoparticles. *Small* 3: 1941-1949.
79. Pernodet N, Fang X, Sun Y, Bakhtina A, Ramakrishnan A, et al. (2006) Adverse effects of citrate/gold nanoparticles on human dermal fibroblasts. *Small* 2: 766-773.
80. Tahara K, Sakai T, Yamamoto H, Takeuchi H, Hirashima N, et al. (2009) Improved cellular uptake of chitosan-modified PLGA nanospheres by A549 cells. *Int J Pharm* 382: 198-204.
81. Davoren M, Herzog E, Casey A, Cottineau B, Chambers G, et al. (2007) *In vitro* toxicity evaluation of single walled carbon nanotubes on human A549 lung cells. *Toxicol In Vitro* 21: 438-448.
82. Mitchell LA, Gao J, Wal RV, Gigliotti A, Burchiel SW, et al. (2007) Pulmonary and systemic immune response to inhaled multiwalled carbon nanotubes. *Toxicol Sci* 100: 203-214.
83. Lin W, Huang YW, Zhou XD, Ma Y (2006) *In vitro* toxicity of silica nanoparticles in human lung cancer cells. *Toxicol Appl Pharmacol* 217: 252-259.
84. Lison D, Thomassen LCJ, Rabolli V, Gonzalez L, Napierska D, et al. (2008) Nominal and effective dosimetry of silica nanoparticles in cytotoxicity assays. *Toxicol Sci* 104: 155-162.
85. Sung JH, Ji JH, Yoon JU, Kim DS, Song MY, et al. (2008) Lung function changes in Sprague-Dawley rats after prolonged inhalation exposure to silver nanoparticles. *Inhal Toxicol* 20: 567-574.