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Revisão

Potencial risks of nanoparticles

Riscos potenciais do nanopartículas

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Abstract

Nanotoxicology is an emergent important subdiscipline of Nanosciences, which refers to the study of the interactions of nanostructures with biological systems giving emphasis to the elucidation of the relationship between the physical and chemical properties of nanostructures with induction of toxic biological responses. Although potential beneficial effects of nanotechnologies are generally well described, the potential (eco) toxicological effects and impacts of nanoparticles have so far received little attention. This is the reason why some routes of expousure, distribution, metabolism, and excretion, as well as toxicological effects of nanoparticles are discussed in this review.
Keywords: *nanotoxicology; nanoparticles; carbon nanotubes; toxicological effects.*

Resumo

A nanotoxicologia é uma subdisciplina importante emergente dentro da nanociência, que se refere ao estudo da interação entre as nanoestruturas e os sistemas biológicos, com ênfase especial na elucidação da relação entre as propriedades físicas e químicas das nanopartículas com a indução de respostas tóxicas biológicas. Embora em geral sejam bem descritos os efeitos benéficos da nanotecnologia, os efeitos potenciais toxicológicos e o impacto das nanopartículas receberam pouca atenção até agora. Por isso, formas de exposição, distribuição, metabolismo e excreção, como também efeitos toxicológicos das nanopartículas são discutidos nesta revisão.
Palavras-chave: *nanotoxicologia; nanopartículas; nanotubos de carvão; efeito toxicológico.*

1 Introduction

Nanotechnology is generally seen as a new and fast emerging field that involves the manufacture, processing, and application of structures, devices, and systems by controlling shape and size at the nanometer scale (BOUWMEESTER et al., 2009). This area of investigation has been widely used in the pharmaceutical industry, medicine, and engineering technology in the last two decades. Over 500 products incorporating nanotechnology are already commercially available. These include simple passive nano-scale particles, compounds, and composites for use in foods, pesticides, sunscreens, cosmetics, stain resistant clothing, automotive paints and coatings, sporting goods, and digital cameras (HODGE; BOWMAN, 2007).

The high speed introduction of NP-based consumer products observed nowadays urges the need to generate a better understanding about the potential negative impacts that NPs may have on biological systems (BOUWMEESTER et al., 2009). Nanotoxicology, as an important emerging subdiscipline of nanotechnology, has been defined as "science of engineered nanodevices and nanostructures that deals with their effects on living organisms" (OBERDORSTER et al., 2005; SMART et al., 2006).

At this scale, the classic laws of physics are no longer applicable resulting in novel properties and functions not found at the macro-scale such as significantly different chemical reactivity, electrical conductivity, strength, mobility, solubility, magnetic, and optical properties (HODGE; BOWMAN, 2007), which might result in unpredictable safety problem and risk (ACTION…, 2006; BOUWMEESTER et al., 2009).

In general, the impact of nanoparticles on the body depends on properties such as particle size, mass, chemical composition, and surface, and also on how the individual nanoparticles aggregate together (OBERDORSTER et al., 2005). The extent to which nanoparticles enter human bodies, the sites of penetration, and possible accumulation and translocation of nanoparticles in the body may also determine the potential risks of nanoscale materials (OBERDORSTER, 2001; OBERDORSTER et al., 2005).

For the assessment of nanotoxicity, several criteria may be considered, such as: exposure assessment of nanoparticles; toxicology of nanoparticles; ability to extrapolate nanoparticle toxicity using existing toxicological databases; environmental and biological fate, transport, persistence and transformation of nanoparticles; and recyclability and overall sustainability of nanomaterials (DREHER, 2004).

Nanoparticles could have a number of possible causes of toxicity: nanostructures have been demonstrated to have electronic, optical, and magnetic properties that are related

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to their physical dimensions, and the breakdown of these nanostructures could lead to a unique toxic effect that is difficult to predict (NEL et al., 2006). In addition, nanostructure surfaces are involved in many catalytic and oxidative reactions (NEL et al., 2006). If these reactions induce cytotoxicity, the toxicity could be greater than a similar bulk material because the surface area-to-volume ratio for nanoscale material is much greater (XIA et al., 2006), and some nanostructures contain metals or compounds with known toxicity; thus, the breakdown of these materials could elicit similar toxic responses to the components themselves.

