# Health Effects of Nanoparticles

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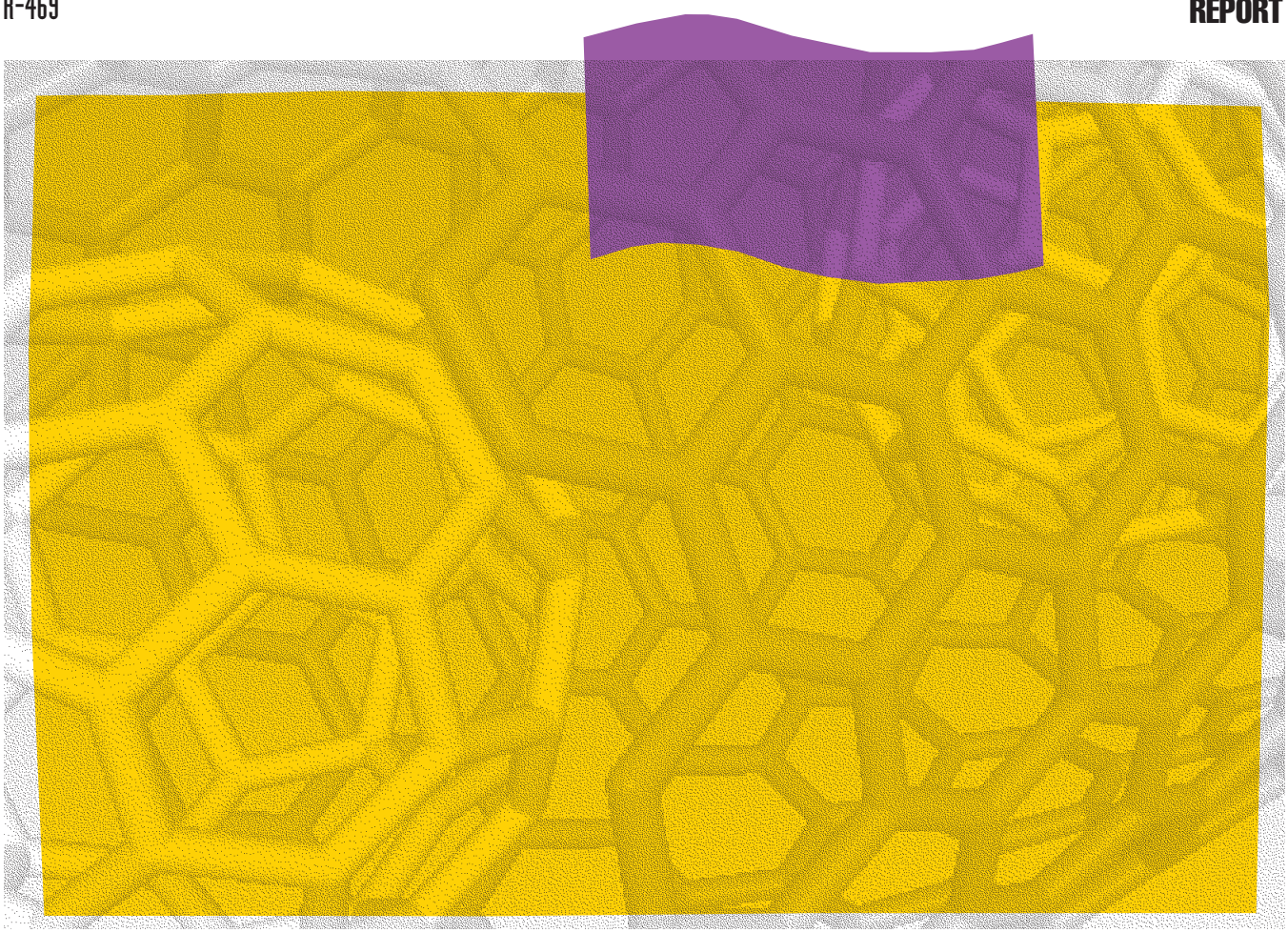
STUDIES AND RESEARCH PROJECTS

R-469

REPORT



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REPORT

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# SUMMARY

The nanoparticle and nanotechnology field is a fast-growing research niche[1]. Research has already led to significant breakthroughs, and several products are available commercially. It is currently anticipated that the number of exposed Quebec workers, both in use and processing and in production, will increase over the next few years. The purpose of this literature review is to determine whether nanoparticles represent health risks for workers. The chosen presentation format has the advantage of covering all the aspects that must be considered in toxic risk assessment. For some nanoparticles, the toxicological data are very partial, so this presentation format reveals the scope, and especially the limits, of the information currently available.

