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Helth effects of nanoparticles

Claude Ostiguy
IRSST

Brigitte Soucy
IRSST, Université de Montréal

Gilles Lapointe
CSST

Catherine Woods
IRSST, Université de Montréal

Luc Ménard
CSST

See next page for additional authors

Auteurs

Claude Ostiguy, Brigitte Soucy, Gilles Lapointe, Catherine Woods, Luc Ménard, and Mylène Trottier

Chemical Substances and Biological Agents

Studies and Research Projects

REPORT **R-589**

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Second Edition

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IRSST – Communications Division
505, De Maisonneuve Blvd West
Montréal (Québec)
H3A 3C2
Phone: 514 288-1551
Fax: 514 288-7636
publications@irsst.qc.ca
www.irsst.qc.ca
Institut de recherche Robert-Sauvé
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REPORT **R-589**

Health Effects of Nanoparticles

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Claude Ostiguy, Research and Expertise Support Department, IRSST

Brigitte Soucy, IRSST

Gilles Lapointe, Répertoire toxicologique, CSST

Catherine Woods, IRSST

Luc Ménard, Direction de la prévention inspection, CSST

Mylène Trottier, Proximeduc

Clic Research
www.irsst.qc.ca

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SUMMARY

Research in the nanoparticle (NP) and nanotechnology field is growing at a breathtaking pace. The reason is simple: the unique properties of NP will allow the development of products with unprecedented characteristics and opportunities in every field of human activity, and with tremendous economic impacts. It is currently anticipated that the number of exposed Quebec workers, not only in manufacturing these products but also in using and processing them, will increase over the next few years. Several products are already available commercially and some Quebec companies now have large-scale NP production capacity.

While technological research is already well established, with many transfers to industrial production, research on occupational health and safety (OHS) risk assessment has lagged behind significantly. Fortunately, the latter has shown strong growth in the scientific community over the last ten years. An initial evaluation of existing knowledge concerning nanoparticle health risks had been published by our team in early 2006 and covered the literature up to 2004. This report is the second edition and incorporates scientific knowledge up to mid-2007.

Insoluble or low-solubility nanoparticles in biological fluid are the greatest cause for concern. Because of their tiny size, several studies have shown behaviour unique to NP. Some of them can pass through our various defence mechanisms and be transported through the body in insoluble form. Thus, some NP can end up in the bloodstream after passing through all the respiratory or gastrointestinal membranes. They are then distributed to various organs and accumulate at specific sites. Others travel along the olfactory nerves and penetrate directly into the brain, while still others pass through cell barriers and reach the nucleus of the cell. These properties, extensively studied in pharmacology, could allow NP to be used as vectors to carry drugs to targeted body sites, including the brain. The corollary is that undesirable NP could be distributed through the bodies of exposed workers and has deleterious effects.

In toxicology the effects are normally correlated to the quantity of product to which individual animals or humans are exposed. The greater the mass absorbed, the greater the effect. In the case of NP, it has been clearly shown that the measured effects are not linked to the mass of the product, which challenges our entire approach to the classical interpretation of toxicity measurement. It is clearly shown that at equal mass, NP are more toxic than products of the same chemical composition but of greater size.

Although several studies find a good correlation between the specific surface and the toxic effects, a consensus seems to be emerging in the scientific community that several factors can contribute to the toxicity of these products and that it is currently impossible, with our limited knowledge, to weight the significance of each of these factors or predict the precise toxicity of a new nanoparticle. The published studies link the observed effects to different parameters: specific surface, number of particles, size and granulometric distribution, concentration, surface dose, surface coverage, degree of agglomeration of the particles and pulmonary deposition site, the “age” of the particles, surface charge, shape, porosity, crystalline structure, electrostatic attraction potential, particle synthesis method, hydrophilic/hydrophobic character and post-synthesis modifications (grafting of organic radicals or surface coverage to prevent aggregation). The presence of certain contaminants, such as metals, can also favour free radical formation and inflammation, while the chemical composition and delivery of surface components, NP colloidal and surface properties, compartmentation in the lung passages and biopersistence are other factors adding a dimension of complexity to the understanding

of their toxicity. The slow dissolution of certain NP or NP components in the body can become a major factor in their toxicity. These various factors will influence the functional, toxicological and environmental impact of NP.

Several effects have already been shown in animals. Among these, toxic effects have been identified in several organs (heart, lungs, kidneys, reproductive system…), as well as genotoxicity and cytotoxicity. For example, some particles cause granulomas, fibrosis and tumoural reactions in the lungs. Thus, titanium dioxide, a substance recognized as having low toxicity, shows high pulmonary toxicity on the nano-scale in some studies and no or almost no effects in other studies. In general, the toxicological data specific to nanoparticles remains limited, often rendering quantitative risk assessment difficult due to the small number of studies for most substances, the short exposure period, the different composition of the nanoparticles tested (diameter, length and agglomeration), or the often-unusual exposure route in the work environment. Additional studies (absorption, biopersistence, carcinogenicity, translocation to other tissues or organs, etc.) are necessary for quantitative assessment of the risk associated with inhalation exposure and percutaneous exposure of workers.

Although major trends may emerge and show numerous toxic effects related to certain NP, it can be seen that each product, and even each synthesized NP batch, can have its own toxicity. Any process or surface modification can have an impact on the toxicity of the resulting product.

Given this context, the authors of this report consider that the IRSST should favour the introduction of strict prevention procedures, which remain the only way to prevent the development of occupational diseases. Thus, the authors strongly recommend that the IRSST concentrate its future research efforts on developing exposure assessment strategies and tools, and on the development and measurement of the effectiveness of control methods for occupational NP exposure.

TABLE OF CONTENTS

1. INTRODUCTION

Our team has produced comprehensive reviews of current knowledge on types of nanoparticles, their synthesis and applications, as well as the associated health risks and the exposure assessment challenges facing OHS specialists. The control and prevention aspects of occupational health and safety associated with nanoparticles were also discussed (Ostiguy *et al.*, 2006a, 2006b, 2008). The chapter dealing with the assessment of knowledge in the area of hygiene pertaining to health risks (Ostiguy *et al*., 2008) is largely based on the present document, which focuses on integration of nanoparticle (NP) toxicity data from the literature. NPs are produced intentionally with the aim of developing new materials that exhibit certain specific properties. These properties are related to at least one of their dimensions, which must be less than 100 nanometers (nm).

Recent studies of the biological effects of NPs show signs that some manufactured nanoparticles display unexpected toxicity to living organisms. Some of these particles can become potentially harmful and even cause deleterious human health effects.

Some characteristics of nanoparticles

With a reduction of their size, nanoparticles reveal unique properties. A size reduction results in a substantial increase in the specific surface and the surface Gibbs free energy. This physical parameter of free energy reflects the fact that chemical reactivity increases rapidly as particle size diminishes. For example, water has a specific surface of 12.57×10^{-3} m^2/g at a diameter of one millimetre but the surface expands to $12.57 \times 10^{+3}$ m^2/g at a diameter of one nanometre. Surface energy also rises by a factor of one million as size decreases from millimetres to nanometres (Zhao and Nalwa, 2007c).

This means that the NP has a very large specific surface, a large proportion of its molecules on the surface and very high reactivity because the reactivity is linked to free energy. This increased NP specific reactivity allows us to understand that the biological behaviour of NPs and their effects on living organisms can become totally different when particle size decreases. The results of toxicity studies have also led to a reconsideration of the traditional concepts of toxic risk assessment, which is normally based on the dose-response ratio, with the dose expressed in terms of mass or concentration. Indeed, the results obtained clearly showed that on the nano-scale, factors such as specific surfaces, surface modifications, number of particles, surface properties (stereochemistry, degree of ionization, oxido-reduction potential, solubility, intermolecular force, interatomic distance between the different functional groups, partition coefficient), concentration, dimensions, structure are all factors that must be considered in toxicity assessment.

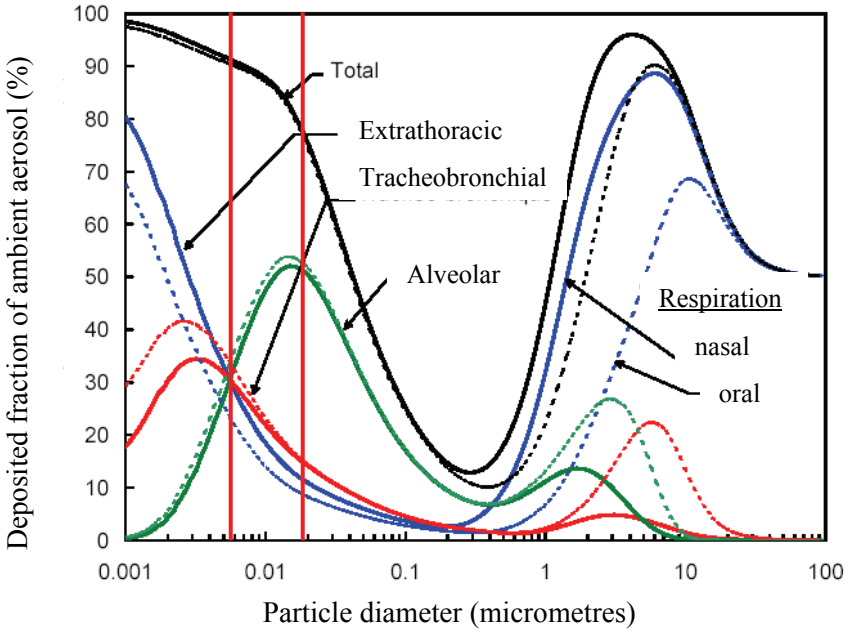
Moreover, contrary to toxicity studies with larger particles, the initial exposure dose can involve a degree of uncertainty because the nanoparticles can agglomerate into larger particles during the emission process, the exposure process outside the organism or during translocation processes within the organism. This is particularly true during inhalation exposure experiments. This means that the form and physicochemical properties of NPs can evolve during experimentation and the biological microenvironment can be extremely sensitive to these changes. This results in the possibility of substantial modification of the interactions between the biological systems and the NPs, making the interpretation of toxicity experimentation results even more complex (Zhao *et al.*, 2007a).

Absorption and pulmonary deposition of nanoparticles

The lungs are the main route for dust entering the human body. Dust deposition in the pulmonary system varies considerably according to the granulometry of ultrafine dusts and their airborne behaviour. Normally, for coarser dusts encountered in work environments, the proportion of dusts deposited in the alveolar region increases as particle diameter decreases, reaching a maximum value of around 20% for 3-micrometre particles. This percentage then diminishes gradually. This situation has led hygienists and occupational health physicians to consider reflexively that the smaller the particle, the deeper it is deposited in the lungs. They should beware of this reflex – the situation is totally different for nanoparticles!

Figure 1, taken from Witschger and Fabriès (2005) and reproduced with the permission of the Institut National de Recherche Scientifique (INRS) in France illustrates the deposition rate in the different pulmonary regions according to particle size. This figure, reproduced in an ISO technical report (ISO, 2007), clearly illustrates that no particle with an aerodynamic diameter of 1 nm, or 0.001 micrometre, reaches the alveoli, while 80% are deposited in nose and pharynx. The other 20% ends up in the tracheobronchial region. At this size, retention of inhaled nanoparticles is nearly 100%.

Figure 1: Prediction of total and regional particle deposition in the airway according to particle size. Reproduced with authorization of INRS-France.



By increasing particle size to 5 nm (vertical line to the left of 0.01 micrometre), 90% of all inhaled particles are retained in the lung and then are deposited in the three regions with relative uniformity. Total pulmonary absorption of 20 nm particles (second vertical line, to the right of 0.01 micrometre) decreases to 80% but more than 50% of 20 nm particles are deposited in the alveolar region. This means that 20% of inhaled particles penetrate the lung but leave it during exhalation. Particle granulomatry thus has a major impact on the pulmonary deposition site (Witschger and Fabriès 2005; Oberdorster 2005a; Zhao and Nalwa, 2007; ISO, 2007). In several

nanoparticle production processes, the granulomatry can also vary considerably according to the stage of production. To understand dust behaviour and aggregation phenomena, see the IRSST reports (Ostiguy *et al*., 2006, 2008).

The three pulmonary regions represent very substantial differences in the surfaces where particles can be deposited. Thus, even though the mass of 20 nm ultrafine particles deposited in the alveolar region represents over 50% of the total mass, the deposited dust concentration, expressed in lung surface units, will still be over 100 times greater in the nasal region and more than 10 times greater in the tracheobronchial region (Oberdörster, 2005a). The quantity of particles and the particle deposition site in the pulmonary system are also influenced significantly by the presence of a pre-existing lung disease (Maynard and Kuempel, 2005; US EPA, 2005). For example, the particle deposition rate in the upper bronchioli is four times higher in persons suffering from chronic obstructive pulmonary disease (COPD) than in healthy individuals exposed to the same particle concentration (US EPA, 2005). These differences in dust distribution in the lungs may have major consequences on the health effects of inhaled ultrafine particles and the elimination mechanisms involved (Schiller *et al.*, 1988; Kim and Jaques, 2000; Jacques and Kim, 2000; Daigle *et al*., 2003; Oberdörster, 2005a, 2005b; Zhang *et al*., 2005b). Moreover, the increase in respiratory volume and the deposited fraction during physical exercise can increase the total number of particles deposited more than 4.5 times compared to the body at rest (Daigle *et al*., 2003) and change the deposition site by significantly increasing particle speed and the possibility of impaction in the airway (Zhao and Nalwa, 2007).

Elimination of dusts deposited in the lungs

The human body has various defence mechanisms to eliminate these undesirable foreign objects. Two processes are involved: chemical dissolution for soluble particles and physical translocation, i.e., transport from one place to another, for insoluble or low-solubility particles. Soluble ultrafine dusts will act at the solubilization site and will not be discussed here, since the effects are highly variable depending on the dust composition and identical to those of larger dusts which are also solubilized.

By translocation, insoluble or low-solubility particles deposited in the pulmonary system are eliminated from the respiratory system by transporting them elsewhere in the body. The mucociliary escalator eliminates the coarsest particles, which normally are deposited in the upper lungs, mainly in the tracheobronchial region. The tracheobronchial mucous membranes are covered with ciliated cells that form an escalator and expel the mucus containing the particles into the digestive system. Normally this is an efficient mechanism that eliminates particles from the respiratory tract in less than 24 hours, even ultrafine particles (Kreyling *et al*., 2002).

In the alveolar region, the macrophages will take up the insoluble particles by phagocytosis, a mechanism whereby the macrophages will surround the particles, digest them if they can and proceed slowly to the mucociliary escalator to eliminate them. This is a relatively slow process, with a half-life of about 700 days in humans (Oberdörster, 2005a). However, the efficiency of phagocytosis is heavily dependent on particle shape and size. Several studies seem to show that unagglomerated ultrafine particles deposited in the alveolar region are not phagocyted efficiently by the macrophages (particularly particles with a diameter of less than 70 nm; Bergeron and Archambault, 2005). However, the macrophages are very efficient for coarser particles in the one to three micrometre range (Tabata and Ikada, 1988; Green *et al*., 1998).

The often inefficient uptake of ultrafine and nanometric dusts by macrophages can lead to a major accumulation of particles if exposure is continued and to greater interaction of these particles with

the alveolar epithelial cells. Studies have shown that some ultrafine particles can pass through the epithelium and reach the interstitial tissues (Ferin *et al.*, 1992; Oberdörster *et al.*, 1992, 1994, 2000; Kreyling and Scheuch, 2000, Kreyling *et al.*, 2002; Borm *et al.*, 2003, 2004). This phenomenon seems more prevalent in higher species, such as dogs and monkeys, compared to rodents (Nikula *et al.*, 1997; Kreyling and Scheuch, 2000). Once the epithelium is crossed, a fraction of the particles can reach the lymphatic nodules.

Other nanoparticle absorption mechanisms

For nano-scaled ultrafine particles, it is now recognized that two other mechanisms contribute to the absorption of these particles. Ultrafine particles and NPs can pass through the extrapulmonary organs via the bloodstream (Oberdörster, 2002, 2005a, 2005b; Nemmar *et al.*, 2001, 2002a; Meiring *et al.*, 2005). Once they reach the bloodstream, the nanoparticles can circulate throughout the body and be distributed to the different organs. Moreover, some particles can be transported along the sensory axons to the central nervous system (Oberdörster *et al.*, 2004; Qingnuan *et al.*, 2002; Mikawa *et al.*, 2001; Wang *et al.*, 2004). These two mechanisms could play a major role in the development of certain cardiac or central nervous system diseases, but these phenomena still have to be demonstrated clearly in humans (Oberdörster, 2005a, 2005b). Katz *et al.* (1984) described neuronal transport from the nose to the brain for 20 to 200 nm microspheres. Inhalation of 35 nm radiomarked carbon particles led to a significant accumulation in the olfactory bulb of rats seven days after exposure. Several studies showed that when rats are exposed to dusts or welding fumes containing manganese, an insoluble manganese fraction could pass through the hematoencephalic barrier, circulating directly from the nose to the brain via the olfactory nerves, thus allowing manganese to accumulate in the brain. Such studies also were performed on various soluble metals and led to the same conclusions (DeLorenzo, 1970; Tjalve et Henriksson, 1999; Brenneman *et al.*, 2000; Dorman *et al.*, 2002; Ostiguy *et al.*, 2003, 2005, 2006c; Oberdorster *et al.*, 2004; Salehi, 2005; Elder *et al.*, 2007). In humans, it is clearly shown that manganism is related to manganese accumulation in the brain, although the exact mechanism of this accumulation is not fully understood (Ostiguy *et al.*, 2003, 2005).

Effects of ultrafine dusts

Several lung diseases related to fine dusts in the work environment have long been known: pneumoconiosis (silicosis, asbestosis), lung cancer, welder's disease, occupational asthma, berylliosis, etc. Donaldson *et al.* (2005) produced a review of the current knowledge in the field. It clearly appears that pulmonary toxicity is related to oxidative stress caused by the presence of transition metals, an organic fraction or a very high specific surface of deposited dusts. This oxidative stress can lead to activation of the epithelial cells. The section on fullerenes will show that toxic effects of these molecules on the cells are also linked to an oxidative stress mechanism. In addition, persons suffering from chronic obstructive bronchitis or asthma (among others) are already subject to oxidative stress (Hervé-Bazin, 2005). Exposure to ultrafine dusts thus could have major health consequences, particularly for susceptible individuals.

Animal studies of ultrafine particles have shown pulmonary inflammation with histopathological change and translocation of particles to extrapulmonary tissues. Translocation of inhaled ultrafine particles in the bloodstream could affect endothelial function and promote thrombosis and other blood system problems, including increased blood coagulation (Elder *et al.*, 2000, 2002, 2004; Nemmar *et al.*, 2002a; Kreyling *et al.*, 2002; Zhou *et al.*, 2003). This phenomenon has been shown in hamsters (Nemmar *et al.*, 2002b, 2003) but the situation in humans remains ambiguous.

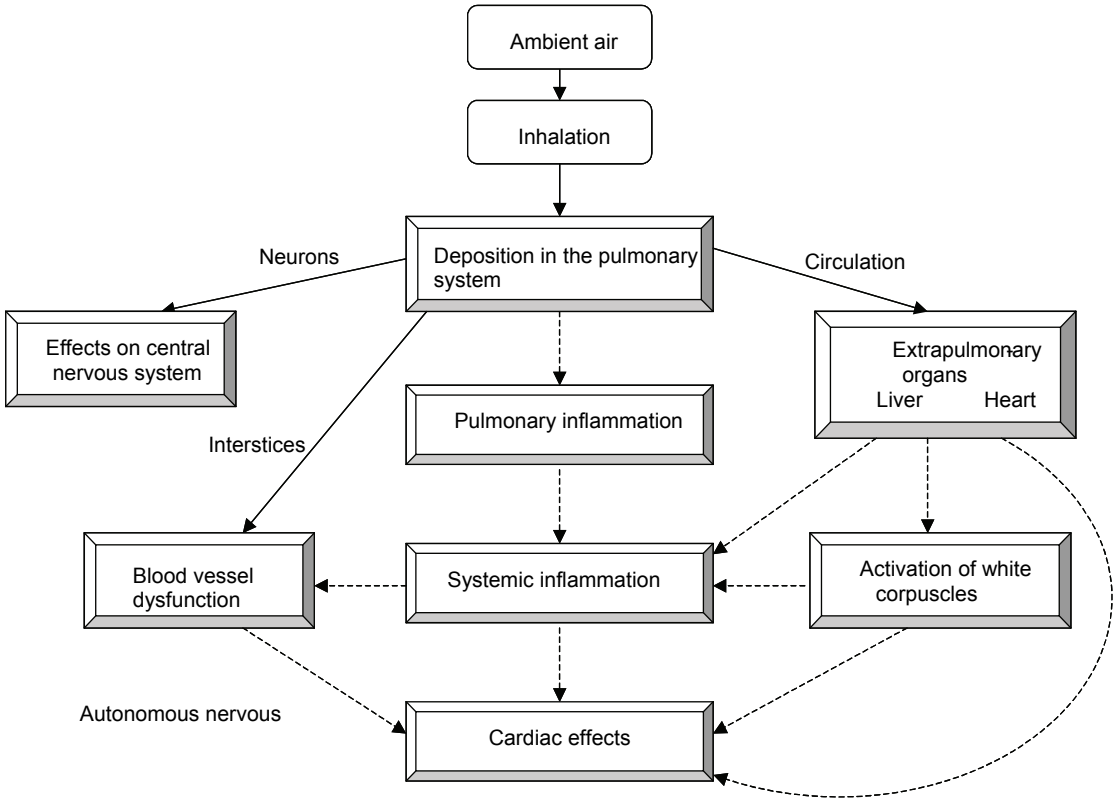
Epidemiological studies and volunteer studies of the human cardiovascular system have shown that the level of inhaled particles has direct effects on cardiovascular physiology, with alterations of cardiac rhythm, arterial diameter and plasma viscosity. Several epidemiological studies (Seaton *et al*., 1995; Peters *et al*., 1997, 2001; Wichmann *et al*., 2000; MacNee and Donaldson, 2000; Samet *et al.,* 2000; Utell and Frampton, 2000; Penntinen *et al*., 2001; Bateson and Schwartz, 2001; Oberdorster 2001; Pekkamen *et al*., 2002; Wichmann 2003a, b; Pope *et al*., 2004; Kreyling *et al.,* 2004; Peters, 2005; Brunekreef *et al.* 2005; Dominici *et al.* 2006) found a direct relationship between exposure to nano-scaled ultrafine dusts and respiratory and cardiovascular effects. Significant relationships were established in several epidemiological studies showing that an increase in fine particle air pollution, mainly due to vehicle emissions, led to an increase in morbidity and mortality of more fragile populations with respiratory and cardiac problems (Dockery, 1994; Schwartz *et al*. 1994, 1995; Bruske-Hohlfeld *et al.*, 2005). Controlled clinical studies in the laboratory showed ultrafine dust deposition throughout the pulmonary system, all accompanied by cardiovascular problems (Brown *et al*., 2002; Daigle *et al*., 2003; Pietropaoli *et al*., 2004; Oberdörster, 2005a, 2005b). A symposium of experts held in October 2005 (Mossman *et al*. 2007) concluded that the pulmonary or cardiac problems related to overexposure to ultrafine dusts are partly related to genetic factors and partly to environmental conditions. Studies of coal miners exposed to ultrafine dusts showed accumulation of such dusts in the liver and spleen (Donaldson, 2005). Accumulation was higher in miners exhibiting severe pulmonary problems, thus suggesting that damaged lungs or lungs with substantial deposits favour the passage of ultrafine particles to the blood system. A preliminary mouse study with a metallofullerene (Zhao *et al*. 2007a) suggests that this nanoparticle induces death by thromboembolism but additional studies are required to understand the mechanism.

Ultrafine particles could also be at the origin of autoimmune reactions. They then would act as haptenes by modifying the structure of the proteins, thus altering their properties and possibly rendering them antigenic (Donaldson *et al*., 2006; Nel *et al*., 2006).

Thus, ultrafine dusts of the same dimensions as nanoparticles mainly penetrate the body via inhalation and are deposited in the lungs. A portion of these dusts can be distributed directly to the brain via the olfactory nerves. The lungs do not necessarily succeed in totally eliminating these undesirable particles, which then cause pulmonary inflammation. This can lead to the development of lung diseases specific to the nature of the dusts that caused them. These very fine dusts can also pass through the different pulmonary protection barriers, reach the blood system and be distributed to every part of the body, where they can cause different kinds of damage. Oberdörster (2005c) summarizes the effects on the body of inhaling nano-scaled ultrafine dusts. Translocated particles then can induce various damages in different parts of the body. Figure 2 summarizes the potential effects of inhaled ultrafine particles.

Many international bodies are concerned about the health risks related to nanoparticles. The available documents prepared for these bodies include Arnall (2003), Bodegal *et al*. (2003), Christiansen (2004), Aitken *et al*. (2004), European Commission (2004), Dreher (2003), Durrenberger *et al*. (2004), Feigenbaum *et al*. (2004), Health and Safety Executive, (2004), Hoet *et al*. (2004b), Lamy (2005), Malsch *et al*. (2004), Mark D (2005), Morrison *et al*. (2003), Oberdörster *et al.* (2000), Royal Society and Royal Academy of Engineering (2004), Ostiguy *et al*., (2006a, 2006b), AFSSET (2006), Zhao and Nalwa (2007a). The present report seeks to update the IRSST report published in 2006 (Ostiguy *et al*., 2006a) by consolidating the main information currently available on the subject, presented by nanoparticle type based on the study of the original articles.

Figure 2: Potential effects of inhaled ultrafine particles (Reproduced with permission of Dr. Gunter Oberdörster, 2005a).



Translocation of particles: ⟶

Mediator: - - - - - - - - ▸

2. OBJECTIVES

The purpose of this report is to summarize and classify the original articles identified in the scientific literature up to summer 2007, pertaining to the study of the toxicity of nanoparticles synthesized for use in nanotechnology. The content was used in updating Chapter 6 of the knowledge assessment on *"Nanoparticles: Current knowledge about occupational health and safety risks and prevention measures"* produced by our team (Ostiguy *et al*., 2008).

3. METHODOLOGY

Analyzing the scientific literature via the approaches commonly used for this type of research in different databases by the IRSST Informathèque and the CSST documentation centre identified peer-reviewed journal articles on nanoparticle toxicity. The literature is covered exhaustively up to summer 2007. Among the main databases and search engines consulted, we should mention MedLine, Toxline, PubMed, Inspec, Coppernic, Embase, Ntis, Ei, Compendex, SciSearch, Pascal, Alerts, Teoma and Scirus. To cover the breadth of the nanomaterial spectrum, the following key words were used: nanotechnologie, nanotechnology, nanoparticules, nanoparticles, nanomatériaux, nanomaterials, nanotoxicity, nanotoxicité, fullerènes, fullerenes, nanotubes, quantum dots, points quantiques, nanocristaux, drug delivery, ultrafine particles, nanomedicine and nanomédecine.

The literature review specifically targeted engineered nanoparticles produced for commercial uses. Then, ultrafine dusts such as diesel fumes or welding fumes were not considered. Titanium dioxide and carbon black were only briefly discussed as important literature reviews already cover these well documented substances.

The contents of the various articles were summarized. In most cases, a comparative analysis of different articles on the same aspect could not be performed, given the lack of information currently published and available. Given the large variety of NPs studied and the limited information available for each one, results are presented by categories: fullerenes, carbon nanotubes, quantum dots, organic nanoparticles, inorganic nanoparticles… Many publications focused on the biopharmacological use of nanomaterials for therapeutic or diagnostic purposes. Although these studies inform us about several toxicological aspects of certain nanomaterials, the integration and generalization of this material requires a prudent approach. These nanomaterials are developed for treatment or diagnostic investigation purposes, specifically to avoid producing toxicity in humans. When publications concerned toxicological aspects that seemed particularly relevant to our research, we included them. The studies selected are also useful for risk assessment of workers in the biopharmaceutical industry.