2 Toxicokinetics

2.1 Important routes of exposure

Currently, the main route of exposure of the general public to nanoparticles comes in the form of air pollution; however, this is not the only route. Nanoparticles have been introduced into epidermal creams such as sunscreens, and they have also been designed for use in a range of therapeutic and diagnostic applications (e.g. drug delivery), which will result in exposure to the digestive tract and possibly the blood stream (DONALDSON et al., 2004).

Several studies describe the exposure of the respiratory system to airborne ultrafine particles in order to test the hypothesis that they cause significant health effects, while other exposure routes, such as skin and gastrointestinal (GI) tract, have not been considered as extensively as the respiratory tract as portals of entry for nanocarrier systems.

Skin

A contact with nanoparticles through the skin can occur due to occupational exposure during the manufacturing of solvents, pesticides, or pharmaceuticals. Skin exposure to nanoparticles can also occur during non-occupational situations from the use of cosmetics and in the intentional application of topical creams and other drug treatments.

Initial studies of nanoparticle absorption through the skin are inconclusive; some demonstrate little penetration into the epidermis, while others using more complex flexing protocols show deep absorption (TINKLE et al., 2003; HAGENS et al., 2007; ROUSE et al., 2007).

Broken skin represents a readily available portal of entry even for large (0.5–7.0 μm) micron size particles (OBERDORSTER et al., 2005). Even intact skin, when flexed, makes epidermis permeable to nanoparticles (OBERDORSTER et al., 2005; HAGENS et al., 2007). A study from Tinkle et al. (2003) showed that fluorospheres (0.5–1 μm) can penetrate the epidermis and reach it by using a skin flexing protocol that is likely to be representative of physiological conditions. Once in the epidermis, nanoparticles reach the lymphatic system and regional lymph, and from there they can translocate to the systemic vasculature. Nanoparticles can also reach sensory skin nerves after the injection of nanoparticles in the tongue and facial muscles of mice. Cationized nanoparticles can reach cell bodies of facial neurons highlighting the importance of electric charge on nanoparticle incorporation and disposition into axons (OBERDORSTER et al., 2005). To better understand dermal absorption of nanoparticles, more research on regular skin, dry skin, and damaged skin is necessary.

Respiratory tract

Exposure to nanosized materials has increased since new anthropogenic sources were developed approximately three decades ago. Inhaled nanoparticles are deposited in all regions of the respiratory tract; however, larger particles may be filtered out in the upper airways, whereas smaller particles reach distal airways (HAGENS et al., 2007). The respiratory tract can be divided into three regions: nasopharyngeal, tracheobronchial, and alveolar (MOGHIMI; HUNTER; MURRA, 2005). Significant amounts of certain particle size ranges can deposit in each region, for example, 90% of nanoparticles of 1 nm in diameter deposit in the nasopharyngeal region, whereas only 10% of these nanoparticles deposit in the tracheobronchial region and almost none reach the alveolar region. In comparison, 15% of nanoparticles of 20 nm in diameter deposit in the nasopharyngeal region, 15% in the tracheobronchial region, and approximately 50% in the alveolar region (MOGHIMI; HUNTER; MURRA, 2005).

After absorption across the lung epithelium, nanoparticles can enter the blood and lymph to reach cells in the bone marrow, lymph nodes, spleen, and heart. Nanoparticles can even reach the central nervous system and ganglia following translocation (OBERDORSTER et al., 2005; HAGENS et al., 2007).

Epidemiologic analysis and controlled clinical trial studies in humans have been used to describe the toxicology of airborne natural nanosized materials. These nanosized materials often have cardiovascular and respiratory effects that result in significant morbidity and mortality in susceptible segments of the population. Subjects with asthma or chronic obstructive pulmonary disease show greater deposition of natural nanosized materials in the respiratory tract than healthy individuals.