Nonetheless, several studies exist on the toxicological properties of nanoparticles. Although the various toxicological aspects and the diversity of the nanomaterials assessed are just beginning, many deleterious effects have been documented, particularly in animals. Insoluble or low solubility nanoparticles are the greatest cause for concern. Several studies have shown that some of them can pass through the various protective barriers of living organisms. The inhaled nanoparticles can end up in the bloodstream after passing through all the respiratory or gastrointestinal protective mechanisms. They are then distributed in the various organs and accumulate at specific sites. They can travel along the olfactory nerves and penetrate directly into the brain, just as they can pass through cell barriers. These properties, extensively studied in pharmacology, could allow organic nanoparticles to be used as vectors to carry medications to targeted body sites. The corollary is that undesirable nanoparticles could be distributed throughout the body of exposed workers. Some of these nanoparticles have shown major toxic effects.

Another special feature of nanoparticles is that their toxicity seems to be linked to their surface. This is a major difference from the usual situations, in which toxicity is normally linked to a product's mass. Nanoparticles are so tiny that small quantities (expressed in terms of mass), could have major toxic effects, because of their large surface. Several studies show much greater toxic effects for the same mass of nanoparticles as compared to the same product with a higher granulometry.

The available studies have shown several effects in animals, depending on the type of nanoparticles. Nephrotoxicity, effects on reproduction and genotoxic effects have been identified so far. Some particles cause granulomas, fibrosis and tumoural reactions in the lungs. Thus, titanium dioxide, a substance recognized as non-toxic, shows high pulmonary toxicity on the nano-scale. Cytotoxic effects have also been reported.

In general, the toxicological data specific to nanoparticles remains insufficient due to the small number of studies, the short exposure period, the different composition of the nanoparticles tested (diameter, length and agglomeration), and the often-unusual exposure route in the work environment, among other factors. Additional studies (absorption, translocation to other tissues or organs, biopersistence, carcinogenicity, etc.) are necessary to assess the risk associated with inhalation exposure and percutaneous exposure of workers.

[1] The IRSST report "Nanoparticles: Current knowledge about occupational health and safety risks and prevention measures" provides a literature review on the development of nanoparticles.

The limitations of the data currently available are especially significant, given that the diversity, quantity, quality, commercial production and use of the available nanomaterials will increase as new applications are developed. Currently, science has no methodologies to assess the toxicity of all of these new products on short deadlines and at reasonable costs.

Given the many unknowns related to nanoparticles, their potential health effects and the documented toxicity risks of ultrafine particles in humans, the establishment of strict prevention procedures is still the only way to prevent any risk of development of occupational diseases. The populations potentially exposed to nanoparticles should be prudent and apply safe measures of source elimination, exposure control and individual protection, both in production and in implementation and use of these products.

The development of Quebec expertise in nanotoxicology should be encouraged and focus primarily on the study of products developed and imported in Quebec. The aim should be to advise and support the various stakeholders in occupational health and safety, businesses and people concerned with research and development of nanotechnology-based products.

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# 1. INTRODUCTION

Our team has produced a comprehensive review of current knowledge on types of nanoparticles, their synthesis and applications, as well as the health risks associated and 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.*, 2006). The chapter that deals with this knowledge review on health risks is largely based on the present document, which focuses on integration of nanoparticle toxicity data from the literature. Nanoparticles 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).

The scientific literature is relatively rich in information on the toxicity of ultrafine particles, which are defined as having an aerodynamic diameter of less than 100 nm. Contrary to nanoparticles, ultrafine particles are undesirable products. They are often linked to atmospheric pollution and mainly come from undesirable products formed by combustion, often through industrial processes. Diesel emissions, welding fumes and fly ash are examples of such ultrafine particles.