4. HEALTH EFFECTS OF FULLERENES

Fullerenes are spherical cages containing from 28 to more than 100 carbon atoms. The most widely studied form, synthesized for the first time in 1985 (Kroto *et al*.), contains 60 carbon atoms, C_{60} (Holister *et al*., 2003). This is a hollow sphere, resembling a soccer ball, composed of interconnected carbon pentagons and hexagons (Holister *et al*., 2003; Hett, 2004). Fullerenes are a class of materials displaying unique physical properties. They can be subjected to extreme pressures and regain their original shape when the pressure is released. When they are not modified, these molecules do not combine with each other. However, when fullerenes are manufactured, certain carbon atoms can be replaced with other atoms and form bondable molecules, thus producing a hard but elastic material. The surface chemical composition can be modified and different organic chains can be added, or they can be incorporated into carbon nanotubes (see Chapter 5). Since fullerenes are empty structures with dimensions similar to several biologically active molecules, they can be filled with different substances and find medical applications (Holister *et al*., 2003). Among the many biomedical applications envisioned for fullerenes, it was shown that fullerenes can be excellent sensors of free radicals (Krusic *et al*. 1991). Chemically modified fullerenes are also envisioned as drug delivery vectors, such as neuroprotective, antiviral, antibacterial, antitumoural, antiapoptic and antioxidant agents (Fiorito 2007).

4.1. Toxicokinetics

4.1.1 Absorption

A Scrivens and Tour (1994) study cited by Monteiro-Riviere and Inman (2006) on absorption of unmodified C_{60} modified with ^{14}C by human keratinocytes showed retention of about 50% of the C_{60} after 6 h. However, it is not known whether the C_{60} was really absorbed or whether it was only adsorbed to the cell surfaces.

In a study intended to establish whether human monocyte-derived macrophages can internalize NP, Porter *et al*. (2006) showed that C_{60} NP were accumulated along the nuclear membrane and in the nucleus. Their work supported the hypothesis that C_{60} toxicity would be due, at least in part, to lipid peroxidation caused by generation of free radicals in the cell membranes. They also found them in certain intracellular organelles, such as lysosomes, free cytoplasm and the nucleus, which suggests that intracellular sites could also play a role in peroxidation. The researchers hypothesized that if the NP penetrate the nucleus, this could damage the DNA.

4.1.2. Distribution

4.1.2.1. Inhalation and airway exposure

No data

4.1.2.2. Cutaneous exposure

No data

4.1.2.3. Ingestion exposure

No data

4.1.2.4. Exposure by other routes

Rajagopalan *et al*. (1996) studied the pharmacokinetics of a water-soluble fullerene, p,p'-bis(2-amimoethyl)-diphenyl-C_{60}, administered intravenously in rats (15 and 25 mg/kg). Injection of 25 mg/kg caused the death of two tested rats in 5 minutes. In five other rats, a 15 mg/kg dose did not result in any death and showed that the compound is greater than 99% bound to plasma proteins and distributes into tissues. It also exposed the absence of a renal clearance mechanism.

Tsuchiya *et al*. (1996) showed that C_{60} (intraperitoneal administration, 50 mg/kg; day 18 of gestation) is distributed throughout the embryo and the yolk sac of mice 18 hours after injection. Thus, it passes through the placental barrier.

A preliminary study by Moussa *et al*. (1997) showed that the C_{60} fullerene could be detected in the blood, liver and spleen in mice one, two and six days after an intraperitoneal injection (0.5 mL/20 g).

In a study by Yamago *et al*. (1995) cited by Oberdörster *et al*. (2005b) on intravenously exposed rats, 90% of the functionalized and radiomarked C_{60} fullerenes (solubilized in water with polyethylene glycol and albumin; 9.6 kBq in ~50 μL) were still present in the body one week after administration, including 73-80% in the liver. Fractions of the dose were also found in the spleen (up to 2%), the lungs (up to 5%), the kidneys (up to 3%), the heart (~ 1%) and the brain (~ 0.84%) (Fiorito *et al*., 2006a, b).

4.1.2.5. In vitro

No data

4.1.3. Metabolism

The C_{60} fullerene can reduce the hepatic enzyme activity of glutathione (glutathione S-transferase, glutathione peroxidase and glutathione reductase) *in vitro* in humans (liver coming from an autopsy), mice and rats (Iwata *et al*., 1998).

4.1.4. Excretion

In the study by Yamago *et al*. (1995), 98% of the same functionalized and radiomarked C_{60} fullerenes (18 kBq in 100 μL), but administered orally, were found in the feces 48 h after administration (Oberdörster *et al*., 2005b). The remaining 2% were eliminated in the urine, indicating a certain absorption in the blood (Oberdörster *et al*., 2005b).

Rajagopalan et al. (1996) studied the pharmacokinetics of a water-soluble fullerene, p,p'-bis(2-amimoethyl)-diphenyl-C60, administered intravenously in rats (15 and 25 mg/kg). The authors reported the absence of a renal clearance mechanism.

4.2. Effects according to routes of exposure (administration)

4.2.1. Inhalation and airway exposure

The National Toxicology Program (NTP) in the United States is currently studying the pulmonary toxicity of C_{60} fullerenes following inhalation exposure or intratracheal instillation (Bucher, 2006). The results of this study were unavailable at the time this document was written.

4.2.2. Cutaneous exposure

4.2.2.1. Effects on the organs

No data

4.2.2.2. Immunological and allergic effects

No data

4.2.2.3. Effects on reproduction and the reproductive system

No data

4.2.2.4. Development effects

No data

4.2.2.5. Genotoxic effects

No data

4.2.2.6. Carcinogenic effects

There was no effect on DNA synthesis in the application of C_{60} fullerenes to mouse skin (200 µg during 72 h), but a slight increase in ornithine decarboxylase activity (enzyme with a role in the promotion of tumours) was noted in the epidermis (Nelson *et al*., 1993). Moreover, no increase in cutaneous tumours was observed in a subchronic study (24 weeks) of initiation and promotion of carcinogenesis.

4.2.2.7. Cellular and humoural effects

No data

4.2.3. Ingestion exposure

Chen *et al*. (1998b) studied the acute and subacute toxicity of C_{60} polyalkylsulfonate in rats. No mortality was observed in an acute oral toxicity (LD_{50}) test (0 and 2500 mg/kg).

4.2.4. Exposure by other routes

4.2.4.1. Effects on the organs

- Oberdörster (2004) exposed largemouth bass (*Micropterus salmoides*) to water-soluble fullerenes (nC_{60}). The author observed lipid peroxidation in the brain and a decrease in glutathione in the fish gills after 48 h of exposition to a 0.5 ppm concentration. However, these results are controversial. According to Andrievsky *et al*. (2005), the negative biological effects observed by Oberdörster (2004) are associated with the presence of a large quantity of impurities. The particles tested by

Oberdörster (2004) were said to contain about 10% organic impurities, particularly tetrahydrofurane used as a solvent. According to Andrievsky *et al*. (2005), pristine C_{60} fullerenes are non-toxic and aqueous solutions of hydrated fullerenes (C_{60}•nH_2O) show positive biological activities (antioxidant activity, for example).

4.2.4.1.1. Effects on the skin and the mucous membranes

No data

4.2.4.1.2. Effects on the respiratory system

Chen *et al*. (2004) studied the antioxidant potential of fullerene derivatives on isolated Wistar rat lungs subjected to ischemic reperfusion. Ischemic reperfusion is responsible for production of reactive oxygen species (Chen *et al*., 2004). The increase in arterial pressure and capillary blood pressure in the lungs induced by sodium nitroprusside during ischemic reperfusion was corrected by 10 mg/kg of polyhydroxylated fullerenes [$C_{60}(OH)_{7\pm2}$] administered by intraperitoneal injection for 3 days before the beginning of the study.

By intratracheal instillation, Sayes *et al.* (2007) exposed rats to 0.2 to 3.0 mg/kg concentrations of nano-C_{60} and $C_{60}(OH)_{24}$ using quartz as a positive control. Analysis of the biomarkers contained in the fluids drawn by bronchoalveolar lavage and by a histopathological evaluation of the tissues after one day, one week, one month and three months showed a transient inflammation and cellular effects identical to the controls one day after exposure. An increase in lipid peroxidation was observed at high doses (1.5 and 3.0 mg/kg) for nano-C_{60} after one and three days post-instillation. No effect on the pulmonary tissues was measured for the two products at the highest concentration after three months. However, quartz produced dose-dependent inflammatory responses characterized by accumulation of neutrophils and alveolar macrophages, as well as thickening of the pulmonary tissues consistent with the development of pulmonary fibrosis. These *in vivo* tests show a completely different behaviour than the results obtained *in vitro* (Sayes *et al*., 2004, 2005).

4.2.4.1.3. Liver effects

No data

4.2.4.1.4. Kidney effects

Chen *et al*. (1998) studied the acute and subacute toxicity of C_{60} polyalkylsulfonate in rats. While no mortality was observed in an acute oral toxicity (LD_{50}) test (0 and 2500 mg/kg), an approximate LD_{50} of 600 mg/kg was determined by intraperitoneal injection (0, 500, 750 and 1000 mg/kg) and kidney impairment was observed in the deceased animals. A study by intravenous injection of 100 mg/kg showed a nephropathy and biochemical impairment (significant decrease in alkaline phosphatase and triacetylglycerol) two weeks after administration, thus corroborating the kidney impairment observed after intraperitoneal injection. Several effects were reported in a 12-day subacute toxicity study by intraperitoneal injection (0, 0.6, 6 and 60 mg/kg). Reduced water and food consumption, a significant decrease in body weight and in the weight of certain organs (thymus and heart), an increase in the weight of the spleen and a significant rise of certain biochemical blood parameters (significant increase in aspartate aminotransferase and a significant decrease in triacetylglycerol) were

observed at 60 mg/kg. A nephropathy was observed at 6 and 60 mg/kg respectively.

4.2.4.1.5. Effects on the gastrointestinal system

No data

4.2.4.1.6. Effects on the heart and blood circulation

No data

4.2.4.1.7. Effects on the blood and the hematopoietic system

Bosi *et al*. (2004) studied the hemolytic effect of different water-soluble C_{60} fullerenes (fulleropyrrolidine derivatives) on human red blood cells *in vitro*. The fullerenes with two cationic chains triggered hemolysis of 40 to 50% of the cells at concentrations ranging from 20 to 60 μM (incubation for 30 min.). The position of the cationic chain on C_{60} seems to have an influence on the hemolytic potential. Indeed, the derivative with a cationic chain in the equatorial position showed a higher hemolytic potential than the other derivatives (at the same concentration). The position of the cationic charges determines their accessibility to the surface of the cells (Hoet *et al*., 2004a). The fullerenes with carboxylic functions or a single cationic chain did not show any hemolytic effect up to a concentration of 80 μM.

4.2.4.1.8. Effects on the nervous system

No data

4.2.4.2. Immunological and allergic effects

The administration of C_{60} (conjugated to thyroglobulin and albumin) by intraperitoneal injection in mice triggered an increase in C_{60}-specific antibodies, IgG (Chen *et al*., 1998a). IgG play a role in the defence against infection.

4.2.4.3. Effects on reproduction and the reproductive system

No data.

4.2.4.4. Development effects

Tsuchiya *et al*. (1996) performed an *in vitro* and *in vivo* study of the effects on development of mice. The presence of C_{60} fullerenes solubilized with polyvinyl pyrrolidone inhibited cellular differentiation and proliferation of mesencephalic cells *in vitro*. Intraperitoneal administration on the eleventh day of gestation caused 100% mortality and body flexion anomalies at 137 mg/kg, malformations (head and tail region) at 50 mg/kg and increased head volume at 25 mg/kg. At 50 mg/kg, C_{60} was distributed throughout the embryo and the yolk sac was impaired. Thus, C_{60} passes through the placental barrier, disrupts the yolk sac and causes intrauterine mortality and malformations.

4.2.4.5. Genotoxic effects

Sera *et al*. (1996) observed *in vitro* mutagenic activity in 3 salmonella strains exposed to the C_{60} fullerene and to visible light in the presence of a metabolic activation system.

Zakharenko et al. (1997) observed no effect of the C_{60} fullerene during an *in vitro* somatic mutation and recombination test (SMART) on *Escherichia coli* and an *in vivo* test on *Drosophila melanogaster* larvae.

Babynin *et al*. (2002) tested the mutagenic activity of three C_{60} fullerene derivatives on *Salmonella thyphimurium*: dimethoxyphosphoryl-carbethoxy-methanofullerene, dimethoxyphosphoryl-carbmethoxy-methanofullerene and 1-methyl-2-(3,5-di-tertbutyl-4-hydroxy-phenyl)-3,4-fulleropyrrolidine. Negative results were obtained for the first and last of these derivatives, while the second proved to be antimutagenic.

Dhawan *et al*. (2006) prepared C_{60} colloidal dispersions in water and in a water-ethanol mixture in the total absence of any other organic solvent. Using human lymphocytes, they measured a dose-dependent genotoxic response at concentrations as low as 2.2 µg/L in water and 4.2 µg/L in water-ethanol.

4.2.4.6. Carcinogenic effects

The fullerene genotoxicity studies are insufficient to produce an adequate assessment of carcinogenicity.

4.2.4.7. Cellular and humoural effects

In vitro exposure to the C_{60} fullerene (12.5 µg C_{60}-cyclodextin) induced oxidative damage in rat hepatic microsomes. This damage can be modulated by antioxidants and free radical scavengers (Kamat *et al*., 1998).

Photoinduced (halogen lamp) cytotoxicity of fullerenes has been reported in several studies. Yang *et al*., (2002) showed that this activity could vary with the number of malonic acid molecules added to the C_{60} fullerene (dimalonic acid, trimalonic acid or quadrimalonic acid). Phototoxic inhibition of cell growth was greater for dimalonic acid than for trimalonic acid and quadrimalonic acid, in descending order.

Sayes *et al*. (2004) studied the cytotoxicity (CL_{50}) of four water-soluble fullerenes in human cells *in vitro* (skin fibroblasts and hepatic carcinoma cells; 0.24 to 2400 ppb). They showed that toxicity varies with the nature of the functional group. The authors observed CL_{50} of 20 ppb with nano-C_{60}[1], 10,000 ppb with C_3, 40,000 ppb with $Na^{+}_{2-3}[C_{60}O_{7-9}(OH)_{12-15}]_{(2-3)}$ and > 5,000,000 ppb with $C_{60}(OH)_{24}$ on skin fibroblasts. The generation of oxygen radicals (superoxide anion) by fullerenes is said to be responsible for cell membrane damage causing cell death (Sayes *et al*., 2004). The addition of functional groups, such as hydroxyls or carboxyls, would make the fullerenes less toxic for the cells (Sayes *et al*., 2004).

Chen *et al*. (2004) studied the antioxidant potential of fullerene derivatives on RAW 264.7 mouse macrophages. The damage induced in the macrophages by exposure to sodium nitroprusside or hydrogen peroxide was corrected dose-dependently by prior exposure to polyhydroxylated fullerenes [$C_{60}(OH)_{7 \pm 2}$] (5-1,500 µM). However, 1 and 1.5 mM concentrations of $C_{60}(OH)_{7 \pm 2}$ triggered cell death (by apoptosis or necrosis, after 24 h).

Bosi *et al*. (2004) studied the cytotoxic effects of different water-soluble C_{60} fullerenes (fulleropyrrolidine derivatives) on human breast cancer cells (MCF-7), rat liver cells (Hep-G2), pig renal proximal tubule cells (LLC-PK_1) and red blood cells. The fullerenes with two cationic chains triggered cytotoxicity at

[1] Nano-C_{60} is a stable colloid formed by the addition of C_{60} in water. It has an orderly crystalline structure formed by unmodified C_{60} (Fortner *et al*., 2005).

concentrations ranging from 1 to 50 μM (after 30 minutes of incubation). Red blood cells seem to be more tolerant of derivatives with a relatively high hydrophobic surface/hydrophilic surface ratio.

Sayes *et al*. (2005) studied nano-C_{60} cytotoxicity in human cells *in vitro* (skin fibroblasts, hepatic carcinoma cells and astrocytes). The cells were exposed to concentrations of 0.24, 2.4, 24, 240 and 2400 ppb for 48 h. Cells treated or not treated with a balanced saline solution served as controls. The authors determined LD_{50} of 20, 50 and 2 ppb for skin fibroblasts, hepatic carcinoma cells and astrocytes respectively. No change in mitochondrial activity and no difference in the cellular DNA concentration were observed between the controls and the cells exposed to 2400 ppb of nano-C_{60}. The authors observed cell membrane damage and an increase in glutathione (natural antioxidant) in the cells exposed to 240 ppb of nano-C_{60}. No oxidative damage was observed following the addition of L-ascorbic acid (antioxidant) with nano-C_{60} in the culture medium. Nano-C_{60} cytotoxicity is said to be attributable to the damage caused to the cell membrane by lipid peroxidation (Sayes *et al*., 2005). The authors assume that nano-C_{60} is at the origin of formation of reactive oxygen species.

No C_{60} effect on human keratinocyte or fibroblast proliferation was observed in the Scrivens and Tour (1994) study cited by Monteiro-Riviere and Inman (2006).

Table 1 summarizes the existing information concerning the health effects of fullerenes. This information remains very limited.

Table 1. Documented health effects of fullerenes [1]

Effect	Route			
	Inhalation[2]	**Cutaneous**	**Ingestion**	**Other**
Toxicokinetics			▲	▲■
Irritation				
Systemic[3]				
Acute			▲	▲
Intermediate				
Chronic				
Neurological				
Immunological				▲
Development				▲
Reproductive				
Genotoxic				▲
Cancer		▲		

[1] Existing human (■) or animal (▲) studies. Adopted from the ATSDR.

[2] Including intratracheal instillation.

[3] Systemic effects: acute (≤ 14 days), intermediate (15 to 364 days) and chronic (≥ 365 days).

5. HEALTH EFFECTS OF CARBON NANOTUBES

Discovered barely a few decades ago, carbon nanotubes are a new crystalline form of carbon molecules. Wound in a hexagonal network of carbon atoms, these hollow cylinders can have diameters as small as 0.7 nm and reach several millimeters in length (Hett, 2004). Each end can be opened or closed by a fullerene half-molecule. These nanotubes can have a single layer (like a straw; they are then known as SWCNT, or Single-Walled Carbon NanoTubes) or several layers (known as MWCNT, or Multi-Walled Carbon NanoTubes) of coaxial cylinders of increasing diameters in a common axis (Iijima, 1991). Multilayer carbon nanotubes can reach diameters of 20 nm (Aitken *et al*., 2004). They are chemically and thermally very stable (Hameed Hyder, 2003). Their manufacturing normally involves the presence of metals, the final content of which in the product will depend on the product's conditions of synthesis and subsequent purification. The presence of these metals (Co, Fe, Ni, Mo, for example), which can represent up to 50% of the content by weight, can have a major influence on carbon nanotube toxicity (Donaldson *et al*., 2006; Lam *et al*., 2006; Maynard *et al*., 2004). Maynard *et al*. (2004) point out that it is possible that the carbon layer invariably coating the particles may be altered during phagocytosis and/or other biological processes. The nanotube dispersion processes used in experimental studies (for example, sonification) can also lead to the release of metals (Lam *et al*., 2006).

It is considered that a 20 x 2000 nm nanotube can have 100 times more surface than a spherical nanoparticle 20 nm in diameter (Donaldson *et al*., 2006). This can represent a significant factor in nanotube toxicity. Moreover, carbon nanotubes have a great propensity to agglomerate, and this could significantly reduce the total surface capable of interacting with the body's cells (Donaldson *et al*., 2006).

Recent developments make it possible to consider several potential applications for chemically modified carbon nanotubes. Among those considered, it is appropriate to mention their use in preparation of orthopedic prostheses, as implants, as biosensors for protein and enzyme detection, in anticancer therapy, in tissue engineering and as a bone growth support material facilitating healing of fractures (Fiorito 2007).

When produced, carbon nanotubes represent a complex matrix composed of carbon nanotubes, nanotube agglomerates (carbon nanowires), non-tubular carbon and metallic catalysts (Maynard *et al*., 2007). The toxicity of the material to which the worker will be exposed will depend on the partition and the arrangement of these particles in the work environment.

5.1 Toxicokinetics

5.1.1. Absorption

In a study by Kam *et al*. (2004) cited by Monteiro-Riviere and Inman (2006), SWCNT and SWCNT conjugated to streptavidin (used as an indicator) were internalized by endocytosis in human acute promyelocytic leukemia cells (HL60) and human Jurkat T cells.

5.1.2. Distribution

5.1.2.1. Inhalation and airway exposure

No data

5.1.2.2. Cutaneous exposure

No data

5.1.2.3. Ingestion exposure

Hydroxylated SWCNT administered by gavage in mice (100 µL of a 15 µg/mL solution) are distributed to most of the organs and tissues, except the brain (Wang *et al*., 2004).

Chrysotile fibrils with a diameter in the same order of magnitude as MWCNT (20 nm) were detected in the blood after ingestion (Donaldson *et al*., 2006). Translocation of fibres to the pleural space was also observed (Donaldson *et al*., 2006).

5.1.2.4. Exposure by other routes

The study by Wang *et al*. (2004) shows in mice that radiomarked hydroxylated SWCNT administered intraperitoneally (100 µL of a 15 µg/mL solution) are distributed throughout the body, except the brain, pass through several compartments and are retained in the bones. This distribution was not affected by the other routes used (intravenous, subcutaneous and gavage).

5.1.2.5. In vitro

Pantarotto *et al*. (2004) studied the intracellular transport of functionalized SWCNT, i.e., conjugated to lysine, on human and mouse fibroblasts *in vitro* (1, 5 and 10 mM). They showed that these carbon nanotubes could pass through the cellular membrane, accumulate in the cell and end up in the cell nucleus.

Cherukuri *et al*. (2004) showed that carbon nanotubes (1 nm in diameter by 1 µm long) could be ingested by mouse peritoneal macrophages *in vitro*.

Monteiro-Riviere *et al*. (2005b) found MWCNT in the cytoplasmic vacuoles of human epidermal keratocytes *in vitro* (up to 3.6 µm long), a decrease in cell viability and a significant increase in an inflammation marker (interleukin-8). MWCNT are capable of penetrating the cell membrane. The authors point out that iron is not involved in the increase of IL-8 because the particle analysis revealed that the MWCNT did not contain any. Iron was used as a catalyst in synthesizing MWCNT (by vapour phase deposition), but analysis of the MWCNT by two different techniques did not allow its presence to be detected in the MWCNT before and after exposure.

5.1.3. Metabolism

No data

5.1.4. Excretion

In the study by Wang *et al*. (2004), 11 days after exposure, about 80% of the radiomarked SWCNT administered intraperitoneally had been excreted (94% in the urine and 6% in the feces).

Muller *et al*. (2005) studied the persistence of MWCNT (5.9 ± 0.05 µm long; 9.7 ± 2.1 nm outer diameter) and ground MWCNT (0.7 ± 0.07 µm long; 11.3 ± 3.9 nm outer diameter) in the lungs of rats (Sprague-Dawley females) exposed by single intratracheal instillation (0.5, 2 and 5 mg). At the lowest dose (0.5 mg), 80% of the MWCNT and 40% of the ground MWCNT ended up in the lungs 60 days after administration. The number of animals was not specified. The authors conclude that carbon nanotubes are persistent in the lungs and that their length influences the kinetics of their clearance. However, Donaldson *et al*.

(2006) point out that the fibres studied by Muller *et al*. (2005) are clearly shorter than the mineral fibres considered to be long and measuring nearly 20 μm.

Macrophages are efficient in phagocyting inhaled carbon nanotubes shorter than 15-20 μm (Donaldson *et al*., 2006). Aggregates are also said to be more easily phagocyted than unaggregated carbon nanotubes (Donaldson *et al*., 2006).

5.2. Effects according to routes of exposure (administration)

5.2.1. Inhalation and airway exposure

5.2.1.1. Effects on the organs

5.2.1.1.1. Effects on the skin and the mucous membranes

No data

5.2.1.1.2. Effects on the respiratory system

Huczko *et al*. (2001b) performed an exploratory study of pulmonary function in Guinea pigs (2 groups of 5 males). A single dose of 25 mg of soot containing carbon nanotubes was administered by intratracheal instillation. No effect on pulmonary function (current volume, respiratory frequency and pulmonary resistance) and on analysis of bronchoalveolar lavage fluid was observed (after 4 months). However, Lam *et al*. (2006) point out that no pathological study was performed on the lungs and that this assessment criterion is crucial in any pulmonary toxicity study involving dusts.

Lam *et al*. (2004a) studied the pulmonary toxicity of acute exposure to three SWCNT preparations in male mice (single intratracheal instillation; 0, 0.1 and 0.5 mg/mouse). No clinical sign (body temperature, piloerection, or other) was observed at 0.1 mg, but inflammation and pulmonary granulomas were recorded for unrefined nanotubes (RNT) and purified nanotubes (PNT). The granulomas were composed of macrophages and administered particles (at 0.1 and 0.5 mg). There was an increase in mortality for Carbolex CNT, but no mortality for RNT and PNT. Clinical signs were observed at 0.5 mg for RNT (hypothermia, inactivity and other), but none concerning PNT. The authors reported an increase in pulmonary granulomas for RNT, PNT and CNT. Carbon black and quartz were used as controls; there was no inflammation and no granuloma for carbon black, as opposed to inflammation and no granuloma for quartz. Only 4 or 5 animals were used per treatment.

Warheit *et al*. (2004, 2005) studied the pulmonary toxicity of acute exposure to a SWCNT preparation in male rats (single intratracheal instillation; 0, 1 and 5 mg/kg). There was no effect at 1 mg/kg. At 5 mg/kg, they reported a high mortality rate (~15%) caused by mechanical blockage of the upper airway, a statistically insignificant increase in pulmonary cell proliferation and an increase in multifocal pulmonary granulomas. A significant increase in lung weight, a significant transient increase in bronchoalveolar lavage anomalies (neutrophilic cells, proteins, lactate dehydrogenase (LDH)) were also observed. There was no effect on the pulmonary macrophages. The number of rats was not mentioned. The duration of post-instillation observation was too short to evaluate the evolution of pulmonary lesions and their eventual regression. The nanotubes also tend to agglomerate, forming larger particles, which could have a different

pulmonary toxicity than unagglomerated nanotubes. No conclusion is possible regarding the inherent toxicity of SWCNT (absence of dose-response ratio, non-uniform distribution and possible regression of the granulomas).

Shvedova *et al*. (2005) exposed mice (C57BL/6) to purified SWCNT (99.7% carbon and 0.23% iron by weight) by pharyngeal aspiration (single dose; 0, 10, 20 and 40 μg/mouse). The authors observed unusual pulmonary effects, including an acute inflammation followed by rapid and progressive formation of granulomas and fibrosis (dose-dependent relationship). Shvedova *et al*. (2005) associated interstitial fibrosis with thickening of the alveolar walls with unagglomerated SWCNT, while granuloma formation was associated with hypertrophied epithelial cells surrounding SWCNT agglomerates. Analysis of he bronchoalveolar lavage fluid showed an increase in protein, LDH and γ-glutamyl transferase activity (cell damage indicators; dose-dependent relationship). The accumulation of an oxidative stress marker (4-hydroxynonenal) and a decrease in glutathione (antioxidant) in the lungs were also observed. Neutrophil accumulation and an increase in proinflammatory cytokines (TNF-α, IL-1β) were observed on Day 1, but the TNF-α and IL-1β levels returned close to the control level 3 days after exposure. Lymphocyte accumulation (Day 3) was followed by accumulation of macrophages and TGF-β1 (growth factor involved in fibrogenesis) (Day 7). Respiratory function disorders, a decrease in bacterial elimination (*Listeria monocytogenes*) and an increase in type II alveolar cells (AT-II) were also observed. Ultrafine carbon black and SiO_2 particles did not trigger granuloma formation or thickening of the alveolar walls at the same dose (40 μg/mouse) and the inflammatory response was less noticeable than with SWCNT (Shvedova *et al*., 2005). The 20 μg SWCNT dose is equivalent to the pulmonary deposition resulting from a worker's exposure to the OSHA permitted exposure limit (PEL) for graphite (5 mg/m^3) for 20 days (8 h per day). The authors conclude that workers exposed to such an SWCNT concentration may be at risk of developing pulmonary lesions.