The presence of natural nanosized particles is associated with the formation of blood markers of coagulation, which has effects on the systemic inflammation and pulmonary diffusion capacity and increases the risk of ventricular disrhythmias (OBERDORSTER et al., 2005).

Translocation, the transport of dissolved materials within the body, has been proposed as a mechanism for nanosized particles to reach extrapulmonary sites and then other target tissues. Nanoparticles can access the systemic vasculature either directly or via lymphatic transfer by transcytosis (crossing the epithelia of the respiratory tract into the interstitium), phagocytosis, endocytosis, or some other transmembrane process (OBERDORSTER et al., 2005; HAGENS et al., 2007). A second target after translocation is suggested to be the sensory nerve endings embedded in the airway epithelia, followed by translocation to ganglia and the central nervous system via axons. In addition to epidemiological and controlled clinical studies, the effects of nanoparticles in the respiratory tract have been studied through inhalation and instillation studies

in rodents and in vitro cell culture systems. In rodents, ultrafine particles cause mild pulmonary inflammatory responses and have effects on extrapulmonary organs (OBERDORSTER et al., 2005). Dosing with both natural and anthropogenic nanosized particles, in vitro studies showed pro-inflammatory and oxidative stress related cellular response (OBERDORSTER et al., 2005; CURTIS et al., 2006; LANONE; BOCZKOWSKI, 2006).

The shape and structure of nanoparticles may also predispose them to inhalational toxicity. For example, carbon nanotubes (CNTs) have distinct pulmonary effects as compared to carbon black and graphite, which are larger structures of similar chemical make-up (LACERDA et al., 2006). When the toxicity of CNTs was compared to that of carbon black after intratracheal instillation in mice, CNTs proved to be significantly more harmful (OBERDORSTER et al., 2005). Carbon black was ingested by macrophages in the alveolar region and resided predominantly in this site. In comparison, macrophages that ingested CNTs migrated to centrilobular locations and caused interstitial granulomas (OBERDORSTER et al., 2005; CURTIS et al., 2006). Pharyngeal aspiration of single wall carbon nanotubes (SWCNTs) caused increased inflammation and cell damage. Two patterns of lung remodeling were present depending on whether SWCNTs aggregate (granuloma) or distribute (interstitial fibrosis) through the lung space (OBERDORSTER et al., 2005; LANONE; BOCZKOWSKI, 2006). Either pro-inflammatory (TNF-α, IL-1β) or anti-inflammatory profibrogenic cytokines (TGF-β, IL-10) are expressed in tissues affected by nanoparticles (KAGAN; BAYIR; SHVEDOVA, 2005; DUFFIN; MILLS; DONALDSON, 2007). When SWCNTs and MWCNTs were compared to C60 fullerenes, they showed greater cytotoxicity to alveolar macrophages (SWCNTNNMWCNTNNC60) (KAGAN; BAYIR; SHVEDOVA, 2005). C60 fullerenes are allotropic forms of carbon that are arranged in clusters and are also used as nanocarrier systems (OBERDORSTER et al., 2005; CURTIS et al., 2006; KAGAN; BAYIR; SHVEDOVA, 2005).

Gastrointestinal tract

Nanoparticles can reach the GI tract after mucociliary clearance from the respiratory tract through the nasal region or can be ingested directly in food, water, cosmetics, drugs, and drug delivery devices (OBERDORSTER et al., 2005; HAGENS et al., 2007).

Acute toxicity of copper particles and nanocopper was measured in mice; LD50 for nanocopper is 413 $mg.kg^{-1}$ compared to 5000 $mg.kg^{-1}$ for copper. Nanocopper was also reported to cause pathological damage to the liver, kidney, and spleen (CURTIS et al., 2006).