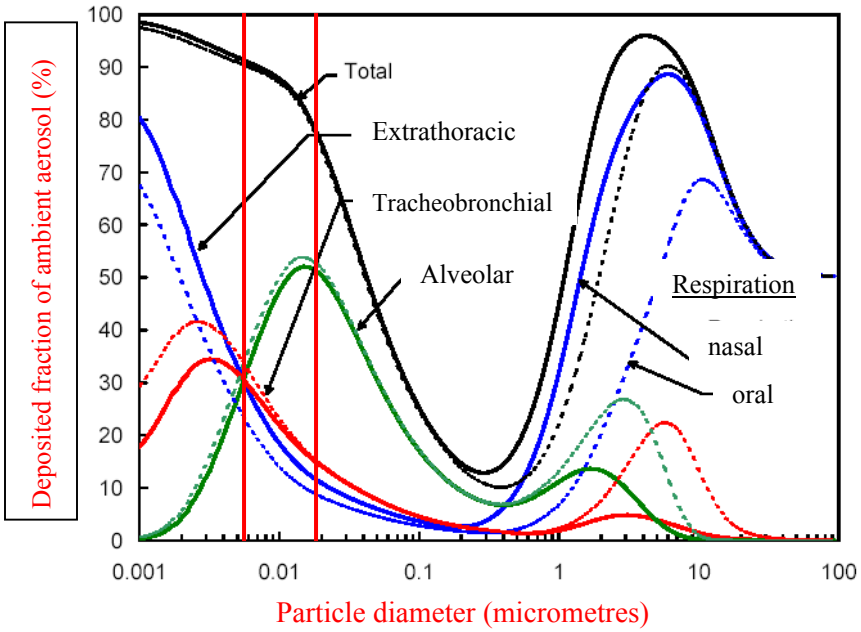
There is much more information on the toxicity of these combustion products, of the same dimensions as nanoparticles, than on the new nanoparticles. We will summarize part of the information available on these products to obtain an overview of the current knowledge regarding toxicity of nano-scaled particles.

## 1.1 Absorption and pulmonary deposition of ultrafine dusts

The respiratory tract system is the main route for dust entering the human body, followed by ingestion. 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 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 deposition of particles in the airway according to particle size (41). 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) 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 granulometry thus has a major impact on the pulmonary deposition site (Witschger and Fabriès 2005; Oberdorster 2005b). In several nanoparticle production processes, the granulometry can also vary considerably according to the stage of production. To understand dust behaviour and aggregation phenomena, see the IRSST report (Ostiguy *et al*., 2006).

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, 2005b). 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 (Kim and Jaques, 2000; Schiller *et al*., 1988; Jacques and Kim, 2000; Daigle *et al*., 2003; Oberdörster, 2005a, 2005b; Zhang *et al*., 2005b).

## 1.2 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 particles or particles with

low solubility. 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.

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, 2005b). 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. However, the macrophages are very efficient for coarser particles in the one to three micrometre range (Tabata and Ikada, 1988; Green *et al*., 1998).

Inefficient uptake of ultrafine 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 (Oberdörster *et al.*, 1992, 2000; Kreyling and Scheuch, 2000). 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 cross the epithelium, a fraction of the particles can reach the lymphatic nodules.

For nano-scaled ultrafine particles, it is now recognized that two other mechanisms contribute to reduce the concentration of particles in the lungs (Oberdörster, 2005a, 2005b). Ultrafine particles can pass through the extrapulmonary organs via the bloodstream. Some particles can be transported along the sensory axons to the central nervous systems. 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, a fraction of manganese 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 (Tjalve and Henriksson, 1999; Brenneman *et al.*, 2000; Dorman *et al.*, 2002; Ostiguy *et al.*, 2003, 2005; Salehi, 2005). 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 proved (Ostiguy, 2003, 2005).