Muller *et al*. (2005) studied inflammation and fibrosis in the lungs of rats exposed to unground MWCNT and ground MWCNT (single intratracheal instillation; 0.5, 2 and 5 mg). The two types of MWCNT triggered an inflammatory reaction (increase in the activity of LDH, proteins, neutrophils and eosinophils) and a fibrotic reaction (increase in collagen deposition), as well as production of proinflammatory cytokines (TNF-α) in the lungs. The inflammatory response was more pronounced with the ground MWCNT, but a 5 mg dose of ground MWCNT induced the same fibrotic response as 2 mg of unground MWCNT. The authors also observed a greater dispersion of ground MWCNT through the pulmonary parenchyma where they caused interstitial granulomas. The accumulation of unground MWCNT agglomerates in the airway triggered partial or complete obstruction of the bronchi, formation of collagen-rich granulomas and alveolar inflammation (after 2 months). The administration of carbon black used as a control did not induce significant inflammation or fibrosis. However, asbestos (Rhodesian chrysotile; 2.4 μm long by 0.17 μm wide on the average) used as a positive control triggered the same effects as MWCNT. The authors conclude that MWCNT are potentially toxic for humans and that strict industrial hygiene measures must be put in place. Moreover, Donaldson *et al*. (2006) point out that the great toxicity of ground MWCNT could be attributable to their

greater dispersion in the lungs or to the activity of metals present in MWCNT and released by grinding.

Huczko *et al*. (2005) exposed Guinea pigs (5 to 10 animals per group) to different types of MWCNT by intratracheal instillation (single dose of 15 mg). 90 days after administration, the authors observed an obliterating bronchiolitis with pneumonic organization accompanied by a non-specific reaction resembling a desquamative interstitial pneumonia, but without fibrosis or with a mild fibrosis around the bronchioli. The authors also observed an increase in resistance to pulmonary dilation and infiltration of the bronchoalveolar space by inflammatory cells in certain animals. In addition to infiltration of inflammatory cells around the vessels, around the bronchioli and in the interstitial space, a central and peripheral atelectasia[2], emphysema and an alveolar exudation were observed. Differences in these effects were observed depending on the type of MWCNT administered. Nanotubes were found within alveolar macrophages. The dimensions of the MWCNT tested were not specified. The authors conclude that the duration of exposure to MWCNT administered by intratracheal instillation is crucial to induce measurable effects in the lungs.

Muller *et al*. (2005) and Lam *et al*. (2006) point out that the absence of lesions observed by Warheit *et al*. (2004, 2005a) may be due to the fact that the agglomeration of SWCNT prevented them from reaching the site where lesions are produced, i.e., the alveoli.

Kagan *et al*. (2006) point out that the unusual effects observed by Shvedova *et al*. (2005) (acute inflammation followed by rapid fibrosis formation) could have been caused by changes in the normal progress of the inflammatory response in which the macrophages are involved. The iron contained in SWCNT is said to modify the redox environment of the macrophages and influence their pro/anti-inflammatory response (Kagan *et al*., 2006).

Grubek-Jaworska *et al*. (2006) studied *in vivo* the effect of single intratracheal instillation (12.5 mg) of four types of commercially available MWCNT and SWCNT with very low iron contents (<0.01 ppm) in groups of 4-5 Guinea pigs. After three months, they noticed that all the CNT lead to organized pneumonitis with an interstitial non-specific focal reaction resembling pneumonia without fibrosis or with a low peribronchiolar fibrosis in all the exposed animals but not in the controls. The concentration of IL-8 in the bronchoalveolar lavage fluids increased for only one of the 4 CNT. The number of macrophages, lymphocytes and neutrophils increased significantly for another of the 4 CNT. Finally, for a third of the 4 CNT, the authors measured an increase in macrophages and eosinophils. It is interesting to note that the carbon deposits were found mainly in the bronchioli and were almost totally absent from the alveolar ducts and the alveoli. The authors did not notice any granulomas in the lung tissue. The authors also noted a tendency of the nanotubes to form aggregates (strings/rods), and mechanical blockages of certain airways were detected in some animals.

The current research suggests that in the case of nanotubes longer than 20 μm and with a sufficient number, one can expect to encounter the same types of effects as those observed following exposure to biopersistent mineral fibers (chrysotile, for

[2] Atelectasia is a "collapse of the pulmonary alveoli when there is an absence of ventilation" (translated from the glossary of the Service du répertoire toxicologique (toxicological directory service) of the CSST, http://www.reptox.csst.qc.ca/Lexique-A.htm).

example), i.e., fibrosis, cancer, changes in the pleura and mesothelioma (Donaldson *et al*., 2006).

Poland *et al*. (2008) showed, in a pilot study, that exposing the mesothelial lining of the body cavity of a mice to long MWCNT results in asbestos-like pathogenic behaviour, including inflammation and formation of granulomas. Then, CNT introduced directly into the abdominal cavity of a mice show asbestos-like pathogenicity.

5.2.1.1.3. Liver effects

No data

5.2.1.1.4. Kidney effects

No data

5.2.1.1.5. Effects on the gastrointestinal system

No data

5.2.1.1.6. Effects on the heart and blood circulation

Li *et al*. (2005) exposed mice (C57BL/6) to carbon nanotubes (CNT) by pharyngeal aspiration (single dose; 0.5, 1 and 2 mg/kg). The authors observed damage to the mitochondrial DNA of the aorta (oxidative stress-dependent parameter; dose-dependent relationship) 7, 28 and 60 days after administration. The oxidative damage was accompanied by an alteration in expression of the genes responsible for the inflammatory response in the heart (MCP-1 and VCAM-1). According to Li *et al*. (2005), the effects observed in mouse hearts could be a direct effect of CNT found in the circulatory system or an indirect effect of inflammation of the lungs. The cardiovascular effects observed following inhalation of particles could also be linked to the autonomic nervous system (Hoet *et al*., 2004a; Seaton and Donaldson, 2005). Li *et al*. (2005) conclude that CNT trigger effects which can be predisposition factors for atherogenesis (formation of atherosclerotic plaque in the arteries).

Li *et al*. (2006, 2007) exposed mice with a high blood cholesterol rate (ApoE-/-) to SWCNT by pharyngeal instillation at concentrations of 10 and 40 µg/mouse. They noted an activation of heme oxygenase-1, an oxidative marker in the lung, the aorta and the cardiac tissue, 7, 28 and 60 days after exposure, and the mice developed aortic DNA damage. The authors (Li *et al.,* 2007) also assessed the effect of repeated exposure in 10 mice (20 µg every 2 weeks for 8 weeks). The percentage of the surface of the aorta covered with atherosclerotic plaque was significantly higher in mice, which had been exposed to SWCNT compared to the controls. A significant increase in atherosclerotic lesions in the brachiocephalic arteries was also observed. The authors conclude that SWCNT exposure accelerates the progression of atherosclerosis in ApoE-/- mice.

5.2.1.1.7. Effects on the blood and the hematopoietic system

No data

5.2.1.1.8. Effects on the nervous system

No data

5.2.1.2. Immunological and allergic effects

No data

5.2.1.3. Effects on reproduction and the reproductive system

No data

5.2.1.4. Development effects

No data

5.2.1.5. Genotoxic effects

No data

5.2.1.6. Carcinogenic effects

No data

5.2.1.7. Cellular and humoural effects

Manna *et al.* (2005) studied SWCNT toxicity for human keratinocyte cells. The results show an increase in oxidative stress and inhibition of cell proliferation following exposure to concentrations of 0.5 to 10 μg/mL for periods of 12 to 72 hours, as well as a dose-dependent decrease in cell viability. The results obtained suggest that SWCNT activate the keratinocyte-specific transcription factor NF-κB dose-dependently and that the activation mechanism is related to activation of kinases due to oxidative stress. The expressive of active proteins would lead to an inflammatory response.

According to Donaldson *et al*. (2006), effects on the mesothelial cells should be envisioned following exposure to carbon nanotubes if the effects of asbestos on these cells are considered. However, this hypothesis will have to be proven scientifically.

Dumortier *et al*. (2006) showed that functionalized carbon nanotubes were taken over by the B and T lymphocytes and by the macrophages *in vitro* without affecting cell viability. Single-walled carbon nanotubes were functionalized with radicals containing ammonium ions and then rendered fluorescent by the addition of fluorescein isothiocyanate on the nitrogen. This highly water-soluble form did not influence the functional activity of the immunoregulating cells. They did not induce cell death or activation of lymphocytes or macrophages. CNT containing polyethylene glycol chains also functionalized subsequently with fluorescein isothiocyanate are less soluble and form a stable suspension in water. They preserve the functionality of the lymphocytes but trigger secretion of proinflammatory cytokines by the macrophages and subsequently alter their capacity to respond to a physiological stimulus. The concentrations used fell within a range of 1 to 10 μg/mL with incubation times of 4 to 24 hours.

Pulskamp *et al*. (2007) showed that CNT can penetrate the cellular membrane of rat macrophages (NR8383) and have an influence on their physiology and their cellular function. In working with these NR8383 macrophages and with human lung cells (A549), the authors did not note any acute toxicity for the viability of cells exposed to purified or unpurified MWCNT or SWCNT. No nanotube induced NO, TNF- and IL-8 inflammation mediators. They observed a dose-dependent and dependent increase in the time of intracellular reactive oxygen species and a decrease in the potential of the mitochondrial membrane with commercial CNT for both types of cells. Purified CNT, which contain a lower metal content, showed few or none of these effects. This led the researchers (Pulskamp *et al*., 2007) to suggest that the metals associated with commercial CNT would be responsible for the biological effects.

Wick *et al*. (2007) exposed human MSTO-211H cells *in vitro* to carbon nanotubes of different degrees of agglomeration. The cytotoxic effects of CNT dispersed by means of a surfactant were less than those of asbestos, but the effects of agglomerated CNT in string form (more voluminous, more rigid and more solid) showed greater cytotoxicity than asbestos.

5.2.2. Cutaneous exposure

5.2.2.1. Effects on the organs

5.2.2.1.1. Effects on the skin and the mucous membranes

Huczko and Lange (2001a) studied the effects on the skin and eyes of exposure to carbon nanotubes. The application of a saturated filter of a solution containing nanotubes did not cause irritation or allergy in volunteers. Ocular instillation of an aqueous suspension of nanotubes in rabbits did not cause irritation.

5.2.2.1.2. Effects on the respiratory system
No data

5.2.2.1.3. Liver effects
No data

5.2.2.1.4. Kidney effects
No data

5.2.2.1.5. Effects on the gastrointestinal system
No data

5.2.2.1.6. Effects on the heart and blood circulation
No data

5.2.2.1.7. Effects on the blood and the hematopoietic system
No data

5.2.2.1.8. Effects on the nervous system
No data

5.2.2.2 Immunological and allergic effects

Huczko and Lange (2001a) studied the effects on the skin and eyes of exposure to carbon nanotubes. The application of a filter saturated with nanotubes did not cause allergies in volunteers.

5.2.2.3. Effects on reproduction and the reproductive system
No data

5.2.2.4. Development effects
No data

5.2.2.5. Genotoxic effects
No data

5.2.2.6. Carcinogenic effects
No data

5.2.2.7. Cellular and humoural effects
No data

5.2.3. Ingestion exposure

No data

5.2.4. Exposure by other routes

5.2.4.1. Effects on the organs

5.2.4.1.1. Effects on the skin and the mucous membranes

Shevedova *et al*. (2003), in an *in vitro* study, reported that SWCNT caused a significant dose-response reduction of cell viability and oxidative stress biomarkers (e.g., antioxidant reserve), and a significant increase in lipid peroxides in human epidermal keratinocytes (0, 0.06, 0.12 and 0.24 mg/mL of SWCNT for 18 hours). The authors mention that, according to their results, exposure to unrefined SWCNT can lead to an increase in cutaneous toxicity in exposed workers.

Yokoyama *et al*. (2005) implanted masses of carbon nanotubes ~ 30 to 100 nm in diameter and ~ 100 nm to 1 μm long) in the subcutaneous tissue of Wistar rats. One week after implantation, the authors observed granulation tissue around the CNT masses and an inflammatory response similar to the one observed during granuloma formation. After four weeks, the CNT masses were surrounded by fibrous tissue. No severe inflammatory response (necrosis, tissue degeneration or neutrophil infiltration) was observed around the CNT during the study. The CNT were observed in the intercellular and intracellular spaces one week after implantation. CNT were also observed in the lysosomes of the macrophages. The results suggest that CNT do not exhibit acute toxicity in subcutaneous tissue (Yokoyama *et al*., 2005). The doses administered were not specified.

5.2.4.1.2. Effects on the respiratory system

Shevedova *et al*. (2003, 2004b), in an *in vitro* study, reported that SWCNT caused a significant dose-response reduction of cell viability and oxidative stress biomarkers (e.g., antioxidant reserve), and a significant increase in lipid peroxides in human bronchial epithelial cells (0, 0.06, 0.12 and 0.24 mg/mL of SWCNT for 18 hours). At a concentration of 0.24 mg/mL, they detected iron in the cells and an increase in apoptosis. The authors mention that their results indicate that exposure to unrefined SWCNT can lead to an increase in pulmonary toxicity in exposed workers due to oxidative stress.

5.2.4.1.3. Liver effects

No data

5.2.4.1.4. Kidney effects

No data

5.2.4.1.5. Effects on the gastrointestinal system

No data

5.2.4.1.6. Effect on the heart and blood circulation

No data

5.2.4.1.7. Effects on the blood and the hematopoietic system

No data

5.2.4.1.8. Effects on the nervous system

No data

5.2.4.2. Immunological and allergic effects

Salvador-Morales *et al*. (2006) studied complement activation[3] by SWCNT and double-walled carbon nanotubes (DWCNT; normally 1-3.5 nm in diameter) in human serum (0.62-2.5 mg of nanotubes in 500 µL of serum). The exact dimensions of the nanotubes were not specified. Both types of carbon nanotubes activated the complement in a similar manner to zymosan used as a positive control (dose-dependent relationship). The authors conclude that complement activation by carbon nanotubes via the classical and alternate routes could promote inflammation and granuloma formation. The C1q protein binds directly to the carbon nanotubes and the proteins binding in the greatest quantity to the nanotubes are fibrinogen and apolipoproteins (AI, AIV and CIII).

5.2.4.3. Effects on reproduction and the reproductive system

No data

5.2.4.4. Development effects

No data

5.2.4.5. Genotoxic effects

Zheng *et al*. (2003) showed that single-stranded DNA (unspecified origin) can wind *in vitro* around a carbon nanotube of appropriate diameter and electrical properties. The consequences of such an interaction, particularly in the replication and transcription processes, still have to be studied.

Shvedova *et al*. (2004a) exposed human epidermal keratinocytes (HaCaT) and bronchial epithelial cells (BEAS-2B) to SWCNT. The authors observed damage in certain genes, including those involved in oxidative stress.

5.2.4.6. Carcinogenic effects

No data

5.2.4.7. Cellular and humoural effects

Shvedova *et al*. (2004a) exposed human epidermal keratinocytes (HaCaT) and bronchial epithelial cells (BEAS-2B) to SWCNT. The authors observed changes in cell ultrastructure and morphology, loss of cellular integrity and cellular apoptosis, as well as oxidative stress (free radical formation, accumulation of peroxidation products and decrease in antioxidants in the cells). The authors conclude that SWCNT exposure can trigger cutaneous and pulmonary toxicity and that oxidative stress is one of the potentially significant mechanisms involved in cell damage.

Cui *et al*. (2005) showed that SWCNT could inhibit cell proliferation, induce apoptosis and reduce adherence of human embryonic kidney cells *in vitro* (25, 50, 100 and 150 µg/mL, for 1 to 5 days).

Jia *et al*. (2005b) performed a comparative study of the cytotoxicity of SWCNT, MWCNT (10-20 nm in diameter) and the C_{60} fullerene on alveolar macrophages in Guinea pigs. No cytotoxicity was observed for fullerenes (up to a dose of 226 µg/cm^2). However, SWCNT showed higher toxicity than MWCNT. The dose of particles necessary to induce a reduction of macrophage phagocytosis was lower

[3] The complement is formed by a group of proteins present in the blood, which interact to defend the body against cells recognized as foreign (Salvador-Morales *et al*., 2006).

for SWCNT (0.38 μg/cm^2) than for MWCNT and fullerenes (3.06 μg/cm^2). SWCNT and MWCNT were more toxic than quartz, which is considered to be a major health risk during chronic inhalation exposure in the work environment (Jia *et al*., 2005b).

In a study by Cunningham *et al*. (2005) cited by Monteiro-Riviere and Inman (2006), the gene expression profile obtained after exposure of human epidermal keratinocytes to SWCNT (1.0 mg/mL) was similar to the profile obtained after exposure to quartz or silica.

Shvedova *et al*. (2005) exposed mouse macrophages (RAW 264.7) to SWCNT (0.1 mg/mL for 6 h). The authors observed production of TGF- β1 (growth factor involved in fibrogenesis) similar to the production induced by zymosan (stimulant of the alveolar macrophages used as positive controls; 0.25 mg/mL for 2 h). SWCNT did not trigger an oxidative response or nitric oxide (NO) production or cellular apoptosis. Production of TNF-α and IL-1β was more noticeable with zymosan. It seems that the macrophages did not actively phagocytate SWCNT.

Potapovich *et al*. (2005) also exposed macrophages (RAW 264.7) to SWCNT containing different proportions of iron. The authors observed phagocytosis of the SWCNT by the macrophages, a decrease in glutathione and an increase in the number of apoptotic cells. No intracellular production of superoxide (O_2^-) or NO was observed after exposure. The iron-rich SWCNT catalyzed conversion of extracellular O_2^- into hydroxyl radicals after activation of the macrophages and stimulated IL-6 production. The iron-deprived SWCNT stimulated TGF-β production. The authors point out that cell and tissue damage can be increased by synergy between the inflammatory response and the oxidizing potential of the iron present in SWCNT. Indeed, the inflammation caused by SWCNT provides an environment conducive to the oxidizing action of iron (Patapovich *et al*., 2005).

Muller *et al*. (2005) observed cytotoxicity (increase in LDH activity) and overproduction of proinflammatory cytokines (TNF-α) in rat peritoneal macrophages exposed *in vitro* to ground MWCNT. Carbon black and asbestos used as controls triggered the same effects.

Li *et al*. (2005) assessed the effect of CNT *in vitro* on human aortic endothelial cells. The authors observed an increase in the RNAm of MCP-1, VCAM-1 (genes involved in the inflammatory response in the heart) and IL-8 in the cells after 2 h of exposure. Oxidation of low-density lipoproteins (LDL) was also observed (dose-dependent relationship).

Ding *et al*. (2005) studied the toxicity mechanisms of MWCNT and multi-walled carbon nano-onions (MWCNO) in human skin fibroblasts (HSF42) *in vitro*. The cells were exposed to MWCNT (0.6 and 0.06 μg/mL) and MWCNO (6 and 0.6 μg/mL) for 24 or 48 h. Exposure of cells to MWCNT and MWCNO triggered stoppage of the cellular cycle and an increase in apoptosis and necrosis. Several intracellular signalling pathways were disrupted after exposure. MWCNT and MWCNO activated genes involved in cellular transport, metabolism, cellular cycle regulation and stress response. The authors also observed differences in the gene expression profile depending on the material tested and the dose. MWCNT introduced genes indicating a marked immune and inflammatory response in skin

fibroblasts while MWCNO had an effect on the genes involved in the response to external stimuli. It seems that MWCNT are more toxic than MWCNO and that the intracellular signalling pathways of interferon and p38/ERK-MAPK are involved in this greater toxicity.

Soto *et al*. (2005) studied the viability of mouse lung macrophages exposed to different nanomaterials. Chrysotile was used as a positive control and a relative cytoxicity index of 1 was assigned to it. The authors assigned the following relative cytotoxicity indices to the tested nanomaterials at a concentration of 5 μg/mL: 1.6 for Ag; ~ 0.4 for TiO_2; 0.7-0.9 for Fe_2O_3, Al_2O_3 and ZrO_2; 0.4 for Si_3N_4; 0.8 for carbon black and 0.9-1.1 for carbon nanotube aggregates (SWCNT and MWCNT). The size of the tested particles ranged from 3 to 150 nm (the length of the nanotubes could exceed 15 μm) and the aggregates formed had dimensions ranging from 25 nm to 20 μm. The authors point out that the cellular response to MWCNT aggregates was very similar to the response to chrysotile.

Monteiro-Riviere *et al*. (2005a) studied the toxicity of the surfactants used to prevent MWCNT agglomeration in the experimental studies. Human epidermal keratinocytes were exposed for 24 hours at different dilutions (0.1 to 10%) of Pluronic L61, L92 and F127 and Tween 20 or 60. Viability of the cells exposed to Pluronic F127 ranged from 27.1 to 98.5% while viability with the other surfactants was less than 10%. The decrease in viability was proportional to the concentration of surfactant. The authors then exposed keratinocytes to 0.4 mg/mL of MWCNT with or without Pluronic F127 (at 1 or 5%). MWCNT showed toxicity (increase in IL-8) independent of the presence or absence of surfactant, but toxicity diminished in the presence of Pluronic F127. Surfactant alone only triggered an increase in IL-8. The authors conclude that Pluronic F127 does not increase the MWCNT cytotoxicity despite better dispersion.

Murr *et al*. (2005) compared the toxicity of SWCNT (aggregates approximately 10 μm in diameter) and two types of MWCNT (aggregates 0.1 to 3 μm in diameter, length of 30 nm to 3 μm and length/diameter ratio of approximately 3 to >100) in mouse alveolar macrophages *in vitro*. Chrysotile (aggregates 0.5 to 15 μm in diameter, length of 0.5 to >15 μm and length/diameter ratio of 50 to >1500) and carbon black (aggregates 10-50 nm) were used for comparison. The cells were exposed to concentrations ranging from 0.005 to 10 μg/mL for 48 hours. All particles triggered cell death starting at 2.5 μg/mL and the cytotoxic response in terms of macrophage viability was similar to the response observed with chrysotile. Carbon black was slightly less toxic than nanotubes and chrysotile. Cell viability diminished according to the concentration. According to the authors, chrysotile and MWCNT cannot be differentiated from a morphological point of view.

Bottini *et al*. (2006) compared the toxicity of pristine MWCNT (diameter 20-40 nm and 1-5 μm long) and oxidized MWCNT in cultured human T lymphocytes. At a dose of 400 μg/mL (~10 million carbon nanotubes per cellule), oxidized MWCNT triggered the loss of 80% of the cells in 5 days. The two forms of MWCNT induced apoptosis of the T lymphocytes (time- and dose-dependent relationship), but the oxidized MWCNT turned out to be more toxic than the hydrophobic MWCNT. At the same concentration (400 μg/mL) carbon black (dimensions not specified) had a minimal effect on cell viability.

Kagan *et al*. (2006) exposed macrophages (RAW 264.7) to SWCNT containing different proportions of iron (average diameter 1-4 nm). No intracellular production of superoxide (O_2^-) or NO was observed after exposure to unpurified SWCNT (26% iron by weight; 950 m^2/g) or purified SWCNT (0.23% iron by weight; 1040 m^2/g) at concentrations of 0.12 and 0.5 mg/mL. Unpurified SWCNT catalyzed the conversion of extracellular O_2^- into hydroxyl radicals after activation of the macrophages (by zymosan). The authors also observed a decrease in glutathione and an accumulation of hydroperoxides (LOOH) in the activated macrophages (activated by zymosan or phorbol myristate acetate) and exposed to unpurified SWCNT.

Donaldson *et al*. (2006) report that the toxicity of SWCNT *in vitro* diminishes when their surface is modified. In a study by Kam *et al*. (2004) cited by Monteiro-Riviere and Inman (2006), SWCNT conjugated to streptavidin showed low toxicity in HL60 cells, but the SWCNT-biotin-streptavidin complex triggered cell death.

Sayes *et al*. (2006a) studied the toxicity of SWCNT exhibiting different functionalization densities (ratio between the number of carbon atoms and bound molecules) in human skin fibroblasts *in vitro*. The cells were exposed to SWCNT-phenyl-SO_3H, SWCNT-phenyl-$(COOH)_2$, SWCNT in 1% ethylene oxide and propylene oxide copolymer (Pluronic F108, dispersing agent) and SWCNT-phenyl-SO_3Na for 48 h at concentrations ranging from 3 μg/mL to 30 mg/mL. Cells exposed to a 1% Pluronic F108 solution served as controls. The authors observed that functionalized SWCNT were less toxic than SWCNT in 1% Pluronic F108. A 10% decrease in viability was observed in cells exposed to the 1% Pluronic F108 solution (controls). The toxicity of SWCNT diminishes according to their functionalization density.

Magrez *et al*. (2006) exposed three human lung tumour cell lines (H596, H446, Calu-1) to MWCNT (diameter 20 nm; length/diameter ratio 80-90), carbon nanotubes (diameter 150 nm; length/diameter ratio 30-40) and carbon black nanoparticles (nanometric size distribution; length/diameter ratio ~1). The authors observed a decrease in viability according to the concentration in the three types of cells exposed to MWCNT (0.002 to 0.2 μg/mL for 4 days). At all concentrations and for all the nanomaterials tested, carbon black showed the highest toxicity while MWCNT were the least toxic. The number of viable cells diminished according to the exposure dose for all nanomaterials. The authors point out that the filaments were less toxic than the particles in their study, while the opposite situation was observed in other studies (including Muller *et al*., 2005 and Bottini *et al*., 2006). Morphological changes indicating irreversible cell damage and cell death were also observed. Moreover, an increase in toxicity was observed after addition of carbonyl (C=O), carboxyl (COOH) and/or hydroxyl (OH) groups to the MWCNT surface. The authors conclude that the tested nanomaterials are toxic and that toxicity depends on size.

A proteomic analysis performed on human epidermal keratinocytes exposed to MWCNT showed a modification in the expression of several proteins, suggesting cellular cycle inhibition, among other phenomena (Monteiro-Riviere and Inman, 2006).

Fenoglio *et al*. (2006) studied production of free radicals by purified MWCNT (9.7 ± 2.1 nm in diameter; 5.9 ± 0.05 μm long; 378 m^2/g surface) in an aqueous

suspension. The results suggest that MWCNT do not generate free radicals in the presence of hydrogen peroxide (H_2O_2) or formiate. MWCNT showed a capacity to neutralize free radicals in the presence of an external source of hydroxyl (HO) or superoxide (O_2^-) radicals. The authors conclude that it is possible that the inflammatory reaction caused by exposure to purified MWCNT *in vivo* is attributable to properties other than free radical formation.