3 Distribution, metabolism and excretion

Nanoparticles have been found to be distributed to the colon, lungs, bone marrow, liver, spleen, and the lymphatic system after intravenous injection (HAGENS et al., 2007). Distribution is followed by rapid clearance from the systemic circulation, predominantly by action of the liver and splenic macrophages (MOGHIMI; HUNTER; MURRA, 2005). Clearance and opsonization - the process that prepares foreign material to be more efficiently engulfed by macrophages - occur under certain conditions for nanoparticles depending on size and surface characteristics (CURTIS et al., 2006).

When inhaled, nanoparticles are found to be distributed to the lungs, liver, heart, and kidney. Nanoparticles are cleared in the alveolar region, via phagocytosis by macrophages, facilitated by chemotactic attraction of alveolar macrophages to the deposition site. The average half-life (t1/2) for nanoparticles in the respiratory tract is 700 days in humans. Nanoparticle clearance from the lungs involves a combination of physical and chemical processes. Physical clearance processes in addition to macrophage phagocytosis include mucociliary movement, epithelial endocytosis, interstitial translocation, lymphatic drainage, blood circulation translocation, and sensory neuron translocation. Chemical clearance processes include dissolution, leaching, and protein binding. Some clearance processes show particle size-dependent differences and nano-selective effectiveness (OBERDORSTER et al., 2005).

After oral exposure, nanoparticles distribute to the kidneys, liver, spleen, lungs, brain, and the GI tract (HAGENS et al., 2007). Few studies have looked at clearance of nanoparticles from the (GI) tract. Some nanoparticles can pass through the GI tract and are rapidly eliminated in feces and in urine indicating that they can be absorbed across the GI barrier and into the systemic circulation. However, some nanoparticle systems can accumulate in the liver during first-pass metabolism (OBERDORSTER et al., 2005).

In regards to excretion, there are many possible routes. These routes include the kidney or the liver/bile duct. Hydroxyl functionalized SWCNT dosed intraperitoneally accumulate in the liver and kidneys, and are excreted in the urine within 18 days; whereas, ammonium functionalized SWCNT dosed intravenously showed no liver uptake and much faster renal excretion (WANG et al., 2004; SINGH et al., 2006).

For nanostructures such as QDs, two initial studies showed they do not excrete and remain intact in vivo. This, however, has very recently been demonstrated to be size and surface chemistry dependent. QDs smaller than 5.5 nm in diameter, which are cysteine coated, are excreted in the urine. If not excreted in this manner, how long they reside and what happens to their long-term behavior in vivo remains unclear (FISCHER; CHAN, 2007).

4 Toxicological effects

Due to their size, nanoparticles have a large specific surface area, which may translate into increased biological activity due to different contact interactions with cells and their components and variable biokinetics. The stability of nanoparticles requires further detailed investigation; however, the possibility of Oswalt ripening and agglomeration exists.

Nanoparticles favor the formation of pro-oxidants, especially under exposure to light, ultraviolet light, or transition metals, thereby, destabilizing the balance between the production of reactive oxygen species (ROS) and the biological

system's ability to detoxify or repair the system. Oxidative stress induced by nanoparticles is reported to enhance inflammation through upregulation of redox-sensitive transcription factors including nuclear factor kappa B (NFκB), activating protein 1 (AP-1), extracellular signal regulated kinases (ERK) c-Jun, N-terminal kinases, JNK, and p38 mitogen-activated protein kinases pathways. Nanoparticles can modify mitochondrial function, as well as cellular redox signaling (VEGA-VILLA et al., 2008).

4.1 Acute toxicity

Acute, subacute, and subchronic toxicity following oral exposure have been investigated in rodents for several different NPs (e.g., copper, selenium, zinc, zinc-oxide, and titanium dioxide NPs). The results of the available oral toxicity studies indicate that, depending on the particle size, coating, and chemical composition of the NPs, acute toxicity at high doses may occur (BOUWMEESTER et al., 2009).