## 1.3 Effects of ultrafine dusts

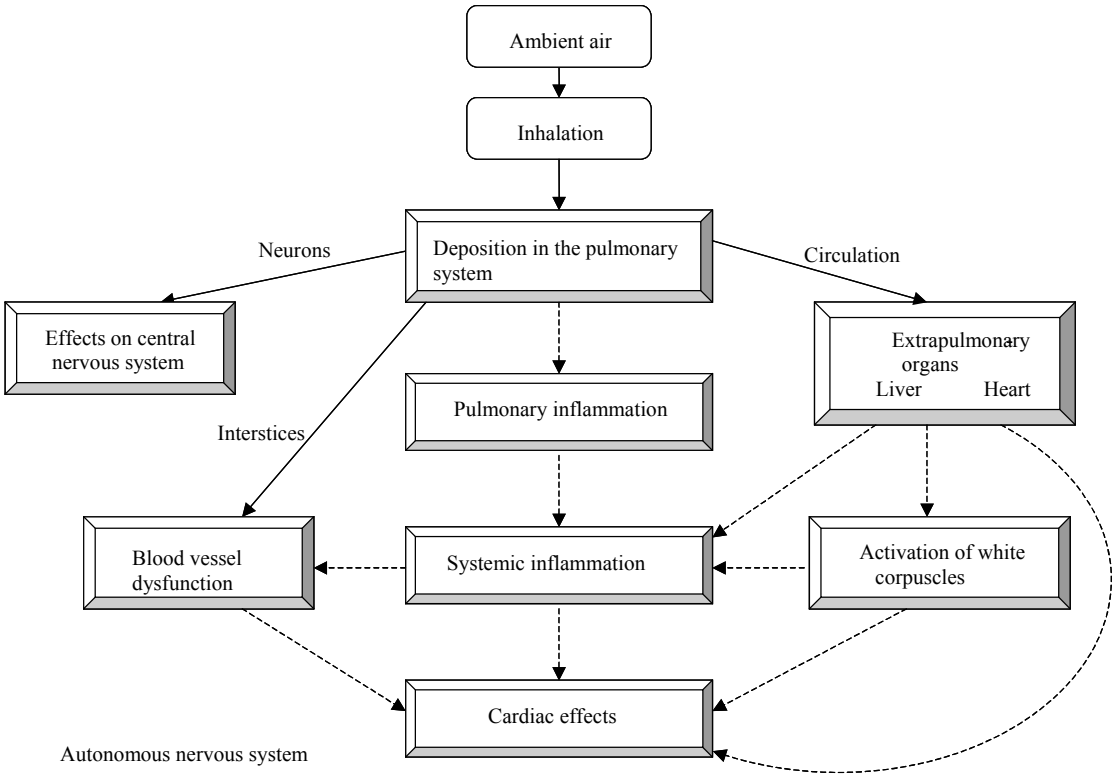
Several lung diseases related to fine dusts in the work environment have long been known: pneumoconiosis (silicosis, asbestosis), lung cancer, welder's disease, occupational asthma, berylliosis, etc. Donaldson (2005) produced a review of the current knowledge in the field. It clearly appears that pulmonary 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.

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 (Nemmar *et al*., 2002a; Elder *et al*., 2000, 2002, 2004; Zhou *et al*., 2003; Kreyling *et al*., 2002). 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 and arterial diameter. Several epidemiological studies (Wichmann *et al*., 2000; Peters *et al*., 1997, Penntinen *et al*., 2001, Pekkamen *et al*., 2002) 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 populations more fragile to respiratory and cardiac problems (Bruske-Hohlfeld *et al.*, 2005). Controlled clinical laboratory studies showed deposition of ultrafine dusts throughout the pulmonary system, accompanied by cardiovascular problems (Daigle *et al*., 2003; Brown *et al*., 2002; Pietropaoli *et al*., 2005; Oberdörster, 2005a, 2005b). 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.

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 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 (2005b) 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 1 summarizes the potential effects of inhaled ultrafine particles.

**Figure 2 : Potential effects of inhaled ultrafine particles according to Oberdörster G, (2005b). (Reproduced with permission of Dr. Gunter Oberdörster)**




Translocation of particles: ⟶

Mediator: ---------▶

Many international bodies are concerned about the health risks related to nanoparticles. The available documents prepared for these bodies include Aitken *et al*. (2004), Arnall (2003), Bodegal *et al*. (2003), Bordé *et al*. (2002), Christiansen (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).

## 2. OBJECTIVES

The purpose of this report is to summarize and classify the original articles identified in the scientific literature up to December 2004, pertaining to the study of the toxicity of nanoparticles synthesized for use in nanotechnology. A number of 2005 articles are integrated. The content was used in writing 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*., 2006).

## 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 the end of 2004. A few articles published in 2005 were also integrated into this knowledge synthesis. 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, puits quantiques, nanocristaux, drug delivery, ultrafine particles, nanomedicine and nanomédecine.

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 little information currently published and available.

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. These molecules are not modified and 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).

## 4.1 Toxicokinetics

### *4.1.1 Absorption*

No data

### *4.1.2 Distribution*

#### 4.1.2.1 Inhalation 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 $C_{60}$ is distributed throughout the embryo and the yolk sac of mice 18 hours after injection. Thus, it passes through the placental barrier (intraperitoneal administration, 50 mg/kg; day 18 of gestation).

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.

#### 4.1.2.5 In vitro

No data

### 4.1.3 Metabolism

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

### 4.1.4 Excretion

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). The authors reported the absence of a renal clearance mechanism.

## 4.2 Effects according to routes of exposure (administration)

### 4.2.1 Inhalation exposure

No data

### 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 Reproductive effects

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, 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 of initiation and promotion of carcinogenesis.

#### 4.2.2.7 Cellular and humoral effects

No data

### 4.2.3 Ingestion exposure

Chen *et al*. (1998) studied the acute and subacute toxicity of $C_{60}$ polyalkylsulfonate in rats. No mortality was observed in an acute oral toxicity test with doses up to 2500 mg/kg.