Fiorito *et al*. (2006a, 2006b) compared the toxicity of different types of SWCNT and C_{60} fullerenes in human and mouse (J 774) macrophages. Cells exposed to highly purified synthetic graphite served as controls. NO production by mouse macrophages was similar with LPS (lipopolysaccharide used as a positive control), pristine SWCNT (2% impurities by weight) and C_{60} fullerenes after 24, 48 and 72 h of incubation. NO production by cells exposed to graphite was significantly higher than by cells exposed only to LPS. All tested nanomaterials were phagocyted by human macrophages. Pristine SWCNT and C_{60} fullerenes induced apoptosis, cell death and metabolic changes in a lower proportion than graphite and unpurified or open SWCNT. The cytotoxic potential of pristine SWCNT was low and very similar to that of C_{60} fullerenes. According to the authors, the results suggest that the C_{60} fullerenes do not induce an inflammatory response in human cells *in vitro* and that they are not cytotoxic. The lowest inflammatory potential of C_{60} fullerenes and pristine SWCNT could be due to the small quantity of catalysts and graphite present in these nanomaterials (Fiorito *et al*., 2006a, b).

Raja *et al*. (2007) studied the impact of rat aortic smooth muscle cell exposure to single-walled carbon nanotubes (SWCNT) aggregated or not aggregated at concentrations of 0 to 0.1 mg/mL for terms up to 3.5 days. Filtered SWCNT, whether aggregated or not, shows a dose-dependent growth inhibition in relation to the control after 2.5 days of incubation. At equal concentrations, carbon black was less inhibiting than SWCNT.

Table 2 summarizes the principal information regarding the toxic effects assessed for carbon nanotubes.

Table 2. Documented health effects of carbon nanotubes [1]

Effect	SWCNT				MWCNT			
	Route				Route			
	Inhalation[2]	Cutaneous	Oral	Other	Inhalation[2]	Cutaneous	Oral	Other
Toxicokinetics			▲	▲■	▲			■
Irritation		■ ▲						
Systemic[3]								
Acute	▲			▲	▲			
Intermediate								
Chronic								
Neurological								
Immunological		■		■				
Development								
Reproductive								
Genotoxic				■				
Cancer								

[1] Existing human (■) or animal (▲) studies. Adopted from the ATSDR.

[2] Including intratracheal instillation.

[3] Systemic effects: acute (≤ 14 days), intermediate (15 to 364 days) and chronic (≥ 365 days).

6. HEALTH EFFECTS OF INORGANIC NANOPARTICLES

Insoluble inorganic nanoparticles can be composed of pure metals or various inorganic products or alloys. Only their nanometric dimensions distinguish them from the same products normally found on a larger scale. However, it is precisely because of their unique properties related to their nanometric scale that these particles are produced. At this scale, they display mechanical, electrical and other properties that do not exist when in larger dimensions.

Among the inorganic nanoparticles, TiO_2 has been studied abundantly. NIOSH (2005) published a preliminary version of its assessment of the health risks related to occupational TiO_2 exposure. The details of the assessment can be consulted in the NIOSH document (2005). To summarize, NIOSH recommends an exposure limit of 0.1 mg/m^3 for ultrafine TiO_2 particles, while the recommended limit for fine TiO_2 particles is 1.5 mg/m^3 (for exposure up to 10 h per day during a 40 h work week). This difference reflects the influence of specific surface in the toxicity of ultrafine particles (NIOSH, 2005). These recommendations should reduce the lung cancer risk to less than 1 in 1000 among workers exposed to TiO_2 (NIOSH, 2005).

6.1. Toxicokinetics

6.1.1. Absorption

In a study by Stearns *et al*. (1994) cited by Hervé-Bazin (2005), copper oxide particles administered by inhalation to hamsters (11 nm, 60 minutes) were found on and in the mucus and on the surface of the alveolar epithelium. The concentrations administered were not specified. The particles also penetrated the epithelial cells, the pulmonary interstitium and the macrophages, as well as the alveolar capillaries and the lymphatic vessels. Oberdörster *et al*. (2005b) point out that translocation to the blood is probable once the particles have reached the lymphatic ganglia.

In a study by Hatch *et al*. (1994) cited by Hervé-Bazin (2005), the study of alveolar macrophages in healthy volunteers (7 workers from an oil-fired power plant, 4 welders from that same plant and 3 university employees without known exposure) revealed the presence of ultrafine particles in the macrophage phagolysosomes of each subject. Metals such as cadmium, vanadium, titanium and iron were found. No link was observed between the presence of ultrafine particles in the macrophages and the subject's occupation. This means that exposure to ultrafine particles can occur outside the work environment (Hervé-Bazin, 2005). Another study performed on healthy non-smoking adults also revealed the presence of ultrafine particles in the alveolar macrophages (Witschger and Fabriès, 2005).

Hussain *et al.* (2001) showed cell capture of microparticulate substances by enterocytes, and their transport between cells. In some cases, the passage of microparticles from the intestinal lumen to the bloodstream led to distribution of substances in the body. Hillyer and Albrecht (2001) exposed this intestinal persorption phenomenon for unconjugated colloidal gold nanoparticles of 4, 10, 28 and 58 nm. In an ingestion study in mice, these researchers showed the capture of nanoparticles by the maturing enterocytes of the small intestine villosities. This effect was inversely proportional to the nanoparticle size.

Limbach *et al*. (2005) studied the absorption of cerium oxide nanoparticles (25-50 nm and 250-500 nm) in human lung fibroblasts *in vitro* (100 ng/g to 100 µg/g of fluid). The authors observed absorption of nanoparticles by the fibroblasts (linear increase in relation to the exposure time). Nanoparticles were found exclusively in agglomerate form and in vesicles within the cell cytoplasm. Small oxide nanoparticles agglomerate quickly in contact with the culture medium unless their surface is modified (Limbach *et al*., 2005). Larger nanoparticles were absorbed in greater quantity than smaller particles. The authors conclude that particle size is the dominant factor (indirectly due to agglomeration of small particles) which determines the absorption rate, while the concentration in number and the total surface are of minimal importance.

6.1.2. Distribution

6.1.2.1. Inhalation and airway exposure

After intranasal instillation, De Lorenzo (1970) reported translocation of 50 nm silver-coated colloidal gold nanoparticles along the axons to the olfactory bulb in monkeys. The average particle displacement speed was around 2.4 mm/h, with some particles appearing in the olfactory bulb 30 to 60 minutes after nasal inoculation. This was confirmed by Oberdorster *et al*. (2004). Wang *et al*. (2005) showed in mice that inhaled 20 and 200 nm titanium oxide particles can both be translocated to the mouse's brain by the olfactory nerve. The translocation speed depended on the size.

Oberdörster *et al.* (1994) published one of the first studies on the comparison of fine (250 nm) and ultrafine (20 nm) TiO_2 particles. In this inhalation study in rats, the authors observed greater pulmonary retention of ultrafine particles three months post-exposure. The concentrations administered were similar for fine and ultrafine particles (respectively 22.3 ± 4.2 and 23.5 ± 2.9 mg/m^3). A greater number of 20 nm particles were found in the lymphatic ganglia, a phenomenon indicating penetration of the interstitial spaces. The ultrafine pulmonary clearance time was lengthened and translocation of these particles in the pulmonary interstitium and the regional lymphatic nodules was significantly higher for the 20 nm particles. The specific surface is the parameter best correlated with the observed effects.

To study the distribution of iridium-192 (Ir^{192}) nanoparticles by inhalation in rats, Kreyling *et al.* (2002) ventilated the anesthetized animals and exposed them to 15 and 80 nm aerosols (at 2.5 µg/cm^{-3}). The thoracic fractions of the particles were 0.49 and 0.28 respectively. They observed radioactive iridium in the animals' liver, heart and brain. This phenomenon was ten times greater for 15 nm nanoparticles. Iridium nanoparticles are insoluble and were not absorbed in the intestine. The authors conclude that these nanoparticles are translocated to the organs, resulting in circulation of nanoparticles by the pulmonary blood vessels. Also using Ir^{192}, Semmler-Behnke *et al*. (2007) tracked the elimination of this substance from rat lungs after intratracheal inhalation over a six-month period. Immediately after inhalation, the authors found Ir^{192} nanoparticles in the bronchoalveolar lavage (BAL) fluids. Subsequently, nanoparticles were mainly associated with alveolar macrophages. After three weeks, the NP content of the BAL was no more than 6% of the pulmonary load, which contrasts strongly with the behaviour of the micrometric particles associated with alveolar macrophages.

Three weeks after inhalation, 80% of the retained Ir^{192} was translocated to the pulmonary epithelium and interstitium. The authors conclude that nanoparticles are phagocyted less efficiently than micrometric particles but are displaced efficiently from the lung surface to the interstitium. From these interstitial sites, they are eliminated in the larynx in the long term via the macrophages (Semmler-Behnke *et al*. 2007).

A study by Mortelmans and Nemery (2002) cited by Hervé-Bazin (2005) confirmed the passage into the human bloodstream with carbon particles (< 100 nm) marked with radioactive technetium. Radioactivity in the blood was maximal 10 to 20 minutes after inhalation and for about one hour. The same study revealed the liver as the principal organ accumulating these particles.

Oberdörster *et al.* (2002) studied the body distribution of 20 - 29 nm carbon-13 nanoparticles (insoluble) in an inhalation study in rats. The animals were placed in an exposure chamber at concentrations of 0, 80 and 170 $\mu g/cm^3$. No increase in concentration was observed in several animal organs (lungs, heart, brain, olfactory bulb and kidneys) up to 24 hours after exposure. However, the researchers observed a large accumulation of carbon-13 in the livers of both groups of animals, 18 and 24 hours after exposure. The authors explain the liver concentration by translocation of nanoparticles from the respiratory system to the circulatory system, and then to the liver.

In a longer-term inhalation study in rats, Oberdörster *et al.* (2004) studied the cerebral distribution of carbon-13 (insoluble). In the exposure chambers, the rats were exposed for 6 hours to concentrations of 0, 150 and 170 $\mu g/cm^3$, then sacrificed on days 1, 3, 5 and 7. The analysis of the animals' brain, the cerebellum and the olfactory bulbs showed significant capture in the exposed rats on day 1, which persisted only in the olfactory bulbs, extending to day 7. To explain cerebral capture of carbon-13, the authors postulate translocation from the lung to the bloodstream, and then passage through the hematoencephalic barrier. Transport from the respiratory zones to the olfactory bulbs and then translocation by axonal migration to the brain may have contributed to transport of nanoparticles.

Mills *et al*. (2006) studied translocation of carbon nanoparticles marked with technetium-99 (4-20 nm) in the blood of 10 healthy volunteers (inhalation of about 100 MBq of Technegas in 3 respirations). Radioactivity was detected immediately in the blood and reached a maximum after one hour. The authors observed 95.6 ± 1.7% retention of Technegas in the lungs after 6 h and no accumulation of radioactivity was detected in the liver or the spleen. The authors conclude that most of the carbon nanoparticles remained in the lungs up to 6 h after inhalation and these results do not support the hypothesis that carbon nanoparticles marked with technetium-99 are translocated rapidly into the circulatory system. A soluble species not linked to carbon nanoparticles (^{99m}Tc-pertechnetate) is said to be responsible for the increased reactivity in the blood (Mills *et al*., 2006).

6.1.2.2. Cutaneous exposure

Titanium dioxide (TiO_2) is a substance contained in sunscreens[4]. Lademann *et al.* (1999) did not observe significant absorption of coated TiO_2 nanocrystals (17 nm), beyond the corneal layer of the skin of human volunteers, except for a small quantity (< 1%), which had penetrated the hair follicles. Since the follicles are also isolated from living tissue by a corneal layer, the authors conclude that cutaneous absorption of TiO_2 is absent in living cutaneous tissues.

Schulz *et al.* (2002) did not observe cutaneous absorption of nanocrystalline TiO_2 in the skin layers below the corneum stratum in human skin, after testing the application of three formulations with different particulate characteristics (T805: 20 cubic nm; Eusolex T200: 10-15 cubic nm, agglomerating into needle-shaped 100 nm nanoparticles; Tioveil AQ-10P: 100 nm, in the form of coated needles of Al_2O_3 and SiO_2 and particulate forms of TiO_2; variable affinities for water and oil; coated or not). These results suggest a low probability of absorption of nanoparticulate TiO_2 beyond the dermis and its transport to the bloodstream.

6.1.2.3. Ingestion exposure

Hillyer and Albrecht (2001) reported blood and tissue distribution of ingested colloidal gold nanoparticles in mice. They noted absorption in the animals' brain, lungs, heart, kidneys, intestines, stomach, liver and spleen, more pronounced for 4 and 10 nm nanoparticles, in comparison with 28 and 58 nm particles.

6.1.2.4. Exposure by other routes

Paciotti *et al.* (2004) studied colloidal gold nanoparticles injected intravenously in mice in which they had implanted colon tumour cells. Nanoparticle distribution occurred preferentially at the tumour site, without significant accumulation in the liver, the spleen or the animals' other organs.

Hainfeld *et al.* (2004) showed that gold nanoparticles in solution, injected intravenously into mice with induced breast tumours, were found in the kidneys 5 minutes after injection (tumour/kidney ratio = 0.4) and then were located preferentially at the tumour site (tumour/healthy tissue ratio = 8) and, to a lesser degree, in the liver (tumour/liver ratio = 1.8).

The preliminary results of a study by Vyvyan Howard evoke the possibility that gold particles injected in gestating female rates are transferred to the fetus (Wootliff, 2004).

6.1.2.5. *In vitro*

No data

6.1.3. Metabolism

Jia *et al*. (2005a) compared the subchronic toxicity of selenium nanoparticles (Nano-Se; 20-60 nm) with sodium selenite (Na_2SeO_3) and proteins with a high selenium content in Sprague-Dawley rats of both sexes (0, 2, 3, 4 and 5 ppm of Se in the food for 13 weeks). The authors observed an increase in alanine aminotransferase (ALT) activity in rats exposed to Nano-Se (5 ppm of Se), to

[4] Researchers' focused their attention on sunscreens in the past few years, particularly due to the potential of some of their components to generate production of free radicals and changes in cellular DNA – and thus potentially cancer. This mainly would be linked to their photoinstability.

sodium selenite (4 and 5 ppm of Se) and the proteins with a high Se content (4 and 5 ppm of Se). An increase in aspartate aminotransferase (AST) activity was observed in rats exposed to proteins with a high Se content (5 ppm of Se). A decrease in total proteins and albumin was observed in rats exposed to sodium selenite (4 and 5 ppm of Se) and to proteins with a high Se content (5 ppm of Se).

6.1.4. Excretion

In their experiment with rat inhalation of radiomarked iridium particles, Kreyling *et al.* (2002) showed that nanoparticles were eliminated in the animals' feces without significant intestinal absorption.

6.2. Effects according to routes of exposure (administration)

6.2.1. Inhalation and airway exposure

The results of a case-control study performed by Robertson and Ingalls (1989) of factory workers of 7 carbon black producers in the United States indicate that carbon black exposure in the work environment does not increase the workers' risk of being affected by circulatory, malignant or respiratory disorders. The exposure level of workers affected by various disorders was no higher than among the disease-free controls (0.06-1.72 mg/m^3 depending on the position held and periods ranging from 7 to 202 months). Two controls were selected for each case according to age (± 2.5 years) and one of the two controls also had a length of employment in the carbon black industry comparable to the case (± 2.5 years).

6.2.1.1 Effects on the organs

6.2.1.1.1. Effects on the skin and the mucous membranes

No data

6.2.1.1.2. Effects on the respiratory system

In their inhalation experiment with rats, Oberdörster *et al.* (1994) and Ferin *et al*. (1992) observed a significant increase in inflammation signs or parameters during administration of 20 nm TiO_2 particles in comparison with the same mass of 250 nm particles. Until these studies performed by the same team, titanium oxide was considered to be a non-toxic dust and served as an inert control in several toxicological studies. Damage to the pulmonary epithelium, obstruction of Kohn's pores, development of sources of interstitial fibrosis and alteration of macrophage functions (inflammation mediators) were significantly greater. These results show that inert particles can become biologically active when nano-scaled.

Zhang *et al.* (2005b) report that Donaldson (2001) and his team had proved that nanoparticulate forms (< 50 nm) of titanium oxide, aluminium oxide and carbon black increased the pulmonary inflammation parameters 10 times more than administration of fine particles of the same products. Borm *et al*. (2004b), in a lung cancer journal, point out that low-solubility particles, such as carbon black and titanium oxide, are recognized to cause fibroses, neoplasic lesions and pulmonary tumours in rats. The quantity of these products required to generate the same effects is much smaller with nanoparticles.

Sayes *et al.* (2006b) exposed human skin fibroblasts and pulmonary epithelial cells with 10 nm spherical nanoparticles of anatase (a crystalline form of TiO_2), 5.2 nm spherical particles of rutile (another crystalline form of TiO_2) and 3.2 nm mixed anatase/rutile spherical particles with specific surfaces of 112 (rutile) to 153 m^2/g (anatase). Cytotoxicity and inflammation were observed starting at 100 µg/mL concentrations, according to classical dose-response behaviour, and the effects increased in relation to time and concentration. The effects observed were not correlated to the particle surface, since anatase (specific surface 153 m^2/g) is 100 times more toxic than rutile (specific surface 123 m^2/g) at equal concentrations. The most cytotoxic particles were the most efficient in generating reactive oxygen species and toxicity increased rapidly with ultraviolet illumination, a factor favouring free radical generation.

Warheit *et al*. (2006) studied acute toxicity of TiO_2 particles and rods in rats. Anatase rods (200 nm x 35 nm; 26 m^2/g surface) and anatase particles (~ 10 nm; 169 m^2/g surface) were administered by intratracheal instillation at doses of 1 or 5 mg/kg. Rutile particles (~ 300 nm; 6 m^2/g surface) served as negative controls while quartz was used as a positive control. The authors observed a transient inflammation and cell damage 24 h after exposure to the anatase rods and the anatase particles. The effects observed were no different than the pulmonary effects observed after exposure to the 300 nm TiO_2 particles, despite the substantial surface differences. Exposure to quartz particles triggered a dose-dependent inflammatory response characterized by an accumulation of neutrophils and macrophages, as well as a thickening of the lung tissue indicating development of a pulmonary fibrosis. The authors conclude that these results are in disagreement with the hypotheses that the specific surface is a major factor associated with the toxicity of nano-scaled particles, but they point out that the causes at the basis of this discrepancy are unclear.

Chen *et al.* (2006b) exposed adult male ICR mice to a single intratracheal dose (0.1 or 0.5 mg) of TiO_2 (19-21 nm). TiO_2 induces pulmonary emphysema, macrophage accumulation, type II pneumocyte hyperplasia and epithelial cell apoptosis. The authors also noted a differentiated expression of hundreds of genes, which, among other effects, disrupted the cellular cycle, caused apoptosis, modified the placental growth factor and led to the expression of certain chemokines, capable of causing pulmonary emphysema and alveolar epithelial cell apoptosis.

In another study, Warheit *et al*. (2007a) conclude that the surface properties are critical to determine the effects of ultrafine TiO_2 particles. By intratracheal instillation at doses of 1 to 5 mg/kg with one fine rutile particle sample, another ultrafine sample and a batch containing 80% anatase and 20% rutile with a quartz positive control, they exposed rats (5 rats per group, per dose and per time) and evaluated them 24 hours, one week and three months post-instillation. Exposure to quartz, and to a lesser degree to the anatase/rutile mixture (only for tests at high concentrations for the mixture) produced pulmonary inflammation, cytotoxicity and clearly different histopathological effects in the pulmonary tissues. However, the fine and ultrafine TiO_2 particles only had a transient inflammatory effect and only represent a low pulmonary risk. The authors conclude that the differences in effects are due to the crystalline structure, the pH of the particles and the surface chemical reactivity, since anatase/rutile particles produce more free radicals than rutile particles, which would represent one of the

sources of inflammation and cytotoxicity. The effects were not totally explicable only by the particle surface.

Grassian *et al.* (2007) exposed mice in an exposure chamber acutely (4 hours) and subacutely) (4 h/day for 10 days) to aggregated crystalline anatase (TiO_2) (elementary particles of 2 to 5 nm; average aggregates of 123 nm) to concentrations of 0.77 and 7.22 mg/m^3. Acute exposure produced minimal pulmonary toxicity or inflammation while subacute exposure led to a higher total cell and macrophage count in the bronchoalveolar fluid. The inflammatory response was moderate but significant. The mice recovered three weeks after exposure.

By intratracheal instillation of 14 nm spherical particles of colloidal silica in mice at concentrations up to 100 µg, Kaewamatawong *et al.* (2006) measured moderate to severe acute pulmonary inflammation at 30 or 100 µg accompanied by tissue damage. The total number of bronchoalveolar fluid cells increased up to 15 days and then returned to a level comparable with the controls after 30 days. The authors noticed a thickening of the alveolar septum walls and some interstitial fibroses after 30 days and conclude that oxidative stress and cell apoptosis can induce the observed tissue impairments.

Warheit *et al*. (2007b) exposed rats (5 rats per group, per dose and per time) by intratracheal instillation to 12, 50, 300 and 500 nm quartz particles, which had specific surfaces of 4.2 to 90 m^2/g, and measured the hemolytic potential and surface activity of the particles. After 24 hours, one week, one month and three months, they evaluated the cell proliferation and performed a histopathological assessment of the lung tissue. Exposure to the different quartz particles produced a sustained and dose-dependent inflammatory response, accumulation of alveolar macrophages with early development of pulmonary fibrosis and cytotoxic effects. The observed effects are not always linked to the particle surface but are well correlated to the hemolytic surface activity of the different particles. The results obtained suggest that the intensity of the measured effects correlates better with the hemolytic surface activity of the *in vitro* test than with surface area, size, crystallinity, metal content (iron) or surface radicals.

Zhang *et al.* (2000) studied the effect in rats of intratracheal instillation of the nanoparticulate form of cobalt (20 nm), in comparison with the administration of 5 µm cobalt particles 1, 3, 7, 15 and 30 days after exposure. The authors observed much greater signs of pulmonary inflammation with the nanometric fraction. Analysis of bronchoalveolar lavage fluid revealed an increase in pulmonary permeability and inflammation (increase in neutrophils and proteins, increase in LDH activity). Cytokines indicating an inflammatory reaction modulated by macrophages or monocytes were also present with the two forms of cobalt, but in greater quantity and on a more sustained basis after administration of the nanometric fraction.

In a study by Stearns *et al*. (1994) cited by Hervé-Bazin (2005), exposure of hamsters to ultrafine particles of CuO, Cu_2O and $Cu(OH)_2$ (11 nm, 60 minutes) triggered an increase (factor of 4) in pulmonary resistance for 24 h. Signs of inflammation, particularly the presence of neutrophils and eosinophils, were also observed.

Zhang *et al.* (2003) studied the effect in rats of intratracheal instillation of the ultrafine form of nickel (20 nm) at doses of 0 to 5 mg in comparison with the

administration of 5 μm nickel particles 1, 3, 7, 15 and 30 days after exposure. The authors report indications of pulmonary inflammation and damage for both nickel sizes and the same intensity of effects for 1 and 5 mg of ultrafine nickel. The effects were much more noticeable for ultrafine nickel, particularly in the release of proinflammatory cytokines for 30 days post-exposure. They also report that the number of neutrophils and LDH activity in the bronchoalveolar lavage fluids were much more noticeable for ultrafine nickel than for micrometric nickel.

In a study by Roth *et al*. (1994) cited by Hervé-Bazin (2005), exposure of 3 healthy men (volunteers) to ultrafine iridium particles marked with indium (average diameter of 18 nm) for 2 or 3 respiratory cycles triggered a slowing of pulmonary clearance up to 8 days after exposure. The concentrations were not specified. Larger particles (> 2 μm) have less persistence in the lungs (Hervé-Bazin, 2005). The presence of ultrafine particles in the lungs was also associated with a decrease in forced expiratory volume (Witschger and Fabriès, 2005).

The bactericidal capacity of the lungs was reduced in Guinea pigs and mice exposed to respective concentrations of 15 and 1.5 mg/m^3 of carbon black (Smith and Musch, 1982). The exposure times were not specified.

Studies conducted with workers showed that inhalation of carbon black can foster the development of chronic bronchitis and a slight decrease in pulmonary function (IARC, 1996, 2006a). However, IARC (1996, 2006a) points out that these effects can be attributed to non-specific caused by exposure to dusts. A reaction in the fibrous tissue was also observed around the carbon black deposits in the pulmonary parenchyma (IARC, 1996, 2006a).

Exposure of rats to carbon black at doses greater than 0.5-1 mg/g of lung triggers a slowing of clearance, which translates into an accumulation of particles in the lungs (IARC, 1996, 2006a). An inflammatory response is then observed and develops into active chronic inflammation. An increase in collagen deposition (coming from fibroblast proliferation) and epithelial cell proliferation, as well as metaplasia, were observed in rats subjected to a high dose of carbon black (IARC, 1996, 2006a). No details were provided on the carbon black used.

In a subchronic study by Driscoll *et al*. (1996), rats were exposed to concentrations of 1.1, 7.1 and 52.8 mg/m^3 of carbon black for 6 hours per day, 5 days a week for 13 weeks. After 13 weeks of exposure, the concentrations of 1.1, 7.1 and 52.8 mg/m^3 corresponded respectively to depositions of 354, 1826 and 7861 μg of carbon black in the rats' lungs. Exposure of the rats to 7.1 and 52.8 mg/m^3 triggered a slowing of clearance, pulmonary tissue damage and inflammation, an increase in chemokine expression (MIP-2 and MCP-1), epithelial hyperplasia and fibrosis. The effects were more pronounced at the highest concentration and no effect was observed at 1.1 mg/m^3.

Li *et al*. (1999) exposed rats to fine (260 nm) and ultrafine (14 nm) carbon black particles by intratracheal instillation (single dose of 125 μg). The authors observed an alveolitis (increase in neutrophils) 6 hours after exposure to the ultrafine particles. The fine particles had a similar effect on the phosphate buffered saline (PBS) used for the controls. An increase in LDH and proteins in the bronchoalveolar lavage fluid and a decrease in glutathione in the pulmonary tissues were also observed with the ultrafine particles. The fine particles had a less noticeable effect on the increase in proteins and the decrease in glutathione. The increase in neutrophils was still observable 7 days after exposure. TNF

production by the leukocytes increased gradually after instillation and NO production remained high up to 7 days.

Brown *et al*. (2000) studied the role of the transition metals in the effects observed after exposure to carbon black. Female Sprague Dawley rats were exposed to fine (320 nm) and ultrafine (14 nm) carbon black particles by intratracheal instillation (single dose of 500 μg). The authors observed an increase (8 times) in the number of neutrophils in the bronchoalveolar lavage fluid with ultrafine carbon black particles 24 hours after exposure. The inflammation caused by fine particles was the same as in the controls. Treatment of ultrafine particles with a chelator (desferal) had no influence on the number of neutrophils. The product of leaching of 1 mg of particles did not trigger any significant inflammation in rat lungs. The authors conclude that inflammation caused by ultrafine carbon black particles is not attributable to the presence of transition metals or other soluble elements released by the particles.

Inoue *et al*. (2005) exposed ICR mice to carbon black nanoparticles (14 and 56 nm; 50 μg/week), ovalbumin (antigen; 1 μg/2 weeks) and a combination of ovalbumin and carbon black (according to the same respective protocols) by intratracheal instillation once a week for 6 weeks. The 14 and 56 nm nanoparticles triggered an aggravation of allergic inflammation in the airway (infiltration of eosinophils, neutrophils and mononuclears and increase in the number of goblet cells in the bronchial epithelium). Simultaneous exposure to the nanoparticles and the antigen triggered an increase in expression of cytokines (IL-5, IL-6, IL-13) and chemokines (eotaxin, MCP-1 and RANTES[5]) in the lungs while administration of the antigen alone did not have this effect. The production of an oxidative stress marker (8-OHdG) was more pronounced with the combination of the nanoparticles and the antigen than with the nanoparticles or the antigen administered separately. In general, the effects were more noticeable with 14 nm nanoparticles than with 56 nm nanoparticles. Finally, the 14 nm particles had an adjuvant effect on the total IgE immunoglobulins (involved in allergic reactions) and on the IgG_1 specific immunoglobulins (involved in the defence against infection) and IgE. The authors conclude that nanoparticles can aggravate allergic inflammation in the airway and that this effect can be caused in part by the local increase of IL-5 and eotaxin (involved in recruiting eosinophils) and by the expression of IL-13, RANTES, MCP-1 and IL-6.