4.2 Long term toxicity

Information from toxicity studies indicate that several systemic effects on different organ systems may occur after long term exposure to NPs including the immune, inflammatory and cardiovascular system. Effects on the immune and inflammatory systems may include oxidative stress and/or activation of pro-inflammatory cytokines in the lungs, liver, heart, and brain. Effects on the cardiovascular system may include pro-thrombotic effects and adverse effects on the cardiac function (acute myocardial infarction and adverse effects on the heart rate). No information on the toxicity after chronic or acute low dose oral exposure is currently available. Furthermore, genotoxicity, possible carcinogenesis, and teratogenicity may occur, but no data on these endpoints are available yet (BOUWMEESTER et al., 2009).

Neurotoxicity

There is evidence from ADME studies that NP may pass the blood–brain barrier following systemic availability of NPs (HILLYER; ALBRECHT, 2001; BORM et al., 2006; SILVA, 2007). It is not clear if this is a generic effect of all NPs or only a subgroup. This emphasizes the need for kinetic studies and increased attention of toxicologists to neurotoxicity in their search for potential effects in target tissues. Toxic effects due to the presence (or even accumulation) of NPs in the brain have not been studied so far, but risk assessors should be aware of possible neurological effects when assessing toxicology experiments. Possibly, current guideline tests will need to be adapted to render these tests more sensitive for neurotoxic effects of NPs.

Reprotoxicity

Transfer of NPs across the placenta cannot be excluded (including excretion via breast milk, the blood milk barrier), which could lead to embryotoxicity as a result of exposure to NPs (FUJIMOTO et al., 2005). Data addressing the distribution of NPs to the reproductive cells are currently unavailable. In addition, no clear data showing the distribution of NPs in the fetus are available (TRAN et al., 2005). This leads to the recommendation that reprotoxicity needs to be carefully considered when there is evidence for NP passage of the placenta.

Mutagenicity

Intracellular NPs do not appear to be membrane bound and might have direct access to the intracellular proteins, organelles and DNA of the cell, which might imply enhanced toxic potential. However, possible interactions of NPs with cell components are poorly understood, and validated assays with meaningful endpoints for genotoxicity are needed (BOUWMEESTER et al., 2009).

Allergenicity (or sensitization)

Even for conventional chemicals, little is known on the induction of food allergy and the type of exposure required inducing such responses. In the case of NPs, this becomes extra prominent for two reasons. First of all, it is the possible adjuvant activity of NPs that introduces additional uncertainty. Secondly, because of the actively charged surfaces of NPs, they can absorb biomolecules as they pass through the GI tract, which might result in changed exposure of the cellular lining of the intestine. In addition, although they might also be a reason for concern, surface properties (e.g., coatings) are important determinants for the active uptake of encapsulates. For example, lectins used for coatings are highly immunogenic, can be cytotoxic, or induce inflammatory responses and gastrointestinal irritation (BOUWMEESTER et al., 2009).

4.3 Toxicity of inhaled ultrafine particles

It has been shown that nanoparticles can translocate from the respiratory tract, via different pathways, to other organs/tissues and induce direct adverse responses in remote organs. Such responses may be particularly initiated through the interaction of nanoparticles with sub-cellular structures following endocytosis by different target cells. Therefore, special attention must be given to such effects, which could have serious consequences in a compromised organism or organ. Most of the toxicological data is based on our knowledge from nanoparticles inhaled during daily life such as carbon black, diesel particulates, silica, and titanium oxide nanoparticles, which are considered ultrafine particles (b100 nm in diameter) (AZARMI et al., 2008).