## 4.2.4 Exposure by other routes

### 4.2.4.1 Effects on the organs

#### 4.2.4.1.1 Effects on the skin and mucous membranes

No data

#### 4.2.4.1.2 Effects on the respiratory system

No data

#### 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 test with doses up to 2500 mg/kg, an approximate $DL_{50}$ of 600 mg/kg was determined by intraperitoneal injection (0, 500, 750 and 1000 mg/kg) and nephropathy sign 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 heart and the hematopoietic system

No data

#### 4.2.4.1.8 Effects on the nervous system

No data

### 4.2.4.2 Immunological and allergic effects

No data

### 4.2.4.3 Reproductive effects

No data

### 4.2.4.4 Development effects

An *in vitro* and *in vivo* study of the effects on development of mice was performed by Tsuchiya *et al*. (1996). 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.

#### 4.2.4.6 Carcinogenic effects

No data

#### 4.2.4.7 Cellular and humoral 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, trimalonic or quadrimalonic acid). Phototoxic inhibition of cell growth was greater for dimalonic than for trimalonic and quadrimalonic acid, in descending order. Sayes *et al*. (2004) studied the cytotoxicity ($CL_{50}$) of four water-soluble fullerenes on human cells *in vitro* (skin fibroblasts and hepatic caricinoma cells). They showed that toxicity varies with the nature of the functional group.

# 5. HEALTH EFFECTS OF CARBON NANOTUBES

Discovered barely a decade ago, carbon nanotubes are a new form of carbon molecule. 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) or several layers (like a poster rolled in a tube) 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.

## 5.1 Toxicokinetics

### *5.1.1 Absorption*

No data

### *5.1.2 Distribution*

#### 5.1.2.1 Inhalation exposure

No data

#### 5.1.2.2 Cutaneous exposure

No data

#### 5.1.2.3 Ingestion exposure

Hydroxylated single-walled carbon nanotubes (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)

#### 5.1.2.4 Exposure by other routes

The study by Wang *et al*. (2004) shows in mice that the radiomarked hydroxylated singled-walled carbon nanotubes 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 with 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 could be ingested by mouse peritoneal macrophages *in vitro*.

Monteiro-Riviere *et al*. (2005) found multi-walled carbon nanotubes (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). This demonstrates the capability of MWCNT to penetrate the cell membrane.

### 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 hydroxylated single-walled carbon nanotubes administered intraperitoneally had been excreted (94% in the urine and 6% in the feces).

## 5.2 Effects according to routes of exposure (administration)

### 5.2.1 Inhalation exposure

#### 5.2.1.1 Effects on the organs

##### 5.2.1.1.1 Effects on the skin and mucous membranes

No data

##### 5.2.1.1.2 Effects on the respiratory system

An exploratory study of pulmonary function in Guinea pigs was performed by Huczko *et al*. (2001b). No effect on pulmonary function (current volume, respiratory frequency and pulmonary resistance) and on analysis of bronchoalveolar lavage fluid was observed at the 25 mg dose. The number of animals is not specified and only one dose was administered.

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, an 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) 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.

Lam *et al*. (2004b) 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.

##### 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

No data

##### 5.2.1.1.7 Effects on the heart 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 Reproductive effects

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 humoral effects

No data

### *5.2.2 Cutaneous exposure*

#### 5.2.2.1 Effects on the organs

##### 5.2.2.1.1 Effects on the skin and 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 heart 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 Reproductive effects

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 humoral 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 mucous membranes

Shevedova *et al*. (2003a), 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.

#### 5.2.4.1.2 Effects on the respiratory system

Shevedova *et al*. (2003a), 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 Effects on the heart and blood circulation

No data

#### 5.2.4.1.7 Effects on the heart 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

No data

### 5.2.4.3 Reproductive effects

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

### 5.2.4.6 Carcinogenic effects

No data

### 5.2.4.7 Cellular and humoral effects

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*. (2005) performed a comparative study of the cytotoxicity of SWCNT, multi-walled carbon nanotubes (MWCNT) and the $C_{60}$ fullerene on alveolar macrophages in Guinea pigs. No cytotoxicity was observed for fullerenes. However, SWCNT showed higher toxicity than MWCNT. The dose of particles necessary to induce a reduction of macrophage phagocytosis was lower for SWCNT than for MWCNT and fullerenes.

# 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.

## 6.1 Toxicokinetics

### *6.1.1 Absorption*

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. This intestinal persorption phenomenon was exposed for unconjugated colloidal gold nanoparticles of 4, 10, 28 and 58 nm by Hillyer and Albrecht (2001). 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.