Several recent studies discussed the toxicity of nano-scaled carbon black and will not be set out in detail here, because they are normally considered to be ultrafine particles, not nanoparticles (Frampton *et al*., 2004). Among others, there is the study by Yamamoto *et al*. (2006) by intratracheal instillation in mice, which concludes that there is greater pulmonary inflammation with smaller carbon black particles. The effects are correlated to the particle surface, as in the studies by Stoeger *et al*. (2006) and Koite *et al*. (2006). Apoptosis and pulmonary epithelial cell proliferation can also be caused by carbon black (Stoeger *et al.,* 2006; Sydlik *et al.,* 2006).

[5] RANTES (*Regulation upon activation, normal T expressed and secreted*) is a cytokine, which induces chemotactism of monocytes and memory T cells (glossary of the CSST occupational asthma site, http://www.asthme.csst.qc.ca/Document/Info_Med/Formation/ApLatence/Latence11.html).

6.2.1.1.3. Liver effects

No data

6.2.1.1.4. Kidney effects

No data

6.2.1.1.5. Effects on the gastrointestinal system

No data

6.2.1.1.6. Effects on the heart and blood circulation

Exposure of monkeys to carbon black (53.0 mg/m$^{3;}$ 1000-10000 hours) triggered minimal changes in the electrocardiogram and low ventricular hypertrophy (Smith and Musch, 1982).

Harder *et al*. (2005) observed a slight increase in cardiac rhythm (4.8%) and a decrease in variability of cardiac rhythm in rats after pulmonary deposition of ultrafine carbon particles (38 nm, 180 µg/m^3, 24 h). According to the authors, these changes are related to an autonomic nervous system imbalance caused by activation of a pulmonary receptor.

6.2.1.1.7. Effects on the blood and the hematopoietic system

No data

6.2.1.1.8. Effects on the nervous system

Pisanic *et al.* (2007) exposed rat PC12 neuronal cells (20 000 cells/mL) to Fe_2O_3 nanoparticles (0.15 to 15 mM) and observed internalization of NP, mainly in the cytoplasm and the endosomes and an accumulation in the perinuclear region. They also noted a decrease in dose-dependent cell viability and a lowering of the cells' capacity to develop neurites. They conclude that Fe_2O_3 exposure reduces the capacity of PC12 cells to respond appropriately to the nerve growth factor.

6.2.1.2. Immunological and allergic effects

No data

6.2.1.3. Effects on reproduction

No data

6.2.1.4. Development effects

No data

6.2.1.5. Genotoxic effects

A significant increase in the frequency of hprt gene mutation was observed in rat alveolar epithelial cells after 13 weeks of exposure to 7.1 and 52.8 mg/m^3 of carbon black (Driscoll *et al*., 1996). The same effect was observed 3 and 8 months after the end of exposure in rats exposed to 52.8 mg/m^3.

Contrary to the extracts obtained with organic solvents, intact carbon black particles did not show any mutagenic activity (Watson et Valberg, 2001). Carbon black does not show mutagenic potential in most of the studies (IARC, 1996, 2006a).

6.2.1.6. Carcinogenic effects

Exposure of mice or monkeys (56.5-84.7 mg/m^3) and of Guinea pigs, hamsters, mice or primates (53.0-113.1 mg/m^3) to carbon black did not trigger any

carcinogenic effect (Smith and Musch, 1982). The exposure times were not specified.

A study of female mice exposed to carbon black by inhalation did not show an increase in the incidence of airway tumours (IARC, 1996, 2006a). However, studies in which rats were exposed to carbon black by inhalation or intratracheal instillation revealed a significant increase in the incidence of malignant pulmonary tumours in females (IARC 1996, 2006a). An increase in the incidence of benign lesions was also observed.

According to Borm *et al*. (2005), the overload of the body's defence mechanisms resulting from a chronic inflammation explains the carcinogenic effect of carbon black in rats. The results obtained by Driscoll *et al*. (1996) support this hypothesis, because the mutagenic effects were observed only at concentrations causing inflammation and hyperplasia.

A study conducted in the United Kingdom revealed excess mortality due to lung cancer (SMR of 1.5) in a cohort of workers assigned to carbon black production (IARC, 1996, 2006a). In a control-case study conducted in Canada (Montreal), in which exposure to several substances was assessed in a population sample, an excess risk of esophageal, kidney and lung cancer was observed in relation to carbon black exposure (IARC, 1996, 2006a). No excess risk was observed for the following sites: stomach, colon, rectum, pancreas, prostate, bladder, melanoma and non-Hodgkin's lymphoma.

IARC (1996, 2006a) considers that carbon black may be carcinogenic for humans (group 2B), even though the evidence is currently insufficient. According to Watson and Valberg (2001), an increased risk of lung cancer could not be proved in workers exposed to carbon black during its production.

Epidemiological studies did not show an increased risk of morbidity or mortality linked to lung cancer in workers assigned to TiO_2 production (NIOSH, 2005). No study has been performed concerning workers handling or using TiO_2. NIOSH considers that there is currently insufficient evidence to designate TiO_2 as a potential carcinogenic agent. IARC (2006b) considers that the evidence of TiO_2 carcinogenicity in humans is insufficient, but that it is sufficient in animals. IARC (2006b) thus considers that TiO_2 is potentially carcinogenic for humans.

6.2.1.7. Cellular and humoural effects

In an experiment conducted by intratracheal instillation in rats, Hohr *et al.* (2002) observed an increase in pulmonary neutrophils, early parameters of inflammation, for 20-30 nm TiO_2 nanoparticles. This effect was not as significant with administration of 180 nm particles. Coating by methylation to render the particles hydrophobic, and thus less soluble, slightly reduced neutrophil production for the 2 particulate dimensions of TiO_2 when the doses were 1 mg, but had little impact on 6 mg doses. The authors conclude that particle surface is the determining factor in pulmonary inflammation, while methylation coating played a marginal role in the inflammation parameters.

6.2.2. Cutaneous exposure

6.2.2.1. Effects on the organs

Acticoat™ is a product consisting of a nylon/polyester mesh, trapping polyethylene and including a silver nanocrystal layer. This product has been used for several years to accelerate healing of wounds and reduce bacterial colonization. In the presence of moisture, the product releases ions and silver radicals that would be responsible for antibacterial action. In an *in vitro* study of cultured human keratinocytes, Lam *et al.* (2004b) observed a substantial decrease in cell viability (0 to 9% cell viability after 30 minutes of incubation) and conclude cytotoxicity of silver nanocrystals (0.005 – 0.01% of silver) released by Acticoat™. Poon and Burd (2004), in an *in vitro* study of human fibroblasts and keratinocytes, observed an LD_{100} at low concentrations, comparable to the therapeutic concentrations ($7 – 55 \times 10^{-4}$%).

Cross *et al*. (2007) conclude that less than 0.03% of the applied zinc oxide nanoparticle used in sunscreen formulations penetrated the human epidermis after 24 hours of exposure.

6.2.2.2. Immunological and allergic effects

No data

6.2.2.3. Effects on reproduction and the reproductive system

No data

6.2.2.4. Development effects

No data

6.2.2.5. Genotoxic effects

Bergeron and Archambault (2005) report the results of two studies in which it was shown that the TiO_2 and ZnO contained in sunscreens damaged DNA by free radical production in skin cells.

6.2.2.6. Carcinogenic effects

No effect of carbon black on skin cancer was observed in a control-case study conducted with rubber industry workers (IARC, 1996, 2006a).

No carcinogenic effect was observed following cutaneous application of carbon black in mice (IARC, 1996, 2006a).

6.2.2.7. Cellular and humoural effects

No data

6.2.3. Ingestion exposure

Jia *et al*. (2005a) observed a more pronounced toxicity with sodium selenite and proteins with a high Se content than with Nano-Se. The authors determined a no-observed-adverse-effect-level (NOAEL) dose of 3 ppm for Nano-Se, which corresponds to doses of 0.22 and 0.33 mg/kg of body weight per day for males and females respectively. For sodium selenite and proteins with a high Se content, the authors determined a NOAEL of 2 ppm, which corresponds to doses of 0.14 and 0.20 mg/kg body weight/day for males and females respectively. In their study, the authors conclude that Nano-Se is less toxic than sodium selenite and proteins with a high Se content. A study cited by Jia *et al*. (2005a) showed that Nano-Se (LD_{50}: 113 mg/kg body weight) has an acute toxicity level 7 times lower than sodium selenite (LD_{50}: 15 mg/kg bw) in mice and that their bioavailability is similar in rats. Jia *et al*. (2005a) also observed a decrease in

body weight in rats exposed to Nano-Se (20-60 nm), sodium selenite and proteins with a high Se content at concentrations of 4 and 5 ppm of Se.

Zhang *et al.* (2005a) observed a lower incidence of retarded growth in mice, after ingestion of the nanoparticulate form of selenium (Nano-Se), compared to animals receiving non-nanoparticulate sodium selenite.

Chen *et al*. (2006a) studied the acute effects of administration of copper nanoparticles by gavage in mice. They compared the effects of nanoparticles (23.5 nm; 108 to 1080 mg/kg) and microparticles (17 μm; 500 to 5000 mg/kg) of copper metal and copper ions ($CuCl_2 \cdot 2H_2O$; 24 to 237 mg/kg) with purity over 99.9%. The specific surface of the nanoparticles and microparticles was 2.95×10^5 and 3.99×10^2 cm^2/g respectively. The number of particles present in 1 μg of matter was 1.7×10^{10} for nanoparticles and 44 for microparticles. 90 mice of both sexes were examined for morphological and pathological changes and 90 other were used for biochemical blood tests. The authors found LD_{50} of 110, 413 and >5000 mg/kg for ions, nanoparticles and microparticles respectively. Copper nanoparticles and ions thus are classified as moderately toxic, while microparticles are classified as practically non-toxic (classes 3 and 5 respectively on the Hodge and Sterner scale) (Chen *et al*., 2006a). The toxic effects of copper nanoparticles were more pronounced in males than in females exposed to the same mass of particles.

Wang *et al*. (2006) studied the acute toxicity of zinc powder nanoparticles by gastrointestinal administration in CD-ICR mice of both sexes. The authors administered a dose of 5 g/kg by body weight of nanoparticles (58 ± 16 nm) and microparticles (1.08 ± 0.25 μm) with purity over 99.99%. A group of 20 mice to which sodium carboxymethyl cellulose were administered served as controls. The mice to which zinc nanoparticles were administered showed severe symptoms of lethargy and anorexia, vomiting and diarrhea in the first days of treatment. The death of two mice (attributable to an intestinal obstruction caused by aggregation of zinc nanoparticles) occurred in the first week (days 2 and 6). A slight lethargy but no deaths were observed in the mice receiving the zinc microparticles. Over the first three days, the authors observed a 22% reduction in weight gain among mice exposed to nanoparticles compared to the controls. No weight difference between the controls and the mice exposed to microparticles was observed in the same period. However, the mice exposed to nanoparticles regained their weight 6 days after treatment.

6.2.3.1. Effects on the organs

Jia *et al*. (2005a) observed an increase in the relative weight of the organs (for example, liver, spleen, brain) in rats exposed to Nano-Se (20-60 nm; 5 ppm), to sodium selenite (4 and 5 ppm) and to proteins with a high Se content (3, 4 and 5 ppm).

Chen *et al*. (2006a) observed a severe atrophy (at doses ranging from 232 to 1080 mg/kg) and a change in spleen colour in mice exposed to metallic copper nanoparticles (23.5 nm). The authors also observed an atrophy of the splenic units, lymphocyte reduction and fibrosis in the interstitium of the spleen. Chen *et al*. (2006a) conclude that the spleen is one of the target organs for metallic copper nanoparticles. The spleen of mice exposed to microparticles (17 μm; 1077 mg/kg) was slightly different from that of mice used as controls and

exposed to a solution containing 1% hydroxypropylmethylcellulose K4M (HPMC). The authors also observed a black coloration of the gall bladder in certain mice exposed to nanoparticles. In these mice, several organs, including the kidneys, the spleen and the liver suffered major damage (at all doses) and the severity of the effects depended on the dose administered. The kidneys and the liver are also target organs for metallic copper nanoparticles.

Chen *et al*. (2006a) also measured certain biochemical parameters that reflect the hepatic and renal functions in the blood of mice exposed to metallic copper nanoparticles (23.5 nm; 108-736 mg/kg). All the parameters measured (blood urea nitrogen, creatinine, total bile acid and alkaline phosphatase) were significantly higher than in the controls. No measurement was taken at the highest dose (1080 mg/kg), given that all the animals died within 72 h.

6.2.3.1.1. Effects on the skin and the mucous membranes

No data

6.2.3.1.2. Effects on the respiratory system

No data

6.2.3.1.3. Liver effects

Jia *et al*. (2005a) observed growth inhibition and degeneration of hepatic cells in mice exposed to sodium selenite and to proteins with a high Se content at a concentration of 3 ppm of Se. Nano-Se (20-60 nm) had no effect at this dose and had a significantly lower effect than sodium selenite and proteins with a high Se content at doses of 4 and 5 ppm of Se.

Zhang *et al.* (2005a) observed less hepatic function alterations in mice that ingested selenium nanoparticles (Nano-Se; 20-60 nm; 2 and 4 mg/kg for 15 days), compared to those to which non-nanoparticulate sodium selenite had been administered.

Chen *et al*. (2006a) observed steatosis around the central veins of the hepatic tissues in mice exposed to metallic copper nanoparticles (23.5 nm; 341 mg/kg).

Mouse livers exposed to zinc nanoparticles (58 nm) or microparticles (1.08 μm) in the study by Wang *et al*. (2006) exhibited edema, hydropic degeneration and a slight necrosis of the hepatocytes around the central vein. An increase in certain biochemical parameters (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and LDH) measured in the blood also indicated liver damage induced by microparticles and nanoparticles. In general, zinc microparticles induced more severe liver damage than nanoparticles.

6.2.3.1.4. Kidney effects

Jia *et al*. (2005a) observed a necrosis of the epithelial cells in the medullar zone of the kidneys in 4 of 24 rats exposed to sodium selenite and to proteins with a high Se content at a concentration of 5 ppm of Se. No pathological change was observed in rats exposed to Nano-Se.

Chen *et al*. (2006a) observed a substantial kidney colour change in mice exposed to metallic copper nanoparticles (23.5 nm; 1080 mg/kg). Damage to the proximal tubule cells and signs of glomerulonephritis (swelling of the glomeruli,

for example) were also observed. At a dose of 341 mg/kg, the authors observed degeneration of the epithelial cells of the proximal convoluted tubule and a dose of 1080 mg/kg triggered massive and irreversible necrobiosis of these same cells. The nuclei of the epithelial cells of the renal tubule became less and less visible (starting at 341 mg/kg) and nearly disappeared at 1080 mg/kg. Finally, protein fluid (indicating a disorder of the protein metabolism) containing a purple deposition (sign of precipitation of copper salts) was found in the renal tubules of mice exposed to doses ranging from 341 to 1080 mg/kg. The kidneys of mice exposed to microparticles (17 μm; 1077 mg/kg) were similar to those of the controls.

Wang *et al*. (2006) observed a slight swelling of the glomeruli in the kidneys of mice exposed to zinc nanoparticles or microparticles. Dilation of the renal tubules and the presence of protein moulds were observed only in mice exposed to nanoparticles. An increase in certain biochemical blood parameters (blood urea nitrogen and creatinine) in mice exposed to microparticles suggested a renal dysfunction consecutive to exposure. Nanoparticles induced more severe kidney damage than microparticles despite the absence of changes in the blood parameters following exposure to nanoparticles. The elevation of blood urea nitrogen in mice exposed to microparticles could be caused in part by a hepatic dysfunction (Wang *et al*., 2006).

6.2.3.1.5. Effects on the gastrointestinal system

A slight inflammation of the stomach and the intestine was observed in mice exposed to zinc nanoparticles and microparticles in the study by Wang *et al*. (2005). No significant pathological change was observed in the other organs.

All the mice that received metallic copper nanoparticles in the study by Chen *et al*. (2006a) exhibited gastrointestinal disorders (appetite loss, diarrhea, vomiting) contrary to those that received microparticles (in the same mass concentrations). Some mice that received nanoparticles or ions also exhibited various symptoms, such as tremors or hypopnea.

6.2.3.1.6. Effects on the heart and blood circulation

Fatty degeneration was observed in the cardiovascular cells of the cardiac tissues in mice exposed to zinc nanoparticles in the study by Wang *et al*. (2006). Such degeneration can be caused by anemia. No histopathological alteration of the cardiac tissues was observed following exposure to microparticles. An increase in certain biochemical blood parameters (creatinine kinase, aspartate aminotransferase, LDH and hydroxybutyrate dehydrogenase) in mice exposed to nanoparticles or microparticles also indicates cardiac impairment.

6.2.3.1.7. Effects on the blood and the hematopoietic system

Jia *et al*. (2005a) observed a decrease in certain hematological parameters (hemoglobin, erythrocytes and platelets) in rats exposed to Nano-Se (5 ppm of Se), to sodium selenite (4 and 5 ppm of Se) and to proteins with a high Se content (4 and 5 ppm of Se).

Wang *et al*. (2006) observed an increase in blood platelets and the red blood cell distribution index, as well as a decrease in hemoglobin and haematocrit in mice exposed to zinc nanoparticles (58 nm) and microparticles (1.08 μm) compared to

the controls. The decrease in hemoglobin and haematocrit was more noticeable with nanoparticles than with microparticles. This indicates that nanoparticles triggered more severe anemia than microparticles. No significant difference was observed in certain coagulation parameters (prothrombin time, fibrinogen and activated partial thromboplastin time) following exposure to nanoparticles or microparticles.

6.2.3.1.8. Effects on the nervous system

No data

6.2.3.2. Immunological and allergic effects

No data

6.2.3.3. Effects on reproduction

No data

6.2.3.4. Development effects

No data

6.2.3.5. Genotoxic effects

No data

6.2.3.6. Carcinogenic effects

No satisfactory study of the carcinogenic potential of carbon black administered by ingestion could be identified in the literature.

6.2.3.7. Cellular and humoural effects

No data

6.2.4. Exposure by other routes

6.2.4.1. Effects on the organs

6.2.4.1.1. Effects on the skin and the mucous membranes

No data

6.2.4.1.2. Effects on the respiratory system

No data

6.2.4.1.3. Liver effects

No data

6.2.4.1.4. Kidney effects

No data

6.2.4.1.5. Effects on the gastrointestinal system

In an analysis of human histological specimens including control cases, Gatti (2004) showed a correlation of the presence of microparticles or nanoparticles with colon cancer and Cröhn's disease, an inflammatory bowel disease. The composition of the inclusions in the intestinal tissues was varied and the author postulates a possible association with ceramics or other dental products, prosthetic alloys, food pollutants or previous exposure to barium-based colourings.

6.2.4.1.6. Effects on the heart and blood circulation

Exposure of human aortic endothelial cells to Fe_2O_3, Y_2O_3 and ZnO at concentrations of 0.001 to 50 µg/mL led to their dose-dependent cellular internalization for the three metallic oxides (Gojova *et al*., 2007). Iron oxide does not trigger an inflammatory response at any of the concentrations tested. Yttrium and zinc oxides trigger a strong inflammatory response at a concentration over 10 µg/mL. At the maximum concentration, zinc oxide nanoparticles are cytotoxic and lead to a high proportion of cell deaths (Gojova *et al*., 2007).

6.2.4.1.7. Effects on the blood and the hematopoietic system

In an experiment intended to assess the blood compatibility of various forms of titanium dioxide (TiO_2), Maitz *et al.* (2003) did not observe any effects of the nanocrystalline form on several parameters of platelet-rich human plasma (platelet aggregation and coagulation time).

6.2.4.1.8. Effects on the nervous system

No data

6.2.4.2. Immunological and allergic effects

No data

6.2.4.3. Effects on reproduction and the reproductive system

Braydich-Stolle *et al*. (2005) studied the toxicity of Ag nanoparticles of different diameters on the cells of the male mouse germ line (C18-4). The authors did not observe any cytotoxicity for nanoparticles of 55, 80 and 130 nm, but cytotoxicity was observed with nanoparticles of 25 nm (EC_{50}[6]: 100 µg/mL) and 30 nm (EC_{50}: 25 µg/mL). The authors also observed a loss of LDH by the membrane of the cells exposed to nanoparticles of 25 nm (5 µg/mL) and 30-80 nm (10 µg/mL). In addition, the authors observed a decrease in activity of Fyn kinase (member of the Src cytoplasmic kinase family involved in the proliferation of normal strain spermatogonia) with nanoparticles larger than 30 nm. According to Braydich-Stolle *et al*. (2005), these results suggest that nanoparticle size plays a role in the toxicity mechanism.

6.2.4.4. Development effects

No data

6.2.4.5. Genotoxic effects

Rahman *et al*. (2002) studied the potential of TiO_2 particles (≤ 20 and > 200 nm) to induce chromosomal changes (signalled by presence of micronuclei) in golden hamster embryonic cells. The authors observed a significant increase in induction of micronuclei in cells exposed to particles 20 nm and smaller (1.0 µg/cm^2) after 12, 24, 48, 66 and 72 h of exposure. The authors also observed apoptosis of the cells.

6.2.4.6. Carcinogenic effects

The one-year survival rate of mice with induced breast tumours in the Hainfeld *et al.* (2004) experiment was high (86%), even at the maximum dose of gold

[6] The effective concentration 50 (EC_{50}) is the concentration which causes toxicity in 50% of the cells.

nanoparticles (concentration of 270 mg/cc) administered intravenously for therapeutic purposes before radiotherapy. These results give reason to believe in the low toxicity of this type of formulation.

Sarcomas were observed in male and female mice after subcutaneous injection of carbon black containing an appreciable quantity of polycyclic aromatic hydrocarbons (PAH) (IARC, 1996, 2006a). Subcutaneous injection of carbon black in which PAH were not detected did not trigger sarcomas.

6.2.4.7. Cellular and humoural effects

Stone *et al*. (2000) observed an increase (1.6 times) in the cytosolic calcium (Ca^{2+}) at rest in monocytic cells (Monomac 6) exposed to ultrafine carbon black particles (14 nm; 66 μg/mL). Exposure to fine carbon black particles (260 nm) did not have this effect. Calcium is involved in activation of proinflammatory transcription factor (such as NF-kB) (Brown *et al*., 2000). According to Stone *et al*. (2000), ultrafine carbon black particles could partially activate the opening of Ca^{2+} channels by a mechanism involving reactive oxygen species and thus allow extracellular Ca^{2+} to enter the cells. The study by Brown *et al*. (2000) confirmed the results obtained by Stone *et al*. (2000).

Wagner *et al*. (2001) exposed rat alveolar macrophages to aluminium oxide nanoparticles (Al_2O_3; 30 and 40 nm) and pristine aluminium nanoparticles (Al; 50, 80 and 120 nm). Toxicity was observed 96 and 144 h after exposure to Al_2O_3 nanoparticles (100 and 250 μg/mL). Al nanoparticles triggered slight toxicity after 24 h at the same doses. The authors also observed a decrease in phagocytosis in cells exposed to 25 μg/mL of Al after 24 h. 50 nm Al particles caused a significant decrease in phagocytosis at a dose of 5 μg/mL. No inflammatory response (production of NO, TNF-α or MIP-2) was observed after exposure to the 2 types of nanoparticles. The authors conclude that aluminium nanoparticles are slightly toxic for rat alveolar macrophages.

Renwick *et al*. (2001) exposed alveolar macrophages (J774.2 MΦ) to fine and ultrafine particles of TiO_2 (250 and 29 nm) and carbon black (260.3 and 14.3) for 8 h. No significant cytotoxicity was observed, but a decrease in macrophage capacity for phagocytosis was observed at a concentration of 0.39 μg/mm^2 for ultrafine carbon black particles and 0.78 μg/mm^2 for all particles. At all concentrations, ultrafine carbon black particles had a more noticeable effect than the other particles on the macrophages. In general, the effects on phagocytosis were manifested at a lower concentration for ultrafine particles (0.39 μg/mm^2 compared to 0.78 μg/mm^2 for fine particles). The authors conclude that the slowing of ultrafine particle clearance in the lungs is caused in part by a decrease in macrophage capacity for phagocytosis resulting from macrophage exposure to particles.

Germain *et al.* (2003) compared the *in vitro* cytotoxicity of nanoparticles of a cobalt-chromium (Co/Cr) alloy[7] and alumina ceramic, which were produced by simulated wear of prosthetic joints. Two concentrations were tested on histiocytes and pulmonary fibroblasts in mice. Cell viability was tested 5 days after exposure. The Co/Cr nanoparticles (5 - 20 nm) triggered high cytotoxicity in

[7] Co and Cr ions have sensitizing and carcinogenic potential.

human histiocytes, which depended on the concentration (respective reductions of cell viability from 97% and 42% to 50 and 5 μm^3) and on the mouse fibroblasts (respective reductions of 95% and 73%). Co/Cr particles of 10 µm had no significant effect on cell viability. Alumina ceramic nanoparticles (5 - 20 nm) only produced low cytotoxicity in human histiocytes (18% reduction), and only at high concentrations.

Researchers have taken an interest in the study of cellular phenomena manifested by induction of various mechanisms or by the production of inflammation mediators. An *in vitro* study by Lucarelli *et al.* (2004) showed that SiO_2 and cobalt (Co) nanoparticles exhibited significant proinflammatory activity for the activity of human marrow monocytes, while TiO_2 and ZrO_2 nanoparticles were less active.

Tkachenko *et al.* (2004) have obtained various degrees of *in vitro* capture of modified gold particles by the nuclei of human cervical tumour cell nuclei liver tumor cells and by mouse fibroblastoma cells. Nanoparticles could pass through the three barriers (cellular, endosomal and nuclear membrane) to reach the nucleus. Extrapolation of these results to healthy human cells remains limited, because only tumour cells have been studied.

Peters *et al.* (2004), studying the behaviour and viability of human endothelial cells *in vivo*, observed that PVC, TiO_2, SiO_2 and Co nanoparticles were incorporated into the cell vacuoles. The Co nanoparticles showed high proinflammatory and cytotoxic potential, while the SiO_2 nanoparticles had low proinflammatory potential and the TiO_2 nanoparticles had even lower potential, although still observable, despite the fact that this substance is often considered biologically amorphous. The PVC and Ni nanoparticles did not generate these effects.

The *in vitro* cytotoxicity of MMPC 1 gold nanoparticles (cationic nanoparticle with a quaternary ammonium complex) and MMPC 2 (carboxylic nanoparticle in its anionic form, recognized as not bonding to DNA) was studied by Goodman *et al.* (2004) in primate cells, human red blood cells and *E. Coli* bacteria. The researchers observed cytotoxicity in the MMPC 1 cationic nanoparticles after one hour of incubation (LD_{50}: 1.0 ± 0.5; 1.1 ± 0.1; 3.1 ± 0.6). The MMPC2 nanoparticles did not cause significant toxicity, even after 24 hours of incubation. The authors postulate an interaction of nanoparticles with the cellular membrane and the presence of electrostatic attraction mechanisms.