It has been shown that the toxicity of nanoparticles increases with a decrease in particle size. Ultrafine carbon black particles are known to produce greater pulmonary toxicity in rats when compared to large-sized carbon black particles. Single wall carbon nanotubes also show some degree of toxicity after inhalation. Warheit et al.(2004), studied the toxicity of SWCNTs. In their study, 15% of the rats who were exposed to high-dose (5 $mg.kg^{-1}$) SWCNTs showed mortality 24 hours post instillation. They related this mortality to the mechanical blockage of the upper airways by the instillate and not the inherent pulmonary toxicity of the instilled SWCNTs particulate. It was shown that pulmonary exposure to SWCNTs in rats produces a non-dose

dependent series of multifocal granulomas, which showed evidence of a foreign tissue body reaction and were non-uniform in distribution and not progressive beyond 1 month post exposure. In addition, aspiration of SWCNTs elicited an unusual inflammatory response in the lungs of exposed mice. This inflammatory reaction is probably triggered by damage to pulmonary epithelial type I cells, which include a strong neutrophilic pneumonia followed by recruitment and activation of macrophages. This early response can switch from the acute phase to fibrogenic events resulting in a significant pulmonary deposition of collagen and elastin. This phase is accompanied by a change in the production and release of proinflammatory (tumor necrosis factor-α, interleukin-1β) to anti-inflammatory profibrogenic cytokines (transforming growth factor-β, interleukin- 10). These inflammatory and fibrogenic responses are accompanied by a detrimental decline in pulmonary function and enhanced susceptibility to infection.

In another study, Xia et al.(2006), compared the cellular effects of ambient ultrafine particles with manufactured titanium dioxide (TiO_2), carbon black, fullerol, and polystyrene nanoparticles on a phagocytic cell line (RAW 264.7) that is representative of a lung target for nanoparticles. It was shown that, among the particles tested, ambient ultrafine particles and cationic polystyrene nanospheres were capable of inducing cellular reactive oxygen species (ROS) production, GSH depletion, and toxic oxidative stress. This toxicity involves mitochondrial injury through increased calcium uptake and structural organellar damage. However, TiO_2 and fullerol did not induce toxic oxidative stress. Additionally, it was also proved that increased TNF-α production could be seen with ultrafine particles induced by oxidant injury, but cationic polystyrene nanospheres induced mitochondrial damage and cell death without inflammation.

Rats that were also exposed to ultrafine TiO_2 particles showed evidence of pigment-laden macrophages and macrophage aggregates in the alveolar spaces up to 6 months post-exposure. Pre-exposure of alveolar macrophages to ultrafine particles also significantly reduced subsequent macrophage phagocytotic abilities and the effect was shown to vary dependent upon the particle properties. It was also demonstrated that ultrafine particles can impair phagosome transport and increase cytoskeletal stiffness at high concentrations, which leads to a reduced phagocytotic capability, inhibited cell proliferation, and decreased cell viability (XIA et al., 2006).

To assess such effects, cell culture models seem to be the best way to proceed. The A549 (alveolar) and BEAS-2B (airways) cell lines have been used to study the nanotoxicological aspects of inhaled environmental pollutants. These two cell lines, which do not form functional tight junctions, are considered suitable for toxicology studies of inhaled nanoparticles. Standard toxicity assays such as cellular metabolic activity, membrane integrity, and the release of proinflammatory and inflammatory mediators can be performed with these cells after nanoparticles uptake (AZARMI et al., 2008).

The adoption of screening methodology from environmental sciences will extend the safety information about the toxicological aspects of inhaled polymeric nanoparticles used for drug delivery. However, there is currently no standard cell culture model available to mimic the epithelium permeability in the alveolar region except for pneumocyte monolayers in primary culture. Nevertheless, such cell models are available for the bronchial epithelium. In this case, 16HBE14o- and Calu-3 cells have shown to be suitable models (AZARMI et al., 2008).

4.4 Toxicity of carbon nanotubes

One of the major concerns about the use of CNT based materials is the unknown impact on workers involved in their manufacture and handling. The majority of the studies published, since the first study in 2001, have demonstrated that CNTs could pose potential health problems (SMART et al., 2006).

Lung toxicity

As mentioned above, SWCNT products induced dose-dependent lung lesions, characterized by interstitial granulomas, regardless of the levels of metal impurities. It has been described that SWCNTs are more toxic than carbon black, and CNTs containing Ni is more toxic than quartz, the recognized positive inducer of lung toxicity (SMART et al., 2006).