### *6.1.2 Distribution*

#### 6.1.2.1 Inhalation exposure

One of the first studies on the comparison of fine (250 nm) and ultrafine (20 nm) $TiO_2$ particles was published by Oberdörster *et al.* (1994). In this inhalation study in rats, the authors observed greater pulmonary retention of ultrafine particles. 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 ganglions, a phenomenon indicating penetration of the interstitial spaces. The ultrafine pulmonary clearance time was lengthened and translocation of these particles in the pulmonary interstitium was higher. The specific surface is the parameter best correlated with the observed effects.

To study the distribution of iridium-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 $\mu g/cm^{-3}$). 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 twice as great for 15 nm nanoparticles. Iridium nanoparticles are insoluble and were not absorbed in the intestine. The authors concluded that these nanoparticles were translocated to the organs, resulting in circulation of nanoparticles by the pulmonary blood vessels.

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 brain, the cerebellum and the olfactory bulbs of animals 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, may have contributed to transport of nanoparticles.

#### 6.1.2.2 Cutaneous exposure

Titanium dioxide ($TiO_2$) is a substance contained in sunscreens[2]. Lademann *et al.* (1999) did not observe significant absorption of coated $TiO_2$ nanocrystals (17 nm), beyond the stratum corneum 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 stratum corneum, 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 humans, 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 cold 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

[2] 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 cell DNA – and thus potentially cancer. This mainly would be linked to their photoinstability.

injection (tumour/kidney ratio = 0.4) and then were located preferentially at the tumour site (tumor/healthy tissue ratio = 8) and, to a lesser degree, in the liver (tumour/liver ratio = 1.8).

#### 6.1.2.5 *In vitro*

No data

### 6.1.3 *Metabolism*

No data

### 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 exposure*

In their inhalation studies, 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 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.

### 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.* (2004a) 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 ($7 – 55 \times 10^{-4}$%).

#### 6.2.2.2 Immunological and allergic effects

No data

#### 6.2.2.3 Reproductive effects

No data

#### 6.2.2.4 Development effects

No data

#### 6.2.2.5 Genotoxic effects

No data

#### 6.2.2.6 Carcinogenic effects

No data

#### 6.2.2.7 Cellular and humoral effects

No data

### *6.2.3 Ingestion exposure*

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 mucous membranes

No data

##### 6.2.4.1.2 Effects on the respiratory system

No data

##### 6.2.4.1.3 Liver effects

Zhang *et al.* (2005a) observed less hepatic function alterations in mice that ingested selenium nanoparticles (Nano-Se), compared to those to which non-nanoparticulate sodium selenite had been administered.

##### 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 intestine 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

No data

##### 6.2.4.1.7 Effects on the heart and the hematopoietic system

In an experiment intended to assess the blood compatibility of various forms of titanium oxide ($TiO_2$), 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 Reproductive effects

No data

### 6.2.4.4 Development effects

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

### 6.2.4.5 Genotoxic effects

No data

### 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 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.

### 6.2.4.7 Cellular and humoral effects

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, by 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.

Germain *et al.* (2003) compared the *in vitro* cytotoxicity of nanoparticles of a cobalt-chromium (Co/Cr) alloy[3] and alumina ceramic, which were produced by simulated wear of prosthetic joints. Two concentrations were tested on histiocytes pulmonary

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

fibroblasts in mice. Cell viability was tested 5 days after exposure. The Co/Cr nanoparticles (5 - 20 nm) triggered high cytotoxicity in human histiocytes, which depended on the concentration (respective reductions of cell viability[3] from 97% and 42% to 50 and 5 µm) and 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.

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 corpuscles 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.

In an experiment conducted by intratracheal instillation in rats, Hohr *et al.* (2002) exposed an increase in pulmonary neutrophiles, 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 neutrophile 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 coating by methylation played a marginal role in the inflammation parameters.

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 neutrophiles and proteins, increase in LDH activity). Cytokins 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.

Barlow *et al*. (2005) exposed bovine fetal serum to carbon black fine 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 carbon black fine particles. The effect seemed to be linked to an oxidative phenomenon, because it was reduced by adding antioxidants.

# 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 dimension.

## 7.1 Toxicokinetics

### *7.1.1 Absorption*

No data

### *7.1.2 Distribution*

#### 7.1.2.1 Inhalation 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 could be detected in the blood and in several organs, such as the liver and spleen but not in the heart and lungs.

#### 7.1.2.4 Exposure by other routes

Douglas *et al*. (1986) 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).