Braydich-Stolle *et al*. (2005) studied the toxicity of different nanoparticles in cells of the male mouse germ line (C18-4). The authors incubated cells with nanoparticles of silver (Ag; 15 nm), molybdenum (MoO_3; 30 nm) and aluminium (Al; 30 nm) at concentrations of 5, 10, 25, 50 and 100 µg/mL for 48 h. Cadmium oxide (CdO; 1000 nm) was used as a positive control at concentrations of 1, 2, 5, 10 and 25 µg/mL. No change cell morphology was observed after 48 h of incubation with MoO_3 (at all concentrations). CdO triggered major morphological changes (cell shrinkage and irregularity) at 1 µg/mL and the cells detached from the culture medium and became necrotized starting at 5 µg/mL. Ag nanoparticles triggered apoptosis and necrosis of certain parts of the cells starting at 10 µg/mL. At a concentration below 10 µg/mL, Al nanoparticles accumulated in the cell cytoplasm, where they formed aggregated incapable of penetrating the nucleus, but they did not trigger necrosis, apoptosis or

morphological changes in the cells. Ag nanoparticles were the most toxic, but all the nanoparticles tested were less toxic than CdO (Braydich-Stolle *et al*., 2005).

In their study, Braydich-Stolle *et al*. (2005) examined the mitochondrial function of cells exposed to nanoparticles. Cadmium oxide inhibited mitochondrial activity at a concentration less than 1 μg/mL (EC_{50} : 0.5 μg/mL). In comparison, cadmium chloride, considered to be toxic, had no significant effect at a concentration less than 5 μg/mL (EC_{50} : 21.3 μg/mL). The toxic effects of silver nanoparticles (15 nm) on the mitochondria began to appear between 5 and 10 μg/mL (EC_{50}: 8.75 μg/mL). In comparison, silver carbonate, not considered dangerous, had no effect on the mitochondria up to a concentration of 100 μg/mL (EC_{50}: 408 μg/mL). Molybdenum nanoparticles triggered toxic effects on cellular metabolic activity starting at 50 μg/mL (EC_{50}: 90 μg/mL). By comparison, sodium molybdate, considered moderately toxic to humans and animals at an EC_{50} of 322 μg/mL. The effect of aluminium nanoparticles on mitochondrial activity could not be assessed given that the particles accumulated and formed aggregates in the cytoplasm of the cells at low concentrations.

Braydich-Stolle *et al*. (2005) also assessed the integrity of the cytoplasmic membrane of cells exposed to nanoparticles. Cadmium oxide caused a dose-dependent loss of LDH by the cellular membrane (EC_{50}: 2.5 μg/mL). Nanoparticles also caused a dose-dependent loss of LDH by the cellular membrane at low concentrations (EC_{50} Ag: 2.5 μg/mL; EC_{50} Al: 4.7 μg/mL; EC_{50} MoO_3: 5 μg/mL). Finally, the authors observed a dose-dependent increase in the number of apoptotic cells at concentrations of 1-5 μg/mL for CdO and 10-50 μg/mL for nanoparticles. The number of necrotized cells also increased according to the concentration.

Barlow *et al*. (2005b) exposed bovine fetal serum to fine carbon black particles (260 nm in diameter; 10 mg/mL) and carbon black nanoparticles (14 nm in diameter; 5 and 10 mg/mL). They showed that substances present in serum exposed to 10 mg/mL of carbon black nanoparticles were responsible for 1.8 times more migration of macrophages (from mouse alveoli) than fine carbon black particles. The effect seemed to be linked to an oxidative phenomenon, because adding antioxidants reduced it.

In another study, Barlow *et al*. (2005a) exposed rat type II alveolar epithelial cells (line L-2) to carbon black nanoparticles (14.3 nm; 6 h). Type II epithelial cells are said to play a major role in the inflammatory process in the lungs by production of proinflammatory cytokines. These are responsible for migration of leukocytes to the inflammation sites (by chemotaxis). The authors observed significant production of chemoattractants by the type II cells, measured by macrophage migration. TiO_2 nanoparticles (29 nm) and fine carbon and TiO_2 particles (260.2 and 250 nm respectively) did not have this effect. A dose-dependent increase in LDH production after exposure to the four types of particles was also observed. The authors conclude that carbon nanoparticles are very likely responsible for production of chemoattractants by type II cells.

Gurr *et al*. (2005) studied the effect of ultrafine TiO_2 particles on human bronchial epithelial cells (BEAS-2B) in the absence of photoactivation. Anatase particles of 10 and 20 nm (10 μg/mL) triggered oxidative DNA damage, lipid peroxidation, formation of micronuclei and increased H_2O_2 and NO production in the cells. Anatase particles of 200 nm (or larger) did not induce oxidative stress

in the absence of light. However, 200 nm rutile particles induced H_2O_2 production and oxidative DNA damage in the absence of light. Finally, the level of oxidative DNA damage was slightly higher after exposure to a mixture of anatase and rutile (200 nm; 10 μg/mL for 1 h in total darkness) compared to exposure to the two forms of TiO_2 separately. The authors conclude that the smaller the size of the particles, the higher their potential to induce oxidative stress (in the absence of photoactivation). Ultrafine TiO_2 particles are usually considered biocompatible in the absence of photoactivation (Gurr *et al*., 2005).

Exposure of mouse microglia *in vitro* to non-photoactivated TiO_2 nanoparticles (2.5-120 ppm) trigged rapid (< 5 minutes) and prolonged (2 h) production of reactive oxygen species (Long *et al*., 2006). The authors also observed phagocytosis of nanoparticles by the microglia. Prolonged production of reactive oxygen species by microglia could cause damage to the neighbouring cells, particularly the neurons (Thrall, 2006).

Gupta and Gupta (2005) exposed human skin fibroblasts to superparamagnetic iron oxide nanoparticles (SPION) (40-45 nm) and to SPION coated with pullulan (Pn-SPION) at concentrations ranging from 0 to 2.0 mg/mL. SPION turned out to be toxic for the cells while Pn-SPION showed no toxicity. The authors observed internalization of both types of particles, but according to different mechanisms. They conclude that endocytosis of particles is influenced by the surface characteristics of the particles.

Human pulmonary epithelial cells were exposed *in vitro* to silica nanoparticles containing iron, cobalt, manganese and titanium, as well as nanometric oxides of these metals. The authors (Limbach *et al*. 2007) used very well characterized particles with the same morphologies, as well as comparable dimensions, forms and degrees of agglomeration. They showed that these particles effectively penetrate the cells and trigger up to eight times more oxidative stress in the case of cobalt and manganese than the aqueous solutions of the same metals. They conclude that the risk assessment should also account for the chemical composition and catalytic properties of nanoparticles.

Table 3 summarizes the documented health effects of certain inorganic nanoparticules.

Table 3. Documented health effects of certain inorganic nanoparticles [1]

Effect	Inorganic nanoparticles[2]			
	Route			
	Inhalation[3]	Cutaneous	Oral	Other
Toxicokinetics	▲■	■	▲	▲■
Irritation				
Systemic[4]				
Acute	▲■		▲	▲
Intermediate	▲■		▲	▲
Chronic	▲■			
Neurological				
Immunological				
Development				
Reproductive				▲
Genotoxic	▲	▲		▲
Cancer	▲■	▲■		▲

[1] Existing human (■) or animal (▲) studies. Adopted from the ATSDR.

[2] Inorganic nanoparticles tested: carbon, copper and some copper compounds ($CuCl_2 \cdot 2H_2O$, CuO, CuO_2, $Cu(OH)_2$), or colloidal, selenium, titanium dioxide (TiO_2), iridium-192, zinc, cerium oxide, aluminium oxide, cobalt, silver, SiO_2, chromium, molybdenum, iron oxide.

[3] Including intratracheal instillation.

[4] Systemic effects: acute (≤ 14 days), intermediate (15 to 364 days) and chronic (≥ 365 days).

7. HEALTH EFFECTS OF ORGANIC NANOPARTICLES

As in the case of inorganic nanoparticles (Chapter 6), insoluble organic nanoparticles can be composed of various organic substances, often insoluble polymers to which different organic radicals can be grafted. Some substances can also be made soluble under specific conditions. Often, only their nanometric dimensions distinguish organic nanoparticles from the same products normally found on a larger scale. However, it is precisely because of their unique nano-scaled properties that these particles are produced. On the nano-scale, they display catalytic, chemical or other properties that do not exist when in larger dimensions.

Organic nanoparticles can also be materials involving entrapment, encapsulation or surface adsorption of an active biological substance (Zimmer, 1999). One of their interests depends on their use for transport and optimal targeting of drugs. Medina *et al.* (2007) report on the use of nanoparticles in pharmacology. Kreuter (2001), Lockman *et al*. (2002, 2003) and Tiwari *et al.* (2006), among others, produced literature reviews concerning the use of nanoparticulate systems for cerebral transport of different drugs. The different systems used (coated or uncoated polymers, etc.) have turned out to be an effective tool to help drugs pass through the hematoencephalic barrier (dalargin, doxorubicin, etc.) in several animal species. Tests were performed with various types of nanoparticles: polymeric nanoparticles (Kante *et al*., 1982; Couvreur *et al*., 1982; Gibaud *et al*., 1996; Sakuma *et al*., 2002), colloidal nanoparticles (Nemmar *et al*., 2002a, 2002b), carbon nanotubes (Kostarelos *et al.* 2007) and lipidic nanospheres (Fukui *et al*., 2003). Recently, Ryan *et al*. (2007) reported that SWCNT can inhibit certain allergic reaction mechanisms. The results are promising regarding the therapeutic aspects, mainly for polymeric nanoparticles, but the data are inadequate concerning their toxicity in the work environment because of the unusual route used and the limited information concerning their toxicological assessment. These particles will not be discussed exhaustively in this document.

7.1. Toxicokinetics

7.1.1 Absorption

Alvarez-Román *et al*. (2004) studied cutaneous absorption of polystyrene nanoparticles containing FITC (fluorescein isothiocyanate-5) on pig skin. The authors observed that the polystyrene nanoparticles (20 and 200 nm) accumulated preferentially in the hair follicles. Nanoparticle accumulation in the hair follicles increased according to time and particle size, with the smallest particles (20 nm) displaying greater accumulation. After application of a saturated FITC solution (not bound to nanoparticles), FITC was observed mainly on the surface of the skin after 30 minutes and 1 h. However, a higher FITC concentration was observed in the hair follicles after 2 h of exposure. The authors also observed an accumulation of 20 and 200 nm particles in the interpapillary ridges, but no absorption was observed at these sites. FITC accumulated in the corneocytes.

7.1.2. Distribution

7.1.2.1. Inhalation and airway exposure

No data

7.1.2.2. Cutaneous exposure

No data

7.1.2.3. Ingestion exposure

Jani *et al*. (1990) showed that polystyrene nanoparticles (30, 100 and 300 nm) administered by gavage in rats (1.25 mg/kg/day for 10 days; Hoet *et al*., 2004a) could be detected in the blood and in several organs, such as the liver and spleen. None were found in the heart and lungs.

7.1.2.4. Exposure by other routes

Douglas *et al*. (1996) studied the biodistribution of poly(butyl 2-cyanoacrylate) nanoparticles, whether polymer-coated or not, in rabbits (intravenous injection of 1 mL of each preparation). About 60% of the nanoparticles were located in the liver and the spleen, while about 30% remained in the bloodstream. The coating had no significant influence on nanoparticle distribution.

Sakuma *et al*. (2002) showed that certain hydrophilic polymeric nanoparticles (poly(N-isopropylacrylamide), poly(N-vinylacetamide), poly(vinylamine) and polymethacrylic acid) administered by perfusion increase the permeability of the rat jejunum (part of the intestine) to salmon calcitonin (hypocalcemic drug).

Lockman *et al*. (2003) showed that nanoparticles to which Brij78 and Brij72/tween 80 emulsions are added have no *in vivo* effect on rats and no *in vitro* effect (bovine brain cells) on the integrity of the hematoencephalic barrier. Koziara *et al*. (2003) showed that nanoparticles to which Brij78 and Brij72 are added could pass through the hematoencephalic barrier in rats without affecting the barrier's biological integrity.

Lockman *et al*. (2004) studied the effect of the nanoparticle load on the integrity and permeability of the hematoencephalic barrier. The authors exposed rats to wax nanoparticles by perfusion in the left carotid. Neutral nanoparticles (74.7 ± 53.4 nm; 10 and 20 μg/mL) and low-concentration anionic nanoparticles (127.1 ± 70.6 nm; 10 μg/mL) had no acute effect on the integrity of the hematoencephalic barrier. However, high-concentration anionic nanoparticles (20 μg/mL) and cationic nanoparticles (97.2 ± 68.9 nm; 10 and 20 μg/mL) had a rupturing effect on the hematoencephalic barrier in less than 60 seconds. The authors also observed higher permeability for anionic nanoparticles ($7.93 \pm 0.98 \times 10^{-3}$ mL/s/g) compared to neutral nanoparticles ($4.10 \pm 0.5 \times 10^{-3}$ mL/s/g) and cationic nanoparticles ($2.73 \pm 0.2 \times 10^{-3}$ mL/s/g) at a concentration of 10 μg/mL.

Ferritin and iron dextran nanoparticles were found in the hypoglossal nucleus after injection in the mouse tongue (Oberdörster *et al*., 2005b). When they were injected in mouse facial muscles, the same particles were absorbed by the synapses. In addition, ferritin cations were detected in the facial neurons, which indicates that the particle load has an influence on absorption of particles by the axons and their transport along the axons (Oberdörster *et al*., 2005b).

Kim *et al*. (2006) studied the toxicity and distribution of magnetic silica nanoparticles containing rhodamine B isothiocyanate. The 50 nm nanoparticles were administered by intraperitoneal injection to ICR mice for 4 weeks (25, 50, 100 mg/kg). A dose of 10 mg/kg was used to assess the effects on the hematoencephalic barrier. Nanoparticles were detected in all organs, including the brain, liver, lungs, kidneys, spleen, heart, testicles and uterus. The highest nanoparticle concentrations were observed in the liver and spleen, while distribution in the lungs was minimal. The nanoparticles passed through the

hematoencephalic barrier without affecting its permeability and were absorbed by the neurons. Distribution in the organs was time-dependent.

7.1.2.5. In vitro

No data

7.1.3. Metabolism

No data

7.1.4. Excretion

No data

7.2. Effects according to routes of exposure (administration)

7.2.1. Inhalation and airway exposure

Positively charged polystyrene nanoparticles (60 nm) administered by intratracheal instillation to hamsters (50 and 500 μg per animal) triggered inflammation in the lungs (increase in neutrophils, LDH and histamine in the bronchoalveolar lavage fluid) and a vascular thrombosis after 1 h (Nemmar *et al*., 2003).

7.2.2. Cutaneous exposure

No data

7.2.3. Ingestion exposure

No data

7.2.4. Exposure by other routes

7.2.4.1. Effects on organs

The biochemical, hematological and histological analyses performed by Kim *et al.* (2006) on mice exposed to magnetic silica nanoparticles containing rhodamine B isothiocyanate did not reveal any apparent toxicity in any organs after 4 weeks.

7.2.4.1.1. Effects on the skin and the mucous membranes

Kante *et al*. (1982) did not observe any irritant effects at the injection site of poly(isobutyl cyanoacrylate) and poly(polybutyl cyanoacrylate) nanoparticles (~0.2 μm in diameter, single intravenous injection; 0, 12.5 to 40 mL/kg) during a Lethal Dose 50 (LD_{50}) determination test in mice.

7.2.4.1.2. Effects on the respiratory system

No data

7.2.4.1.3. Liver effects

Fernandez-Urrusuno *et al*. (1997) showed that single or repeated intravenous injection of 214 nm poly(isobutyl cyanoacrylate) nanoparticles or 128 nm polystyrene nanoparticles can temporarily reduce the antioxidant defence of isolated rat hepatocytes.

7.2.4.1.4. Kidney effects
No data

7.2.4.1.5. Effects on the gastrointestinal system
No data

7.2.4.1.6. Effects on the heart and blood circulation
No data

7.2.4.1.7. Effects on the blood and the hematopoietic system
No data

7.2.4.1.8. Effects on the nervous system
No data

7.2.4.2. Immunological and allergic effects

Meng *et al*. (2004), in a biocompatibility assessment, did not observe any harmful effects in animals (inflammation, etc.) during muscle implantation of a material composed of hydroxapatite and polyamide nanocrystals.

Tomazic-Jezic *et al*. (2006) injected mice (the route is not specified) with intact or antigen-coated 50 and 500 nm polystyrene particles. The concentrations are not specified. The authors observed monocyte and macrophage infiltration at the injection site with both types of particles. No toxicity in the liver, kidneys or lungs was observed. Both types of particles had an adjuvant effect on IgG production, but the 50 nm nanoparticles also increased IgE antibody production.

7.2.4.3. Effects on reproduction and the reproductive system
No data.

7.2.4.4. Development effects

Bosman *et al*. (2005) exposed mouse embryos with stage 2 cells and at the blastocyte stage to polystyrene nanoparticles (40-120 nm; 11.0 million/mL). The authors did not observe any difference in the hatching, implantation and degeneration of the embryos compared to the controls. The nanoparticles did not inhibit embryo development up to the blastocyte stage. Smaller nanoparticles were internalized by endocytosis or pinocytosis, but no effect on embryo development was observed. The nanoparticles were located mainly in the trophoblasts.

7.2.4.5. Genotoxic effects

Kante *et al*. (1982) did not observe any mutagenic effect of poly(butyl cyanoacrylate) and poly(methyl cyanoacrylate) nanoparticles and their degradation products during the Ames test (5 *Salmonella typhimurium* strains).

Leong-Morgenthaler *et al*. (1997) showed that benzo(a)pyrene dissolved in sunflower oil and encapsulated in lipid nanoparticles exercised a mutagenic action on human cells *in vitro* similar to benzo(a)pyrene dissolved in dimethyl sulphoxide. A single dose was tested.

Kim *et al*. (2006) observed an increase in the number of reverse mutations during the Ames test performed with magnetic silica nanoparticles containing rhodamine B isothiocyanate (50 nm). However, the changes observed were not

concentration-dependent and were not reproducible. Thus, no conclusion can be drawn as to the toxicity of these nanoparticles.

7.2.4.6. Carcinogenic effects

No data

7.2.4.7. Cellular and humoural effects

No data

Table 4 summarizes documented health effects of certain organic nanoparticles that have been considered for this study. It puts emphasis on the very limited and incomplete data existing on health hazards related to these compounds.

Table 4. Documented health effects of certain organic nanoparticles[1]

Effect	Organic nanoparticles[2]			
	Route			
	Inhalation[3]	Cutaneous	Oral	Other
Toxicokinetics		▲	▲	▲
Irritation				▲
Systemic[4]				
Acute	▲			▲
Intermediate				
Chronic				
Neurological				
Immunological				▲
Development				▲
Reproductive				
Genotoxic				▲
Cancer				

[1] Existing human (■) or animal (▲) studies. Adopted from the ATSDR.

[2] Organic nanoparticles tested: fluorescein isothiocyanate-5 (FITC), polystyrene, poly(butyl 2-cyanoacrylate, poly(N-isopropylacrylamide), poly(N-vinylacetamide), poly(vinyl-amine), polymethacrylic acid, ferritin, iron dextran, magnetic silica, poly(isobutyl cyanoacrylate), poly(polybutyl cyanoacrylate), poly (methyl cyanoacrylate) hydroxyapatite, polyamide, wax.

[3] Including intratracheal instillation.

[4] Systemic effects: acute (≤ 14 days), intermediate (15 to 364 days) and chronic (≥ 365 days).

8. HEALTH EFFECTS OF NANOCAPSULES, NANOSPHERES AND NANOSHELLS AND DENDRIMERS

Nanocapsules, nanospheres and nanoshells can be composed of a wide variety of insoluble organic polymers. Some of these structures are developed to be capable of integration with other substances, often medications. The surface of these nanoparticles can also be modified to interact specifically with certain sites of the body. Because of their nanometric dimensions, these particles can circulate in a living organism, serve as a drug vector or fix to specific cells. They represent a very active research sector with potentially major medical spin-offs.

8.1. Toxicokinetics

8.1.1 Absorption

In 1987, Aprahamian *et al.* showed the intestinal absorption of a drug (Lipiodol) transported by polymeric nanocapsules of about 300 nm in dogs. Within less than one hour after intraintestinal injection of the drug and laparotomy of the animals, the nanocapsules were observed in the lumen of the jejunum (small intestine) and then in the intracellular spaces, in the lamina propria, and finally in the intestinal capillaries.

8.1.2. Distribution

8.1.2.1. Inhalation and airway exposure

No data

8.1.2.2. Cutaneous exposure

No data

8.1.2.3. Ingestion exposure

No data

8.1.2.4. Exposure by other routes

In a study by Malik *et al*. (2000), PAMAM cationic dendrimers (marked with iodine-125) administered by intravenous or intraperitoneal injection to Wistar rats were eliminated rapidly from the bloodstream. Only 0.1-1.0% of the dose of approximately 10 µg/mL was found in the blood one hour after injection. The liver was identified as the organ of accumulation with 60-90% of the dose. Anionic dendrimers showed a longer residence time in the blood.

In a study conducted in rats, Cahouet *et al.* (2002) intravenously injected nanocapsules (20 to 100 nm) with a lipid core and a shell composed of 2-hydroxy-polyethylene glycol (PEG) stearate and lecithin. The nanocapsules were marked with iodine-125 and technetium-99. The authors observed a longer-than-expected persistence of the nanocapsules in the blood compartment. They attributed the longer persistence to the PEG coating. The nanocapsules were distributed in the animals' liver, intestines, stomach and penis, but there was no significant cerebral distribution.

Nigavekar *et al*. (2004) studied the distribution of PAMAM dendrimers (poly(amidoamine); 5 nm) in male C57BL/6J mice injected with mouse melanoma cells and in male nude (nu/nu) mice injected with human prostate

carcinoma cells (DU145). 500 µL of a solution containing 8.8 µg of positively charged dendrimers and 17.5 µg of neutral dendrimers were administered to the C57BL/6J mice by intravenous injection. The nude mice only received the positively charged dendrimers. The authors observed a homogenous distribution of the dendrimers in the mouse liver, indicating that uniform nanoparticle distribution occurred in the vascular organs after administration. The dendrimers were eliminated rapidly from the bloodstream and nanoparticles were found in all the major organs and in the tumour tissues. Deposition of positively charged dendrimers exceeded deposition of neutral dendrimers. The greatest dendrimer absorption occurred in the lungs, kidneys and liver one hour after injection, while the lowest absorption occurred in the brain. No acute toxicity was observed.

8.1.2.5. In vitro

No data

8.1.3. Metabolism

No data.

8.1.4. Excretion

Digestive elimination of nanoparticles radiomarked with iodine-125 and technetium-99 was noted in the Cahouet *et al.* (2002) study of rats. After 24 hours, iodine-125 was still excreted in the animals' urine.

Nigavekar *et al*. (2004) observed rapid initial elimination in the urine of dendrimers administered to mice within 24 h after injection. Subsequently, the nanoparticle level remained relatively stable and decreased very slowly over time. 48.3% of the neutral dendrimers and 29.6% of the positively charged dendrimers were excreted in the urine after 7 days. There was less substantial excretion of dendrimers in the feces over the same period (5.41% of the neutral dendrimers and 2.96% of the positively charged dendrimers). The authors assume that the unexcreted dendrimers are deposited in the microvasculature of various organs and internalized by the endothelium or the cells adjacent to the endothelium.

8.2. Effects according to routes of exposure (administration)

8.2.1. Inhalation and airway exposure

8.2.1.1. Effects on the organs

8.2.1.1.1. Effects on the skin and the mucous membranes

No data

8.2.1.1.2. Effects on the respiratory system

Dailey *et al*. (2006) exposed Balb-C mice to biodegradable nanoparticles of poly (lactic-co-glycolic acid) and DEAPA-PVAL-g-PLGA (a PLGA derivative) and to 75 and 220 nm polystyrene nanospheres (intratracheal instillation of 1 and 2.5 µg/mL). The authors observed an increase in inflammation markers (LDH, proteins, MIP-2 RNAm, neutrophils) in the bronchoalveolar lavage fluid of mice exposed to the 75 nm nanospheres. After 24 h, the neutrophils represented 41% and 74% of the cells present in the bronchoalveolar lavage fluid of the mice exposed to the 75 and 220 nm nanospheres respectively. Biodegradable particles of comparable diameter triggered a lower inflammatory response and neutrophil

recruiting was comparable to that of mice exposed to an isotonic glucose solution (control).

8.2.1.1.3. Liver effects
No data

8.2.1.1.4. Kidney effects
No data

8.2.1.1.5. Effects on the gastrointestinal system
No data

8.2.1.1.6. Effects on the heart and blood circulation
No data

8.2.1.1.7. Effects on the blood and the hematopoietic system

Rabbits exposed to 70 μg/m^3 of nanospheres (unidentified) for 3 h showed a coagulation time twice as fast as rabbits exposed to air not containing nanospheres (Raloff, 2005). Blood vessels in the rabbits' ears had been damaged previously by researchers with a laser.

8.2.1.1.8. Effects on the nervous system
No data

8.2.1.2. Immunological and allergic effects
No data

8.2.1.3. Effects on reproduction
No data

8.2.1.4. Development effects
No data

8.2.1.5. Genotoxic effects
No data

8.2.1.6. Carcinogenic effects
No data

8.2.1.7. Cellular and humoural effects
No data

8.2.2. Cutaneous exposure
No data

8.2.3. Ingestion exposure
No data

8.2.4. Exposure by other routes

8.2.4.1. Effects on the organs

8.2.4.1.1. Effects on the skin and the mucous membranes
No data

8.2.4.1.2. Effects on the respiratory system
No data

8.2.4.1.3. Liver effects
No data

8.2.4.1.4. Kidney effects
No data

8.2.4.1.5. Effects on the gastrointestinal system
No data

8.2.4.1.6. Effects on the heart and blood circulation
No data

8.2.4.1.7 Effects on the blood and the hematopoietic system

In a study by Malik *et al*. (2000), PAMAM cationic dendrimers, DAB (poly(propylene imine)) and DAE (diaminoethane) caused hemolysis of rat red blood cells (in a saline solution) after one hour at a concentration greater than 1 mg/mL. For PAMAM dendrimers, the hemolytic effect depended on the generation[8] (those of generation 1 had no effect). Changes in red blood cell morphology were observed at a concentration of 10 µg/mL with PAMAM and DAB dendrimers (after one hour).

8.2.4.1.8. Effects on the nervous system
No data

8.2.4.2. Immunological and allergic effects
No data

8.2.4.3. Effects on reproduction and the reproductive system
No data

8.2.4.4. Development effects
No data

8.2.4.5. Genotoxic effects
No data

8.2.4.6. Carcinogenic effects
No data

8.2.4.7. Cellular and humoural effects

A study by Roberts *et al*. (1996) cited by Duncan and Izzo (2005) revealed a decrease in the viability of Chinese hamster lung fibroblasts (V79) exposed to PAMAM cationic dendrimers. A concentration of 1 nM (~ 7 ng/mL; generation 3) triggered the death of 90% of the cells while concentrations of 10 nM (~ 280 ng/mL) for generation 5 and 100 nM for generation 7 were necessary to trigger the death of so many cells. Cytotoxicity of the dendrimers with an NH_2 group in a terminal position is concentration-dependent and usually generation-dependent (Duncan and Izzo, 2005).

Torres-Lugo *et al.* (2002) studied the *in vitro* cytotoxicity of hydrogel nanospheres, substances that can bypass the upper digestive tract and act as pharmacological vectors directly in the intestine. Using cultures of human

[8] The generations correspond to the successive layers of molecules added during dendrimer production. The addition of these layers has the effect of increasing the size of the molecules and the number of surface groups. For example, growth of PAMAM dendrimers between generations 1 and 10 has the effect of increasing their diameter from 1.1 to 12.4 nm (Duncan and Izzo, 2005).

intestinal cells to which methacrylic acid ethylene glycol nanospheres have been added, the authors conclude that this nanomaterial has low toxicity. However, the authors observed a reversible alteration of the electrical resistance of the epithelial cells, as well as opening of the junctional membrane complexes. This raised the possibility of cellular transport of the nanocomplex.