Compact SWCNT aggregates (>500 nm in diameter) have also been associated with acute inflammation and granuloma formation in particle deposition sites, and dispersed SWCNT structures (delicate fiber-like structures with average fiber diameter <50 nm in diameter) have been associated with diffuse interstitial fibrosis and alveolar wall thickening in areas distant from SWCNTs aggregate deposition (the proximalalveolar region) and in the absence of persistent local inflammation. These observations of a fibrogenic response to disperse aerosol SWCNT particles are unique and disturbing, suggesting that SWCNT exposure poses significant health risks for workers (SMART et al., 2006).

Animal inhalation studies have been proposed to mimic respiration of airborne CNT particles, but only one investigation into this area has been conducted. Like intratracheal instillation techniques, pharyngeal aspiration showed evidence of large CNT agglomerations in the proximal alveolar regions of the lungs, as well as, fine fiber-type structures in the more distal alveolar regions. However, this inhalation technique still does not mimic physiological respiration bypassing the nose and delivering the CNTs as a bolus dose. There is also evidence that it takes significant energy and agitation to release fine CNT particles into the air. Current handling procedures employed by nanotube manufacturers do not produce significant quantities of airborne CNTs. However, the possibility of cumulative effects, especially if increased quantities are handled, justifies the introduction of safety measures (MAYNARD et al., 2004).

Skin irritation

Studies on skin irritation by CNTs are extremely limited in number. Huczko and Lange (2001) evaluated the potential of CNTs to induce skin irritation by conducting two routine dermatological tests. Initially, 40 volunteers with allergy susceptibilities were exposed to a patch test (filter paper

saturated with water suspension of unrefined CNTs, synthesized via the arc discharge process) for 96 hours. Secondly; a modified Draize rabbit eye test (using a water suspension of unrefined CNTs) was conducted with four albino rabbits monitored for 72 hours after exposure. Both tests showed no irritation in comparison to a CNT-free soot control and it was concluded that "no special precautions have to be taken while handling these carbon nanostructures" However, more recent in vitro studies involving human epidermal keratinocytes have raised concerns over this assessment. Clearly, this is an area requiring further scientific evaluation despite evidence of low skin exposure rates (estimated at 0.2–6 mg of CNTs material per glove) during manufacturing and handling of CNTs to SWCNTs, which resulted in accelerated oxidative stress (increased free radical and peroxide generation and depletion of total antioxidant reserves), loss in cell viability, and morphological alterations to cellular structure (SMART et al., 2006).

Similar dermal toxicity warnings were echoed in 2005 in a study which found that MWCNT initiated an irritation response in human epidermal keratinocyte (HEK) cells. Purified MWCNT (synthesized via CVD) incubated at doses of 0.1–0.4 $mg.mL^{-1}$ with HEK cells for up to 48 hours were observed to localize within cells, elicit the production of the pro-inflammatory cytokine (IL-8) release, and decrease cell viability in a time- and dose-dependent manner. The lack of catalyst particles led these authors to conclude that CNTs themselves were a potential dermatological hazard urging a full toxicological assessment before widespread public exposure (SMART et al., 2006).

In addition, in vitro evaluation of the inflammatory potential of CNTs on peritoneal and alveolar macrophages has been reported. Ground MWCNT has showed a similar capacity for inducing dose-dependent cytotoxicity and up-regulating TNF-a expression as asbestos and carbon black. In contrast, the unground MWCNT has showed lower cytotoxicity and TNF-a expression than those of the ground sample. It has been postulated that the increased agglomeration observed in the unground sample led to a decrease in MWCNT availability to the cells, accounting for the lowered cytotoxic and proinflammatory response (SMART et al., 2006).

5 Regulatory issues

Currently there is no international regulation on nanotechnology or nanoproducts. Only a few government agencies or organizations from different countries have established standards and regulations to define and regulate the use of nanotechnology (CHAU; WU; YEN, 2007).