A literature review concerning the use of nanoparticulate systems in the cerebral transport of different drugs was produced by Kreuter (2001) and Lockman *et al*. (2002). The different systems used (coated or uncoated polymers, etc.) proved to be an effective tool in helping drugs pass through the hematoencephalic barrier (dalargin, doxorubicin, etc.) in several animal species after intravenous injections.

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.

#### 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 exposure

No data

### 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 the organs

##### 7.2.4.1.1 Effects on the skin and 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 an acute toxicity ($DL_{50}$) lethal dose 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 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 heart 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.

#### 7.2.4.3 Reproductive effects

No data

#### 7.2.4.4 Development effects

No data

#### 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 with 5 different *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.

#### 7.2.4.6 Carcinogenic effects

No data

#### 7.2.4.7 Cellular and humoral effects

No data

# 8. HEALTH EFFECTS OF NANOCAPSULES, NANOSPHERES AND NANOSHELLS

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 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 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.

#### 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.

## 8.2 Effects according to routes of exposure (administration)

### *8.2.1 Inhalation exposure*

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

No data

#### 8.2.4.2 Immunological and allergic effects

No data

#### 8.2.4.3 Reproductive effects

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 humoral effects

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

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.

# 9. HEALTH EFFECTS OF QUANTUM DOTS

A major field of research for about the past five years, quantum dots (also called nanocrystals or artificial atoms) represent a special form of spherical nanocrystals from 1 to 10 nm in diameter. They have been developed in the form of semiconductors, insulators, metals, magnetic materials or metallic oxides. 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).

## 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.

### *9.1.2 Distribution*

#### 9.1.2.1 Inhalation 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

In an intravenous study in 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.

#### 9.1.2.5 In vitro

No data

### *9.1.3 Metabolism*

No data

### 9.1.4 Excretion

No data

## 9.2 Effects according to routes of exposure (administration)

### 9.2.1 Inhalation exposure

No data

### 9.2.2 Cutaneous exposure

No data

### 9.2.3 Ingestion exposure

No data

### 9.2.4 Exposure by other routes

#### 9.2.4.1 Effects on the organs

No data

#### 9.2.4.2 Immunological and allergic effects

No data

#### 9.2.4.3 Reproductive effects

No data

#### 9.2.4.4 Development effects

Dubertret *et al.* (2002) injected (CdSe)ZnS quantum dots coated with *n*-poly(ethylene glycol) phosphatidyl ethanolamine (PEG-PE) and phosphatidylcholine (PC) into Xenopus frog embryo cells. They conclude an absence of significant toxicity for embryo development.

#### 9.2.4.5 Genotoxic effects

No data

#### 9.2.4.6 Carcinogenic effects

No data

#### 9.2.4.7 Cellular and humoral effects

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 cellular viability significantly and comparably for the three concentrations (6% cell viability). 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.

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. Polymer-coated inert gold nanoparticles also had a cytotoxic effect comparable to that of CdSe / ZnS nanoparticles. The authors conclude that the toxic effect may be linked to the direct effect of precipitated particles on the cells and not only to production of $Cd^{+2}$ ions.

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.

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.

## 10. HEALTH EFFECTS OF OTHER NANOMATERIALS

No study could be found on the toxicity of nanorobots, nanodevices, nanofilaments or other nanomaterials.

# 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 manufactured particles. Their production relies on their unique properties, based on quantal effects, which allow consideration of new industrial and commercial perspective. The introduction and the other report on nanoparticles (Ostiguy *et al*., 2006) summarize the main conclusions on toxicity of ultrafine particles. It clearly emerges that ultrafine particles, which have granulometric properties similar to 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 shown in animals and measured by different clinical and epidemiological studies in humans.

This study focuses on the different nanoparticles synthesized for the purpose of industrial use. They include fullerenes, single-walled and multi-walled carbon nanotubes, organic and inorganic nanoparticles, quantum dots, nanoshells, nanocapsules and nanospheres.

Soluble nanoparticles toxic effects are linked to their different components, regardless of the particle's initial size. These effects are well known and are not described here. 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 allow a quantitative risk assessment or extrapolation to humans for any of the synthesized nanoparticles. 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 only 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 very preliminary, already allows us to conclude that nanoparticles must be handled with care and that workers' exposure must be minimized, because several toxic effects have been documented.

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 micrometric particles. Because of their size, nanometric particles can enter the extrapulmonary organs. This involves migration of solid particles through the epithelial layers and through the nerve endings, along the neuronal axons to the central nervous system.