The results of a study by Hong *et al*. (2004) reported by Duncan and Izzo (2005) showed that generation 7 PAMAM dendrimers (10-100 nM) triggered formation of holes 15 to 40 nm in diameter in a DMPC lipid bilayer (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) while generation 5 PAMAM dendrimers only enlarged the holes at already damaged sites. Generation 5 PAMAM dendrimers were not cytotoxic up to a concentration of 500 mM (Duncan and Izzo, 2005). Moreover, generation 5 PAMAM dendrimers with an acetamide (C_2H_5NO) terminal group had no effect on the integrity of the cellular membrane up to a concentration of 500 nM.

In an *in vitro* study, Zhou *et al.* (2005) showed that application of a nanosphere formulation to administer arsenic trioxide reduces the blood toxicity of this product, used against bladder cancer, and renders its action more specific to cancer cells.

Duncan and Izzo (2005) compared cytotoxicity of PAMAM, DAB and DAE dendrimers on 3 cell lines (mouse melanoma cells (B16F10), human lymphoblastic leukemia cells (CCRF) and human hepatoma cells (HepG2)) after 72 h of incubation. The authors observed IC_{50} ranging from 50 to 300 μg/mL for generation 4 of the PAMAM, DAB and DAE dendrimers. Generations 1 to 4 of the PAMAM cationic dendrimers were cytotoxic and exhibited IC_{50} similar to the PLL (poly(L-lysine) used as a control. The PAMAM dendrimers were slightly less toxic than the DAB dendrimers, which had an equivalent surface functionality. Considerable changes in the morphology of the B16F10 cells were observed after exposure to the DAB and DAE dendrimers (1 h; 1 mg/mL). Incubation of the B16F10 cells with PAMAM dendrimers for 1 h (generation 4; 5 μg/mL) did not have an effect on cell morphology, but signs of cell membrane damage were observed after 5 h of incubation.

Duncan and Izzo (2005) point out that dendrimer cytotoxicity depends more on the nature of their surface than on the chemistry of the molecular centre. In a study by Chen *et al*. (2004) cited by Duncan and Izzo (2005), cationic dendrimers exercised greater toxicity than anionic or PEG-coated dendrimers in rat liver cells (Clone 9) exposed for 3 h. PAMAM-OH dendrimers were less toxic than PAMAM-NH_2 dendrimers due to coating of the internal positive charges by the surface hydroxyl groups (Duncan and Izzo, 2005).

Table 5 summarizes actually documented health effects of certain nanocapsules, nanosphères, nanoshells and certain dendrimères.

Table 5. Documented health effects of certain nanocapsules, nanosphères, nanoshells and certain dendrimers[1]

Effect	Nanocapsules, nanospheres nanoshells and dendrimers[2]			
	Route			
	Inhalation[3]	Cutaneous	Oral	Other
Toxicokinetics				▲
Irritation				
Systemic[4]				
Acute	▲			
Intermediate				
Chronic				
Neurological				
Immunological				
Development				
Reproductive				
Genotoxic				
Cancer				

[1] Existing human (■) or animal (▲) studies. Adopted from the ATSDR.

[2] Nanocapsules, nanospheres, nanoshells and dendrimers tested: nanospheres (polystyrene 75, hydrogel, methacrylic acid ethylene glycol), dendrimers (poly(amidoamine), poly(propyleneimine, diaminoethane), poly (lactic-co-glycolic acid) (PLGA)).

[3] Including intratracheal instillation.

[4] Systemic effects: acute (≤ 14 days), intermediate (15 to 364 days) and chronic (≥ 365 days).

9. HEALTH EFFECTS OF QUANTUM DOTS

Quantum dots (or nanocrystals) are heterogeneous nanoparticles consisting of a colloidal nucleus surrounded by one or more surface coatings (Ryman-Rasmussen *et al.*, 2006). Their size ranges from 2 to 100 nm (Hardman, 2006). The nuclei of quantum dots may be composed, among other materials, of semiconductors, noble metals or magnetic transition metals (Hardman, 2006). The number of atoms in quantum dots, which can range from 1,000 to 100,000, makes them neither an extended solid structure nor a molecular entity (Aitken *et al*., 2004). The principal research studies have focused on semiconductor quantum dots, which display distinctive quantal effects depending on the dimensions. The light emitted can be adjusted to the desired wavelength by changing the overall dimension (Aitken *et al*., 2004).

Because of their diversity, quantum dots cannot be considered a uniform group of nanomaterials and their toxicity will have to be characterized individually according to each type of quantum dot (Hardman, 2006).

9.1. Toxicokinetics

9.1.1 Absorption

Quantum dots are used as fluorescent probes in diagnostic medical imaging and in therapeutics, because of their optical properties and their capacity to form covalent bonds with peptides, antibodies, nucleic acids or other low-weight molecules (Smith *et al.* 2004). Chan and Nie in 1998, cited by Smith *et al.* (2004), were the first to demonstrate *in vivo* that CdSe / ZnS quantum dots coated with mercaptoacetic acid could bond to blood transferrine. This fluorescent complex was absorbed selectively by cancer cells.

Ryman-Rasmussen *et al*. (2006) studied the absorption of different types of quantum dots in intact pig skin. The authors observed that spherical quantum dots coated with polyethylene glycol (PEG; 35 nm), carboxylic acid (14 nm) and PEG-amine (15 nm) were absorbed by the skin after 8 h of exposure. Quantum dots coated with PEG and carboxylic acid were found mainly in the epidermis, while quantum dots coated with PEG-amine were found mainly in the dermis. Ellipsoidal quantum dots coated with PEG (45 nm) and PEG-amine (20 nm) were found mainly in the epidermis after 8 h. However, ellipsoidal quantum dots coated with carboxylic acid (18 nm) were only absorbed after 24 h. The authors conclude that the skin is permeable to nanomaterials and can serve as a gateway for quantum dots and other nanomaterials, in both producers and users of these materials. Moreover, the results indicate that no mechanical stress (such as abrasion) is necessary for nanomaterials to penetrate the skin. The mechanism involved is said to be passive diffusion through the intercellular spaces. However, this conclusion does not seem to be applicable to all substances. Thus, Tinkle *et al*. (2003) showed that 0.5 and 1.0 μm beryllium particles penetrated the corneal layer to reach the epidermis and occasionally the dermis in human skin samples subjected to flexion simulating a wrist movement (20 flexions per minute). The authors suggested that the movement was necessary for the beryllium particles to penetrate the skin.

9.1.2. Distribution

9.1.2.1. Inhalation and airway exposure

No data

9.1.2.2. Cutaneous exposure

No data

9.1.2.3. Ingestion exposure

No data

9.1.2.4. Exposure by other routes

Dubertret *et al*. (2002) revealed the transfer of CdSe/ZnS quantum dots (coated with PEG-PE and PC and conjugated to an oligonucleotide) to daughter cells during cell division in Xenopus frog embryos. The authors observed quantum dots in the daughter cells after several days of development (up to the tadpole stage). Quantum dots were present in the cytoplasm and in the nuclear envelope of the embryonic cells.

In an intravenous study of mice, Akerman *et al.* (2002) report that the nature of the CdSe / ZnS quantum dot coating could alter the distribution of these nanomaterials in the tissues and organs. It was found that PEG coating reduced capture by the liver and spleen by about 95% and prolonged the half-life of the quantum dots in the bloodstream. Other types of peptide coatings increased distribution in the lungs or in breast tumours induced during the experiment. The authors note the absence of quantum dots in the skin covering the tumour site, in the brain and in the kidneys of the animal subjects.

A study by Kim *et al*. (2004) cited by Oberdörster *et al*. (2005b) confirmed that quantum dots administered by intradermal injection were found in the lymphatic ganglia after penetrating the dermis. The study was performed on mice and pigs. The macrophages and the Langerhans cells of the skin are possibly responsible for transport to the lymphatic ganglia (Oberdörster *et al*., 2005b).

In a study by Hoshino *et al*. (2004a) cited by Hardman (2006), EL-4 cells containing CdSe/ZnS-SSA quantum dots were observed in the kidneys, liver, lungs and spleen of mice up to seven days after their administration by injection. The spleen and the lungs showed a greater accumulation.

In another study cited by Hardman (2006), Ballou *et al*. (2004) observed a differential deposition in the tissues and organs of mice exposed to quantum dots with coatings of different molecular weights. Deposition depended on time and quantum dot size. The quantum dots with the highest molecular weight (5000) remained in the bloodstream for at least 3 h, while the quantum dots with the lowest molecular weight (750) were eliminated from the blood in less than one hour and were found in the lymphatic ganglia, the liver and the bone marrow 24 hours after injection. The quantum dots with the lowest molecular weight were still present in the bone marrow lymphatic ganglia 133 days after injection.

9.1.2.5. In vitro

Parak *et al*. (2002) cited by Hardman (2006) observed endocytosis and storage and vesicular transport of silicon dioxide-coated CdSe/ZnS quantum dots in the perinuclear region of human mammary tumour cells.

Dubertret *et al*. (2002) also revealed endocytosis and active transport of CdSe/ZnS quantum dots coated with *n*-poly(ethylene glycol) phosphatidylethanolamine (PEG-PE) and phosphatidylcholine (PC) after their injection in Xenopus frog embryos.

A study by Kloepfer *et al*. (2003) cited by Hardman (2006) revealed an increase in fluorescence (attributed to intracellular oxidation of the quantum dots) and intracellular Se concentration in *Staphylococcus aureus* cultures after two weeks of exposure to quantum dots conjugated to transferrine. The authors observed cellular internalization of free Cd and Se, but internalization of quantum dots.

Jaiswal *et al*. (2003), cited by Hardman (2006), observed incorporation by endocytosis of CdSe/ZnS quantum dots conjugated to avidine and DHLA by the *Dictyostelium discoideum* (AXS) amoeba and HeLa cells.

Incorporation of CdSe/ZnS quantum dots conjugated to methacrylic acid (MAA) in rat hepatocytes was observed by Derfus *et al*. (2004).

Hoshino *et al*. (2004a) cited by Hardman (2006) observed adhesion to the surface of EL-4 cells by CdSe/ZnS quantum dots conjugated to sheep serum. The authors then observed incorporation of quantum dots by endocytosis and a time-dependent increase in cytosolic concentration.

In a study cited by Hardman (2006), Chen and Gerion (2004) observed CdSe/ZnS quantum dots conjugated to a nuclear localization signal peptide of the SV40 virus in the nuclear compartment of HeLa cells.

In a study by Lidke *et al*. (2004) cited by Hardman (2006), quantum dots conjugated to epidermal growth factor (CdSe/ZnS-EGF) were internalized rapidly in the endosomes of Chinese hamster ovarian cells. Quantum dots thus can be internalized through membrane receptors when the quantum dots carry specific molecules for a type of receptor (for example, erbB1 for CdSe/ZnS-EGF quantum dots) or surface proteins (Hardman, 2006).

Lovric *et al*. (2005) exposed rat pheochromocytoma cells (PC12) and mouse microglia cells (line N9) to CdTe quantum dots (coated with mercaptopropionic acid or cysteamine). Red cationic quantum dots (5.2 nm; 3.75 and 37.5 μg/mL) were distributed in the cytoplasm of the PC12 cells but were not detected in the nucleus. The same phenomenon was observed in the N9 cells after one hour of exposure. However, green cationic quantum dots (2.2 nm) were found mainly in the nucleus (after 1 h). Green quantum dots conjugated to bovine serum albumin were observed mainly in the cytoplasm. The authors conclude that quantum dot distribution in the cell is influenced in part by their size.

9.1.3. Metabolism

In a study by Hoshino *et al*. (2004a) cited by Hardman (2006), about 10% of the EL-4 cells incubated with CdSe/ZnS-SSA quantum dots still contained quantum dots after 10 days of culture. The fluorescent intensity was concentrated in the endocytotic vesicles, which suggests intracellular degradation of the quantum dots (Hardman, 2006). Hoshino *et al*. (2004a) also observed a decrease in fluorescence, possibly caused by a low pH, oxidation of the quantum dot surface structures or the presence of intracellular factors adsorbed to the quantum dot surfaces.

In a study cited by Hardman (2006), Hoshino *et al*. (2004b) observed that the surface coating of quantum dots could detach under acidic or oxidizing conditions in the endocytotic vesicles and be released into the cell cytoplasm.

The results of a study by Gao *et al*. (2004) cited by Hardman (2006) suggest that quantum dot ligands and surface coatings are slowly degraded *in vivo*, leading to an alteration of their surface and a decrease in fluorescence. However, Gao *et al*. (2004) observed that quantum dots coated with a high molecular weight polymer and having an 8-carbon alkyl side chain showed greater stability *in vivo* than quantum dots coated with a single polymer or amphiphilic lipids. In another study cited by Hardman (2006), Aldana *et al*. (2001) observed that the photochemical stability of CdSe nanocrystals was linked to the thickness and density of the ligand monolayer.

9.1.4. Excretion

Akerman *et al*. (2002) observed that the PEG coating of CdSe/ZnS quantum dots was almost entirely suppressed by the lymphatic system's non-specific elimination of quantum dots.

In general, quantum dots are eliminated by the primary excretion organs and systems, such as the liver, the spleen and the lymphatic system (Hardman, 2006).

PEG-coated CdSe/ZnS quantum dots administered to mice by subcutaneous injection were eliminated from the injection site and accumulated in the lymphatic ganglia (Hardman, 2006).

9.2. Effects according to routes of exposure (administration)

9.2.1. Inhalation and airway exposure

According to Hardman (2006), inhalation exposure can pose potential risks, given that studies have shown that quantum dots were incorporated by endocytosis into different types of cells and that they can reside there for weeks, and even months. It is not known whether quantum dots form aggregates in the ambient air (Hardman, 2006).

9.2.2. Cutaneous exposure

The risks related to cutaneous absorption of quantum dots are not currently known, according to the literature review performed by Hardman (2006).

9.2.3. Ingestion exposure

The risks related to accidental ingestion of quantum dots are not currently known, according to the literature review performed by Hardman (2006).

9.2.4. Exposure by other routes

In a study by Larson *et al*. (2003) cited by Hardman (2006), mice given 20 nM and 1 μM solutions of CdSe/ZnS quantum dots by intravenous injection showed no sign of disease.

A study by Ballou *et al*. (2004) cited by Hardman (2006) showed that mice given amphiphilic polymer-coated quantum dots of polyacrylic acid (amp-QDs) and amp-QDs conjugated to PEG-amine groups (intravenous injection of 20 pmol/g

by body weight) exhibited no sign of necrosis at the particle deposition site. The mice were viable for 133 days.

9.2.4.1. Effects on the organs

9.2.4.1.1. Effects on the skin and the mucous membranes

No data

9.2.4.1.2. Effects on the respiratory system

No data

9.2.4.1.3. Liver effects

The release of cadmium after surface alteration of quantum dots by photoactivation and oxidation triggered hepatic cytotoxicity *in vitro* (US EPA, 2005).

9.2.4.1.4. Kidney effects

No data

9.2.4.1.5. Effects on the gastrointestinal system

No data

9.2.4.1.6. Effects on the heart and blood circulation

No data

9.2.4.1.7. Effects on the blood and the hematopoietic system

No data

9.2.4.1.8. Effects on the nervous system

No data

9.2.4.2. Immunological and allergic effects

No data

9.2.4.3. Effects on reproduction and the reproductive system

No data

9.2.4.4. Development effects

In their study of Xenopus frog embryos exposed to CdSe/ZnS quantum dots coated with PEG-PE and PC, Dubertret *et al.* (2002) conclude the absence of significant toxicity for embryo development (at a concentration lower than 5×10^9 quantum dots per cell).

9.2.4.5. Genotoxic effects

In a study by Hoshino *et al*. (2004b) cited by Hardman (2006), reversible DNA damage was observed in human lymphoblast cells (WTK1) after 2 h of exposure to quantum dots conjugated to carboxylic acid at a concentration of 2 μM.

In the same study cited by Hardman (2006), Hoshino *et al*. (2004b) assessed the cytotoxicity of three quantum dot surface coatings (MUA, cysteamine and thioglycerol) and two impurities (tri-*n*-octylphosphine oxide (TOPO) and ZnS) in human lymphoblast cells (WTK1). Exposure to MUA caused cytotoxicity (12 h; > 100 μg/mL) and DNA damage (2 h; 50 μg/mL). Low genotoxicity was

observed after 12 h of exposure to cysteamine. Thioglycerol toxicity was negligible. According to Hardman (2006), the results of Hoshino *et al*. (2004b) suggest that genotoxicity was caused by the hydrophilic surface coatings and not by the core of the quantum dots. Moreover, Chen and Gerion (2004), cited by Hardman (2006), attributed the absence of quantum dot genotoxicity to a silica coating, which prevented interaction of Cd, Se, Zn and sulphur with the proteins and DNA in the nucleus.

9.2.4.6. Carcinogenic effects

No data.

9.2.4.7. Cellular and humoural effects

In a study by Jaiswal *et al*. (2003) cited by Hardman (2006), CdSe/ZnS quantum dots coated with DHLA (400-600 nM) had no effect on the growth and development of HeLa cells and the *Dictyostelium discoideum* amoeba. The cells remained marked by the quantum dots for more than one week.

In an *in vitro* study, Derfus *et al.* (2004) assessed the cytotoxicity of CdSe quantum dots. The viability of hepatocytes incubated in a solution containing the quantum dots decreased according to the concentration (0.0625 <0.25 <1 mg/mL) and diminished further if the quantum dots had been subjected to ultraviolet (UV) radiation for periods of 1, 2 and 4 hours. The quantum dots that had been exposed to UV for 8 hours reduced the cell viability significantly and comparably for the three concentrations (6% viable cells). Exposure of the quantum dots to air 30 minutes before coating with MAA also reduced cell viability, which fell from 98% to 21% at a concentration of 0.0625 mg/mL (Hardman, 2006). The authors conclude that there is significant cytotoxicity of CdSe quantum dots, secondary to oxidation of their surface and the release of Cd^{2+} ions, recognized as carcinogenic in humans. Encapsulation of quantum dots with ZnS tended to reduce this effect (66% cell viability), but fell to almost zero if the quantum dots were encapsulated with 98% bovine serum albumin.

Shiohara *et al.* (2004) studied the *in vitro* cytotoxicity of CdSe / ZnS quantum dots coated with mercaptoundecanoic acid and sheep serum albumin. They produced three forms of quantum dots, which differed according to their photoluminescence. Primate kidney cells, human hepatocytes and cervical cancer cells were exposed to 0, 0.05, 0.1 and 0.2 mg/mL for 24 hours. The authors observed a decrease in the viability of the 3 cell lines at concentrations of 0.1 and 0.2 mg/mL, which increased with the concentration.

A study by Hoshino *et al*. (2004a) cited by Hardman (2006) revealed the toxicity of the materials used to coat quantum dots. The authors observed cytotoxicity after exposure of mouse T lymphoma cells (EL-4) to mercaptoundecanoic acid (MUA) (12 h; 100 µg/mL). However, Hardman (2006) points out that it is unlikely that the effects observed in the study by Lovric *et al*. (2005) are attributable only to mercaptopropionic acid (MPA) and to the cysteamine coating the quantum dots.

In the same study by Hoshino *et al*. (2004a) cited by Hardman (2006), the viability of EL-4 cells incubated with CdSe/ZnS-SSA quantum dots diminished dose-dependently at a concentration greater than 0.1 mg/mL, and most of the cells incubated at a concentration of 0.4 mg/mL were not viable beyond 6 h. Moreover, no toxicity was observed after administration to nude mice by

intravenous injection of EL-4 incubated in 0.1 mg/mL with quantum dots conjugated to sheep serum albumin (Hardman, 2006).

A study by Voura *et al*. (2004) cited by Hardman (2006) showed no difference between the growth of melanoma cells (B16F10) exposed to CdSe/ZnS quantum dots coated with dihydroxylipoic acid (DHLA) at a concentration of 5 µL/mL and the growth of unexposed cells.

No cytotoxicity was observed in a study by Chen and Gerion (2004) cited by Hardman (2006) of cells HeLa transfected by CdSe/ZnS quantum dots conjugated to a nuclear localization signal peptide of the SV40 virus. Quantum dots had a minimal impact on cell survival at a concentration of about 100 nM (100 pmol/10^6 cells).

Kirchner *et al.* (2005) exposed the cytotoxicity of CdSe nanocrystal solutions and of CdSe / ZnS for tumour cells and human fibroblasts. This effect was greater if the nanocrystal coating was made of mercaptopropionic acid, an unstable coating, while more stable coatings (PEG-silica) reduced toxicity in the concentrations used. Phosphosilicate coatings increased the effect, producing Cd^{+2} ions within the cell. It must be noted that polymer-coated inert gold nanoparticles also had a cytotoxic effect comparable to that of CdSe / ZnS nanoparticles. The authors conclude that the toxic effect can be linked to the direct effect of precipitated particles on the cells and not only to production of Cd^{+2} ions.

Green and Howman (2005) conducted an *in vitro* experiment in which they incubated coiled double-stranded DNA in a cadmium selenide solution encapsulated in zinc sulphite functionalized with surface biotin. Ultraviolet (UV) radiation was also used. The results of this study show that the quantum dots altered the DNA by producing SO_2 free radicals, resulting from ZnS oxidation. The proportion of DNA alterations varied according to the presence (56%) or absence (29%) of UV. According to the authors, these results suggest that the mechanism by which quantum dots damage DNA is not only a free radical generation process linked mainly to light. The authors also observed DNA damage at time 0, which signifies that quantum dots have an immediate effect on DNA. DNA incubated alone in darkness or under UV light showed no damage.

Lovric *et al*. (2005) exposed rat pheochromocytoma cells (PC12) to red cationic CdTe quantum dots (5.2 nm; 37.5 µg/mL). After one hour of exposure, the authors observed a modification of the shape of the cells, chromatin condensation and formation of vesicles on the nuclear membrane (cellular toxicity indicators). After 24 h of exposure, 50% of the cells had a nuclear morphology indicating chromatin condensation. Lovric *et al*. (2005) also observed a decrease in cellular metabolic activity after 24 h of exposure to quantum dots (10-100 µg/mL). Green quantum dots (2.2 and 2.3 nm) were significantly more toxic than red quantum dots (5.2 and 5.7 nm) at the strongest concentration tested on the PC12 cells (100 µg/mL). The authors conclude that cytotoxicity of CdTe quantum dots is linked in part to particle size. Red and green quantum dots also triggered a decrease in metabolic activity in mouse microglia (line N9) after 24 h of exposure. The presence of serum in the medium protected the PC12 and N9 cells from the effects of both types of quantum dots. No decrease in PC12 cellular metabolic activity was observed after addition of *N*-acetyl cystein (NAC, a cadmium toxicity inhibitor) in the medium 2 h before exposure to red cationic quantum dots. Bovine serum albumin, whether conjugated or not to anionic

quantum dots (green 2.3 nm and red 5.7 nm) protected the PC12 cells against cytotoxicity. The protection provided by NAC and bovine serum albumin suggests that toxicity is partially attributable to the presence of Cd^{2+}. However, Trolox (vitamin E analog) had no protective effect at the same concentrations, which suggests that the quantum dot toxicity mechanism is not only linked to free radical formation (Lovric *et al*., 2005).

CdTe quantum dots tend to form aggregates in the biological fluids and can possibly form complexes with cellular proteins (Lovric *et al*., 2005).

A study by Hanaki *et al*. (2003) cited by Hardman (2006) showed no effect of CdSe/ZnS quantum dots coated with MUA and conjugated to SSA on the viability of Vero cells exposed to a concentration of 0.24 mg/mL (2 h of exposure followed by lavage of the cells and a new incubation). Quantum dots were observed in the endosomes and lysosomes near the perinuclear region of the cells 5 days after exposure. However, the authors point out that they do not know whether quantum dots affect cell viability when they are found distributed in the cytosol of the cells (Hardman, 2006).

Table 6 summarizes the documented health effects of quantum dots.

Table 6. Documented health effects of quantum dots[1]

Effect	Quantum dots[2]			
	Route			
	Inhalation[3]	Cutaneous	Oral	Other
Toxicokinetics		▲		▲
Irritation				
Systemic[4]				
Acute				
Intermediate				
Chronic				
Neurological				
Immunological				
Development				▲
Reproductive				
Genotoxic				▲
Cancer				

[1] Existing human (■) or animal (▲) studies. Adopted from the ATSDR.

[2] Quantum dots tested: CdSe/ZnS, CdTe, polyacrylic acid.

[3] Including intratracheal instillation.

[4] Systemic effects: acute (≤ 14 days), intermediate (15 to 364 days) and chronic (≥ 365 days).

10. HEALTH EFFECTS OF OTHER NANOMATERIALS

The diversity of the nanoparticles that can be synthesized is almost infinite. We have covered the substances to which most workers may potentially be exposed. However, with the rapid increase of new publications and new journals specializing in nanotoxicology, an exhaustive review of the risks and benefits related to these substances goes far beyond the objectives of this study. Some recent reviews covering these aspects are available (Zhao and Nalwa, 2007; Medina *et al*., 2007).

11. DISCUSSION

Several studies were performed with different animal species and humans to determine whether nano-scaled particles, ultrafine dusts, have harmful health effects. In most of the existing studies, the documented ultrafine dusts are undesirable secondary reaction products, such as welding fumes, diesel emissions, etc. However, nanoparticles essentially are new engineered particles. Their production relies on their unique properties, based on their small size, their large specific surface and the quantal effects, which allow consideration of new industrial and commercial perspectives. The introduction summarizes the current knowledge of toxicity of ultrafine particles. It clearly emerges that ultrafine particles, which have granulometric properties similar to engineered nanoparticles, show toxic effects of various natures in many organs, even if they are absorbed almost exclusively by the pulmonary route. These effects have been measured in animals and shown by different clinical and epidemiological studies in humans.

This literature review, the first edition of which had been published in 2006 (Ostiguy *et al.,* 2006a), focuses on the different nanoparticles synthesized intentionally for large-scale commercial use. They mainly include single-walled and multi-walled carbon nanotubes, organic and inorganic nanoparticles, quantum dots and dendrimers.

Soluble nanoparticles dissolve and their toxic effects are linked to their different components, regardless of the particle's initial size. These effects are well known depending on the chemical composition and are not described in this review. However, the situation is totally different for insoluble or very low-solubility nanoparticles (Oberdörster, 2005a, 2005b). The data currently available on the toxicity of insoluble nanoparticles is very limited and does not always allow a quantitative risk assessment (Kuempel, 2006) or extrapolation to humans for any of the engineered nanoparticles, except possibly for TiO_2 (NIOSH, 2005). In reading this report, it is easy to note the large number of situations for which there is absolutely no information currently available. Moreover, the information is presented by product class, and these classes include many products, each of which can display unique properties. For example, in the case of fullerenes, these nanoparticles may contain from 28 to over 100 carbon atoms. Information is available almost exclusively on the form containing 60 carbon atoms and with a purity, which, most of the time, is undocumented.

Nonetheless, the data currently available on some products reveal various information that, while preliminary, already allows us to conclude that engineered nanoparticles must be handled with care and that workers' exposure must be minimized, since these effects are extremely variable from one product to another. Accordlingly, the IRSST will publish, in 2008, a guide of safe practices suggesting different approaches to work safely with nanoparticles in occupational settings.

Absorption of manufactured nanoparticles

The first feature of nanoparticles is their pulmonary deposition mode, whereby the particles, even though they are very small, will be deposited throughout the pulmonary system and not only in the alveolar region. Oberdörster (2005a, 2005b) clearly shows that mucociliary clearance and phagocytosis are well-documented pulmonary clearance mechanisms for ultrafine or micrometric particles. Because of their extremely small size, nanometric particles can enter the extrapulmonary organs. This involves migration of solid particles, translocation, through the pulmonary epithelial layers to the blood and lymphatic systems and through the olfactory nerve

endings, along the neuronal axons to the central nervous system. Nemmar *et al*. (2002a) present three major hypotheses concerning translocation of ultrafine particles to the blood-air barrier in the alveoli. Transport is accomplished via active processes, such as phagocytosis and endocytosis of the alveolar macrophages and the endothelial cells respectively: passive transport by diffusion; passive or active transport through pores in the cytoplasm of endothelial cells or interstices between the alveolar epithelial cells.