The US Food and Drug Administration (FDA) is among the first government agencies around the world to have a definition of nanotechnology and nanoproducts. However, the FDA has not established its own formal definition although the agency participated in the development of the National Nanotechnology Initiative (NNI), in which the term itself was defined. This organization states that it regulates "products, not technologies". The regulatory consideration of an application involving a nanotechnology product may not occur until well after the initial development of that nanotechnology, as well as the establishment of its statutory classification. This may affect the stage at which the FDA becomes engaged in the regulation of nanotechnology. The FDA only regulates certain categories of products and anticipates that many nanotechnology products may span the regulatory boundaries among different agency centers within the FDA and will be regulated as "Combination Products" by the Office of Combination Products. The FDA has traditionally regulated many products with particulate materials of same size range as that of cells and molecules (nano in size); hence, the existing FDA requirements as well as the existing battery of pharmacotoxicity tests may be adequate for most nanotechnology products. Accordingly, the FDA states that particle size is not the issue. If new toxicological risks that derived from new materials or manufacturing techniques are identified, new safety tests will then be required (CHAU; WU; YEN, 2007).

A close coordination of knowledge and policies among different government agencies is important for the development of a harmonized regulation on the use of nanotechnology. The FDA has been collaborating with the National Institutes of Health, National Institute of Environmental Health Sciences (NIH/NIEHS) on the development of nanotoxicity studies and contributes directly to the evaluations of the toxicity of materials.

In 2006, the NNI – which is a federal research and development programme established in 1996 to coordinate governmental multiagency efforts in nanoscale science, engineering, and technology – invested about 4% (42 million dollars) of the total budget (1054 million dollars) in research that addresses the potential risks caused by nanotechnology to environment, health, and safety (NATIONAL..., 2007).

Three main areas of research focus on understanding the effects of nanotechnology in the environment, health, and safety. They are i) basic research to understand the behavior of nanomaterials in the environment and the human body, ii) research to develop instruments and methods to measure, characterize, and test nanomaterials and to monitor exposure, and iii) research to assess the safety of technology that use nanoparticles (NATIONAL..., 2007).

The ETC, an international organization that "researches and organizes for democratic and transparent technology assessment, greater cultural and biological diversity, and strengthened human and Farmer's Rights in the framework of Food Sovereignty", proposes the evaluation of social implications of all nanotechnologies and a partial moratorium, in addition to the assessment of toxicity of nanoparticles per se (NATIONAL..., 2007).

Other groups, like the Natural Resources Defense Council, the American Federation of Labor and Congress of Industrial Organizations, Beyond Pesticides, the Center for Environmental Health, the Center for Food Safety, Corporate Watch, Edmonds Institute, the Institute for Agriculture and Trade Policy, the International Center for Technology Assessment, the Project on Emerging Nanotechnologies, and the Environment Defense criticize industry influence on the decision making process and ensure that as nanotechnologies advance, possible risks

are minimized, public and consumer engagement remains strong, and the potential benefits of these new technologies are provided. Academia, industry, and regulatory governmental agencies need to consider the unique biological properties of nanoparticles and the related potential risks that may differ from bulk material with the same chemistry (CHAU; WU; YEN, 2007).

6 Conclusions

In spite of the scientific knowledge gained in recent years in nanotoxicology, scientists have not been able to precisely anticipate the behavior and biokinetics of nanoparticles. Medical, academic, and regulatory communities, both at the national and international level, need to determine the potential threats in the workplace for manufacturers and the environment. It should be kept in mind that results are often obtained for only one type and size of NPs. Furthermore, test animals are generally exposed to high concentrations under artificial conditions. Moreover, in vivo systems are extremely complicated, and the interactions of the nanostructures with biological components, such as proteins and cells, could lead to unique biodistribution, clearance, immune response, and metabolism. This limits the usefulness of the data obtained for risk assessment. Extrapolation from one type of NPs to another, or from one size to another, is, on the basis of the present knowledge, still impossible. Presently, there are very few data to determine which type of effects are to be expected for which type of NP.

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