Another potential important route of exposure for workers is cutaneous exposure. In fact, cutaneous absorption could be an even more important route of exposure than inhalation for workers handling nanomaterials prepared and used in colloidal form (Colvin, 2003). Liposoluble nanoparticles could possibly travel in the intercellular space of the corneal layer and pass through the cells, the hair follicles or the sweat glands (Monteiro-Riviere and Inman, 2006). The unvascularized zone of the skin could also serve as a reservoir for nanoparticles, from which they could not be eliminated by macrophages (Monteiro-Riviere and Inman, 2006). Nanoparticles absorbed by the skin can end up in the circulatory system after passing through all the skin layers (Monteiro-Riviere and Inman, 2006). Absorption can be facilitated if the corneal layer of the skin is damaged. In addition, the conditions of exposure in the work environment (the humidity rate, for example) can influence cutaneous absorptions. In the case of nanoparticles weakly absorbed by the skin, an allergy and/or contact dermatitis could be observed (Monteiro-Riviere and Inman, 2006). Warheit *et al*. (2007c) conclude that, in the vast majority of situations, potential pulmonary absorption in the work environment would be at least one order of magnitude greater than cutaneous absorption.

Good personal hygiene practices in the work environment should greatly limit ingestion of nanoparticles. Nonetheless, nanoparticles can be found in the gastrointestinal tract after elimination in the digestive system of nanoparticles coming from the respiratory system via the mucociliary escalator. Also, nanoparticles are now used as additives in the food industry, medications and certain associated products (Zhao *et al*., 2007b). When nanoparticles will be in widespread use in different industrial, agricultural or other products, a certain quantity will end up in the environment, among other places, due to industrial releases, wastewater or disposal of nanomaterials or products containing nanoparticles. In the environment, they may be absorbed by different bioorganisms and eventually enter the food chain.

The scientific studies examined to produce this literature review suggest a very different intestinal behaviour for nanometric particles compared to microparticles. Some experiments with rats or mice showed specific effects unique to nanoparticles following ingestion: severe symptoms of lethargy, anorexia, vomiting, diarrhea, and substantial loss of body weight and death of certain animals by intestinal obstruction (Wang *et al*., 2006). Translocation from the intestine to the blood and lymph is possible for certain nanoparticles and depend on size, surface charge, hydrophilicity/hydrophobicity, biological coating and bonds to different ligands, chemical coating with surfactants, etc. (Zhao *et al*., 2007a). For example, translocation would be greater for hydrophobic particles and for small particles.

Thus, insoluble nanoparticles can be found in the blood after passing through the respiratory, cutaneous or gastrointestinal protective mechanisms and distributing to the different organs throughout the body, including the brain, and being stored in the cells. Oberdorster *et al*. (2007), clearly show the scientific consensus regarding the propensity of nanoparticles to pass through the cell barriers. Once they have penetrated the cells, nanoparticles interact with the subcellular structures, leading to induction of oxidative stress as the principal nanoparticle action mechanism. These properties are currently very studied in pharmacology because they could allow use of nanoparticles as vectors to route drugs to targeted sites in the body. In the United States, the Food

and Drug Administration (FDA) has already approved the therapeutic use of certain NP (http://www.fda.gov/nanotechnology). On the downside, workers in some companies will be exposed by inhalation or by cutaneous contact and nanoparticles could be distributed throughout the body after absorption. Some of these nanoparticles have shown major toxic effects in studies of laboratory animals.

Principal parameters influencing nanoparticle toxicity

The toxicity of microscopic particles is normally well correlated to the mass of the toxic substance. The first nanoparticle studies clearly showed that toxicity, for a specific substance, varied substantially by size for the same nanoparticle mass and that toxicity was correlated to the surface of these particles rather than their mass (Oberdörster 2005b; Monteiller *et al*., 2007). Moss (2006) shows that the number of particles is just as important as their surface when assessing toxicity. Indeed, the functional changes in the macrophages indicated by the reduction in their ability to eliminate nanoparticles has already been shown to be well correlated to the surface of these particles. Moss (2006) maintains that these effects are not only related to the NP surface but to the number of particles because the NP deposited on the macrophages mask their surface. The author concludes that the number and the surface must be considered and help predict the potential obstruction of molecular mechanisms. It is considered that the macrophages are overloaded when the volume occupied by foreign bodies exceeds 6%. Moss (2006) studied the percentage coverage of macrophages by nanoparticles. He concludes that the cellular responses potentially linked to the NP surface may be due not only to the effects of surface chemistry but to obstruction of the cellular processes by the presence of particles or a combination of the two. If the quantity of particles present allows the cell to be covered more than twice, this means an overload and biologically significant physical obstruction. These two parameters (specific surface and number of particles) can be extraordinarily high for nanoparticles and thus directly influence their toxicity. Moreover, a large proportion of nanoparticle atoms are found on their surface, conferring great reactivity (Borm *et al*., 2006a; Fiorito *et al*., 2006a, 2006b; Powell and Kanarck, 2006). However, it must not be forgotten that most studies concern rats, a species particularly sensitive to the development of pulmonary reactions to low-solubility particles (Warheit 2006).

Nanoparticle toxicity can also be modified by surface properties, such as charge and reactivity (Hoet *et al*., 2004a; US EPA, 2005; Haasch *et al*., 2005). The charge of particles influences absorption and distribution of nanoparticles in the body, in particular (US EPA, 2005). For example, the study by Lockman *et al*. (2004) revealed greater permeability of the hematoencephalic barrier to anionic nanoparticles. Thus, the greater photocatalytic activity of Nano TiO_2 anatase is not the result of greater surface area, but instead depends on surface characteristics. Sayes *et al*. (2006b) hypothesize that the ability of Nano TiO_2 to produce free radicals is the critical parameter governing photoreactivity, toxicity and cellular inflammation. Individual susceptibility, interaction of particles with biological components and evolution of particles once they have entered the body are other factors that must be considered (AFSSET, 2006). Dobrovolskaia and McNeil (2007) clearly show that any surface modification can affect toxicity and that the compatibility of a nanoparticle with the immune system is largely dependent on surface chemistry.

Wallace *et al*. (2007) highlight the fact that NP entering the lungs will interact with the pulmonary fluids, particularly by adsorbing the surfactants present in the lungs to the particle surfaces. This conditions the particle surfaces and affects their expression of genotoxicity or cytotoxicity *in vitro*. These effects can be specific to NP surface composition.

Warheit and his team (2005, 2006) performed two pulmonary inflammation studies in rats with fine and nanometric quartz particles. A first study (2005) showed that nanometric particles (50 nm) produced a less intense and sustained pulmonary inflammatory response than micrometric particles (1600 nm). The second study led to the opposite conclusion. The authors conclude that many factors can influence the toxicity of these particles: number of particles, size, area and concentration, surface dose, surface coverage of the particles, degree of agglomeration of the particles and pulmonary deposition site, "age" of the particles, surface charge of the particles, their form, potential electrostatic attraction and capacity for agglomeration, method of synthesis of the particles and post-synthesis modifications. The nanotube studies also showed that the presence of metals could foster free radical formation and favour pulmonary inflammation. Moreover, several studies led to rat mortality, due to overloading and mechanical blockage of the lungs and not to specific toxicity of the tested product. Other researchers, including Borm *et al*. (2006b) point out that chemical composition and delivery of surface components, colloidal and surface properties of NP, compartmentation in the lung passages and biopersistence are other factors contributing another dimension of complexity to NP toxicity. The slow dissolution of certain NP or certain NP components in the body can become a major factor in their toxicity. Porosity and crystalline structure should also be considered (Oberdorster *et al*., 2005c). Warheit *et al*. (2007c) presented the conclusions of an expert workshop organized by ECETOX in 2005. They concluded that, for the time being, it is neither possible nor desirable to try to characterize exposure or toxicity based on a single parameter, such as specific surface, number of particles or any other parameter. However, Wittmaack (2007), exploiting the results already published in the literature regarding the inflammatory response of rat and mouse lungs subjected to different nanoparticles, concludes that several parameters give a linear dose-response ratio with the toxicity measured: number of particles, product of the number of particles by average size, and real specific surface. The specific surface estimated from the particle size did not provide a very good correlation, however.

Beyond all these parameters capable of influencing nanoparticle toxicity, Sayes *et al.* (2007a, 2007b) conclude that when the toxicity of different nanometric particles is considered, the comparison of results between the *in vivo* and *in vitro* tests often shows little correlation, particularly when different variables are considered. These authors conclude that the cellular systems *in vitro* will have to be developed better, standardized and validated in relation with the *in vivo* effects if they are to be useful as toxicity detection tools for different types of inhaled particles.

There now seems to be a consensus in the scientific community that the next nanoparticle toxicity studies will have to be based on extremely well characterized products (Hurt *et al*., 2006 ; Department for Environment, Food and Rural Affairs, 2006). For example, CNT contain a mixture of CNT, non-fibrous carbon products and metals, with different lengths and degrees of agglomeration, etc. The purification and suspension mode to perform toxicological studies can also lead to major modifications of the surface properties of these products, and their interaction with fluid and biological tissues.

The metals used during the synthesis of carbon nanotubes could thus contribute to the toxicity of these products in a variable way, depending on their degree of solubility and biological availability.

Table 7 summarizes the parameters most commonly identified in the literature as contributing to the toxicity of NPs. Proper characterization of NPs would later lead to the availability, once the toxicity mechanisms have been identified, of all of the information required to interpret the results. The only parameter that is currently the subject of a consensus is that the mass does not

allow the dose of NPs to be correlated with the measured effects. The mass should be documented as well, because it is often the only parameter that is known or that has been reported in earlier studies.

Table 7: Main parameters for proper characterization of nanoparticles for toxicological studies

Parameters
▪ Mass, concentration ▪ Chemical composition (purity and impurities) ▪ Solubility ▪ Specific area ▪ Number of particles ▪ Particle size and distribution ▪ Surface properties (charge/zeta potential, reactivity, chemical composition, functional groups, redox potential, potential to generate free radicals, presence of metals, surface covering, etc.) ▪ Shape, porosity ▪ Degree of agglomeration/aggregation ▪ Biopersistence ▪ Crystalline structure ▪ Hydrophilicity/hydrophobicity ▪ Site of pulmonary deposition ▪ Age of particles ▪ Producer, process and source of material used

Scope and limitations of the literature review

The number of publications on the toxicity of NPs is growing rapidly, and our literature review is interested only in the NPs synthesized for commercial use and for which the reviews remain fragmentary. This is why several natural and industrial pollutants, such as welding fumes, diesel motor emissions or even blast furnace fumes have not been considered, or have not been extensively documented, and why information on carbon black (IARC, 1996, 2006a; Frampton et al., 2004) or titanium dioxide (NIOSH, 2005; IARC, 2006b) has only been briefly presented, these products having already been the subject of detailed reviews.

In fact, the lack of current toxicological data is explained by the little information available, the complexity of NP-human body interactions, and the difficulty arriving at a definitive conclusion about the toxicity of a specific NP. The two main limiting factors seem to us to be related to the few studies on identical products by using methodologies and absorption pathways that are representative of occupational exposure, and to the often incomplete characterizations of NPs that do not ensure that identical NPs are being used. The upcoming availability of international reference products will at least allow identical products to be used in different laboratories and will therefore contribute to a better identification of the variables that affect toxicity.

With the high level of uncertainty relating to the toxicity of the majority of available NPs, as well as the almost complete lack of information on occupational exposure, it is difficult to evaluate quantitatively the specific risk of each NP. It is essential, in such a context, to take a preventive

and even precautionary approach, and to consider NPs as being very toxic. In order to facilitate the development and implementation of means of controlling exposure in a context of risk-related uncertainty, we will publish a good practices guide in 2008 that promotes safe work with NPs (Ostiguy *et al*., 2008b), as well as a review of different aspects that can be taken into consideration in evaluating risks and in selecting preventive measures (Ostiguy *et al*., 2008a).

Fullerenes

Several fullerene toxicity studies have been produced. Although no carcinogenic effects were observed after cutaneous application in mice, several toxic effects were reported following ingestion and injection in rats. Intraperitoneal injection disrupted reproduction in mice (mortality, malformation, etc.). Nonetheless, the existing information concerning the health effects of fullerenes remains very limited, as shown in Table 1. Contradictory results were obtained in genotoxicity tests of non-mammalian cells. The same is true of the studies by Sayes *et al.* (2005, 2007a), which conclude much greater toxicity of C_{60} than of $C_{60}(OH)_{24}$ in pulmonary epithelial cells *in vitro* but find an absence of toxicity of these two products *in vivo*. Workers' main potential exposure is pulmonary, yet Table 1 clearly reveals the total absence of information on inhalation absorption, and the total absence of human data. The cytotoxicity induced by C_{60} fullerenes is said to be mainly due to molecular surface modification by irradiation, derivation or functionalization (Fiorito *et al*., 2006a, 2006b). It seems probable that only modified fullerenes would be cytotoxic (Andrievsky *et al*., 2005; Fiorito *et al*., 2006a, 2006b).

Carbon nanotubes (CNT)

Carbon nanotubes can be single-walled (SWCNT) or multi-walled (MWCNT). Their purity can vary widely.

Huczko and Lange (2001a) did not observe any cutaneous irritation in humans and rabbits, or any in rabbit eyes. However, Shvedova *et al*. (2003) note the possibility of cancer and dermatological disorders associated with excess iron (alteration of pigmentation, inflammation, porphyria, etc.), among other consequences.

The exploratory study by Huczko *et al*. (2001b) could not show impairment of pulmonary function or any anomaly in bronchoalveolar lavage fluid in Guinea pigs exposed to SWCNT. Shvedova *et al*. (2003b) conclude that exposure to unrefined SWCNT can lead to increased pulmonary toxicity in workers due to oxidative stress, which in turn is related to the iron associated with SWCNT. Two studies showed that the types of SWCNT tested were capable of causing granulomas in rats and mice after acute exposure (Warheit *et al*., 2004; Lam *et al*., 2004a).

In the Quebec context, two hypotheses have been raised concerning the health risks of CNT exposure. At present they are exclusively speculative and will need to be proved:

1) formation of pulmonary granulomas and similarity with the effects of certain agents, particularly beryllium;
2) cancer due to the similarity with asbestos.

Formation of pulmonary granulomas was shown in rats and mice after a single exposure by intratracheal instillation. Their formation was observed upon exposure to several agents of occupational origin, particularly metals, and in certain diseases (tuberculosis, sarcoidosis, etc.). In the Quebec context, beryllium is receiving special attention among metals because of the recent significance of berylliosis in Quebec. Berylliosis is a disease that appears in the form of systemic

granulomatosis, with predominant pulmonary impairment, although several other organs can also be affected. Berylliosis can be attributed to an immunoallergic mechanism (Service du répertoire toxicologique, 2005). However, currently there is not enough evidence of the role of carbon nanotubes in the inflammation and the immunological component involved in berylliosis to allow adequate extrapolation from one to the other. The hypothesis has yet to be confirmed.

According to several authors (The Royal Society, 2004; Hoet *et al*., 2004a, 2004b; Harris, 1999; Donaldson *et al*., 2006; Poland *et al*., 2008) some carbon nanotubes (CNT) are similar to asbestos fibres. Contrary to nanoparticles, the toxicity of carbon nanotubes increases with their agglomeration (Swiss Engineering, 2006). These agglomerates resemble asbestos fibres both in appearance and toxicity (Swiss Engineering, 2006). From the toxicological point of view, carbon nanotubes are fibres and their toxicity will be correlated to their persistence in the lungs (Lam *et al*., 2006). However, Muller *et al*. (2006) point out that inhalation exposure to carbon nanotubes in industrial environments is very low, given their propensity to form agglomerates with an aerodynamic diameter above the respirability threshold (> 5 μm). However, from the study of Maynard *et al*., (2004), one could conclude that in most situations, generation of free airborne carbon nanotubes is difficult and then, the level of exposure in occupational settings should be extremely low in most situations. This does not mean that the respiratory toxicity risk is negligible, particularly in cases where nanotubes undergo any transformation (Muller *et al*., 2006). In a recent study, Poland *et al*. (2008) showed that exposing the mesothelial lining of the body cavity of a mice to long MWCNT results in asbestos-like pathogenic behaviour, including inflammation and formation of granulomas. Several types of CNT contain iron, e.g., 26.9% in raw CNT and 2.14% in refined CNT (Lam *et al*., 2004). Iron is also found in amphibolic asbestos fibres, accounting for about 30% of their weight. According to Shvedova *et al*. (2003), several researchers observed that an excessive quantity of iron accelerated the growth of neoplasic cells and that, in humans and animals, primary neoplasms develop at sites with rich iron deposits. They also mention that high exposure to ferrous materials in the work environment has been associated with the increased risk of lung cancer in workers. Harris (1999) mentions that chrysotile and CNT have a different tubular structure. The hypothesis of cancer based on the similarity of structure with asbestos seems plausible but has yet to be confirmed. This comparison recalls the asbestos problem in Quebec, with an increased incidence of pleural mesothelioma during the period from 1982 to 1996 (De Guire *et al*., 2003). In 2007, asbestos was still the substance causing the most death by occupational disease in Quebec (CSST, 2008).

In their review of the available data on toxicity of carbon nanostructures, Panessa-Warren et al. (2006) clearly show that the experimental protocols used sometimes have limited physiological meaning due to pulmonary overload or obstruction, as well as instillation-related stress in animals. Nonetheless, CNT seem to have greater pulmonary toxicity than carbon black or ultrafine quartz. It was also observed that non-biodegradable CNT pass rapidly through the alveolar walls to lodge in the living tissues (Hoet et al. 2004b; Oberdörster 2002). Table 2 summarizes the principal information regarding the toxic effects assessed for carbon nanotubes.

Inorganic nanoparticles

Cutaneous studies of TiO_2 in various sunscreen formulations did not show absorption beyond the dermis (in healthy skin) in human subjects. We did not find any other study of cutaneous exposure to nanoparticles that could be transposed to the work environment. However, in a study of crystalline silver nanoparticles in therapeutic application, Lam *et al.* (2004b) and Poon and Burd (2004) raise the possibility of cytotoxicity in lesioned skin or in growing human fibroblasts or keratinocytes.

Shimada *et al*. (2007), in a study of translocation of 14 nm carbon black particles by tracheal instillation, showed nanoparticle accumulation in the spaces between the alveolar epithelial cells with passage into the bloodstream, possibly resulting in cytoplasm shrinkage due to stimuli generated by nanoparticle mediated bonding with the alveolar epithelial cells, followed by occasional NP penetration of the alveolar membrane.

In a rat inhalation study intended to examine the role of the size of particles found in polluted air, Cassee *et al.* (2002) observed that the signs of pulmonary toxicity and pulmonary absorption of soluble $CdCl_2$ particles was greater for nanoparticles than for fine or coarse particles. The comparative study of other fine and ultrafine particles also seems to indicate that the effects of the same substance on the lungs, such as inflammation, fibrosis and cancer, are greater as the particulate dimensions decrease. Some authors postulate that nanoparticles could escape surveillance of alveolar macrophages and migrate to the interstitial compartment, the most vulnerable zone of the respiratory system (Oberdörster *et al.,* 1994; Warheit, 2004). Hohr *et al.* (2002) also had observed an increase in pulmonary inflammatory reaction in rats after inhalation of the nanoparticulate form of TiO_2, in comparison with the microparticulate form.

At the pulmonary level, rapid translocation of several types of nanoparticles to the bloodstream has been observed in several animal experiments (Kreyling *et al.,* 2002; Oberdörster *et al.,* 1994, 2002, 2005a, 2005b). This phenomenon can lead to redistribution of nanoparticles in organs such as the liver. Attempting to explain a link between air pollution and the incidence of cardiovascular diseases, Nemmar *et al.* (2002a) had observed rapid translocation to the bloodstream of 5 to 10 nm radioactive carbon particles inhaled by 5 human subjects. However, a recent study by Mills *et al*. (2006) casts doubt on the conclusions of Nemmar *et al*. (2002a). The authors studied translocation of 4 to 20 nm radioactive nanoparticles in 10 healthy volunteers after inhalation of the same gas used by Nemmar *et al*. (2002a), Technegas. Their results suggest that soluble Technetium-99 species are responsible for the increase in blood radioactivity. The authors conclude that the results reported by Nemmar *et al*. (2002a) evoke a high degree of contamination by Pertechnegas, a by-product of oxidation of Technegas.

In a recent study, Nemmar *et al*. (2005) did not observe translocation of modified polystyrene particles (24, 110 and 190 nm) from the alveolar space to the vascular compartment. However, the study was performed on the lungs of isolated and perfused rabbits and the relevance of these results for *in vivo* exposure has yet to be established (Nemmar *et al*., 2005).

Alternative translocation mechanisms have also been revealed. Some inhaled nanoparticles have passed through the hematoencephalic barrier to be identified in the cerebral zones. This phenomenon could also be secondary to migration of nanoparticles along the axonal routes, from the olfactory bulbs. According to Gatti and Rivasi (2002), ingested particles smaller than 20 µm (20,000 nm) can pass through the intestinal barrier and enter the bloodstream. Intestinal persorption has been revealed for colloidal gold nanoparticles (Hillyer and Albrecht, 2001).

One case study (Gatti, 2004) showed that certain nanoparticles and microparticles were found in the intestinal walls and seemed to be associated with inflammatory bowel diseases and intestinal cancer. Although it is recognized that the toxicity of nanoparticles is often linked to their small size, this factor does not always check out. Peters *et al.* (2004) show in their *in vitro* study that Ni, a recognized sensitizing agent, did not trigger a proinflammatory cellular reaction when administered in nanoparticulate form. Nanoparticulate TiO_2 induces such an effect, however, contrary to its microparticulate form. Several studies on the development of biopharmacological applications reveal a decrease in the general toxicity or cytotoxicity of colloidal gold (Hainfeld *et al.,* 2004; Paciotti *et al.,* 2004), selenium (Zhang *et al.,* 2005a) or arsenic trioxide (Zhou *et al.,* 2005) in nanoparticulate formulations, compared to non-nanoparticulate forms.

Regarding cellular effects, some studies report the cytotoxicity of nanoparticles (Peters *et al.,* 2004; Germain *et al.,* 2003) or their passage through the different cellular membranes (Tkachenko *et al.,* 2004). Table 3 summarizes the documented health effects of some inorganic nanoparticles.

Organic nanoparticles

Generally, organic nanoparticles are materials in which an active biological substance is trapped, encapsulated or adsorbed to the surface (Zimmer, 1999). Their main interest is in their use for transport and optimal targeting of medications.

Tests have been performed with various types of nanoparticles: polymeric nanoparticles (Kante *et al*., 1982; Couvreur *et al*., 1982; Gibaud *et al*., 1996; Sakuma *et al*., 2002), colloidal nanoparticles and spherical lipid nanoparticles (Fukui *et al*., 2003). The therapeutic results are promising, primarily for organic nanoparticles, but the data are insufficient concerning their toxicity in the work environment. Table 4 summarizes the documented health effects of some organic nanoparticles.

Nanocapsules, nanospheres, nanoshells, quantum dots and dendrimers

Nanocapsules, nanospheres and nanoshells are used primarily as pharmacological vectors in biopharmacology. Quantum dots are particularly used in medical imaging. Dendrimers can be used in production of nanotubes and nanocapsules and as diagnostic and therapeutic agents.

The published studies of these nanomaterials deal with the development of products with low toxicity or very specific properties (targeting an organ or tumour cells, bypassing the upper digestive tract, passing or not passing through the hematoencephalic barrier...). This subject goes beyond the scope of this document. There were few toxicity studies relevant to exposure of workers in the work environment.

The studies reveal that coating these nanomaterials can alter their charge, affinity for oil or water, or physiological stability. The results of the comparative studies of various forms of coatings vary widely and depend on the biomedical application developed.

The toxicity of certain quantum dots could be linked to the release of cytotoxic ions, oxidative mechanisms (Kirchner *et al.,* 2005) and other less well-elucidated phenomena (Shiohara *et al.,* 2004). According to Hardman (2006), several studies suggest that quantum dot cytotoxicity is caused by photolysis or oxidation. The study by Derfus *et al*. (2004) showed a greater reduction in the hepatocytic viability of rats exposed to quantum dots after subjection to UV radiation or air. In his review of quantum dot toxicity, Hardman (2006) points out that the exposure times were generally short (15 minutes to 8 h) in the studies in which no cytotoxicity was observed. Moreover, cadmium and selenium are both constituents the most often used in quantum dots and are recognized to cause acute and chronic toxicity in vertebrates (Hardman, 2006). Quantum dot toxicity is said to be linked to the stability of the surface coating (Hardman, 2006; Powell and Kanarck, 2006). According to Lovric *et al*. (2005), commercial quantum dots conjugated to proteins are larger than uncoated and unconjugated CdTe or CdSe quantum dots and are considered non-toxic at concentrations between 20 and 40 nmol. Table 5 summarizes the documented health effects of nanocapsules, nanospheres, nanoshells, and dendrimers. Table 6 summarizes the documented health effects of quantum dots.

12. CONCLUSION

This second edition of the literature review regarding the health risks of nanoparticles reveals the scope of current research in this field and observes that current knowledge of the toxic effects of nanoparticles is relatively limited. Nonetheless, the available data indicate that some insoluble NPs can pass through the different protective barriers, be distributed through the body and accumulate in certain organs and within cells. Toxic effects have already been documented at the pulmonary, cardiac, reproductive, renal, cutaneous and cellular levels. Significant accumulations have been shown in the lungs, brain, liver, spleen and bones.

While a first review concluded that toxicity was well correlated to the surface of the particles rather than to their mass, it now clearly appears that the situation is enormously more complex and that many other factors can influence the toxicity of these products. These factors include, in particular, size, number of particles, form and crystalline structure, tendency to aggregation, surface reactivity, chemical composition and solubility. Synthesis and surface coverage methods rank among other factors potentially affecting toxicity. Obviously, the risk assessment will also have to consider the route of exposure, its duration and its concentration, as well as individual susceptibility and interaction of particles with biological components and their biological destiny.

The development of new materials in this field is being pursued intensively and the health effects cannot all be studied (Won Kang and Hwang, 2004). Moreover, toxicological tests will have to consider that nanoparticles surfaces normally are altered to prevent aggregation. Currently, about 90% of TiO_2 nanoparticles go through a post-production treatment and are coated with mineral or organic substances (Borm, 2005). This treatment radically alters the toxicological properties of nanoparticles.

Although major trends can emerge and show many nanoparticles-related toxic effects, each product, and even each synthesized nanoparticles batch, may have its own toxicity. Any modification of the process or surface can have an impact on the resulting product's toxicity. The documented toxic effects on animals and the physicochemical characteristics of nanoparticles justify immediate application of all necessary measures, based on the preventive approach and the precautionary principle, to limit exposure and protect the health of potentially exposed individuals. In this context, the introduction of strict preventive procedures is still the only way to prevent any risk of occupational disease in researchers and students who develop these products and in workers who synthesize, transform or use nanoparticles.

Given the context, the authors will publish, in 2008, a guide on safe practices to work safely with nanoparticles in occupational settings. The authors also consider that the IRSST should favour the introduction of strict preventive procedures, which are still the only way to prevent occupational exposure. The authors strongly recommend that the IRSST concentrate its future research efforts on the development of exposure assessment strategies and tools and on the development and measurement of the effectiveness of methods for controlling occupational exposure to nanoparticles.

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