Thus, insoluble nanoparticles pass through the respiratory or gastrointestinal protective mechanisms and are distributed to the various organs throughout the body, including the brain. They are stored in the cells and end up in the bloodstream. These properties are currently being studied extensively in pharmacology, because nanoparticles could be used as vectors to transport

drugs to targeted sites in the body. However, nanoparticles can also be distributed throughout the body when workers inhale them. Some of these nanoparticles have exhibited highly toxic effects.

The toxic dose for microscopic particles is often expressed in mass units. However, for nanometric particles, this notion is no longer sufficient, because it does not account for the number of particles or the specific surface for a given mass of particles (Oberdorster 2005a). These two parameters can be extraordinarily high for nanoparticles and influence their toxicity directly. It is wiser to state the dose according to the characteristics of the nanoparticles (number and surface) instead of their mass. Several studies have shown clearly that the toxic effects measured are directly related to the surface of the nanoparticles put in play, and not their mass.

Nanoparticles have a natural tendency to agglomerate, meaning that they group together to form much larger particles (Ostiguy *et al*., 2006). Most industrial applications require unagglomerated particles. In such a context, producers use different post-synthesis strategies to prevent aggregation or stimulate deaggregation. The nanoparticle surface is modified for the intended application, often by coating the particle with a chemical (Borm, 2003). These surface changes can have a major impact on nanoparticle toxicity or safety. The manufacturer and the user must consider this reality, which can totally alter the toxicity of a specific product. Little information exists on the impact of these surface properties, except in pharmacology.

**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.). Contradictory results were obtained in genotoxicity tests of non-mammalian cells. Nonetheless, the existing information on the health effects of fullerenes is still very limited, as Table 1 shows. Worker's 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.

**Carbon nanotubes (CNT)**

Carbon nanotubes can be single-walled (SWCNT) or multi-walled (MWCNT). Their purity can vary widely. Toxicological studies are listed only for SWCNT.

Huczko and Lange (2001a) did not observe any cutaneous irritation in humans and rabbits, or any in rabbit eyes. However, Shvedova *et al*. (2003a) 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. Shevedova *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*., 2004).

**Table 1 : Existing information on the health effects of fullerenes**

| Effect | Route | | | |
|---|---|---|---|---|
| | Inhalation[2] | Cutaneous | Ingestion | Other |
| Toxicokinetic | | | | ▲ |
| Irritation | | | | |
| Systemic | | | | |
| Acute | | | ▲ | ▲ |
| Intermediate | | | | |
| Chronic | | | | |
| Neurological | | | | |
| Immunological | | | | |
| Development | | | | ▲ |
| Reproductive | | | | |
| Genotoxic | | | | ▲ |
| Cancer | | ▲ | | |

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

[2.] Including intratracheal instillation.

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) some carbon nanotubes (CNT) are similar to asbestos fibres. 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*. (2003a), 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*., 2004). In 2004, asbestos was still the substance causing the most death by occupational disease in Quebec (CSST, 2005).

**Table 2 : Documented health effects of carbon nanotubes**

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

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

[2] Including intratracheal instillation.

**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 on 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.* (2004) and Poon and Burd (2004) raise the possibility of cytotoxicity in lesioned skin or growing human fibroblasts or keratinocytes.

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 the 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.

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).

Gatti (2004) showed that certain nanoparticles and microparticles were found in the intestinal walls and seemed to be associated with inflammatory intestine 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).

**Organic nanoparticles**

Generally these are materials in which an active biological substance is trapped, encapsulated or adsorbed to the surface (Zimmer, 1999). Their 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.

**Nanocapsules, nanospheres, nanoshells and quantum dots**

Nanocapsules, nanospheres and nanoshells are used primarily as pharmacological vectors in biopharmacology. Quantum dots are particularly used in medical imaging.

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).

# 12. CONCLUSION

Nanotechnology is a fast-growing field of activity that will allow development of materials with brand-new properties. The number of Quebec workers exposed to nanoparticles should increase over the next few years, in a context in which the impact of nanoparticles on occupational health and safety is currently difficult to predict.

The current knowledge of the toxic effects of nanoparticles is relatively limited. Nonetheless, the available data indicate that some insoluble nanoparticles can pass through the different protective barriers, be distributed in the body and accumulate in several organs. Toxic effects have already been documented at the pulmonary, cardiac, reproductive, renal, cutaneous and cellular levels, while nanoparticles can be distributed throughout the body, including the interior of cells. Significant accumulations have been shown in the lungs, brain, liver, spleen and bones.

The pulmonary route is still the most likely exposure route in the work environment. It is important to realize that the nanoparticle deposition site in the lungs will be affected greatly by nanoparticle dimensions, which can change substantially throughout the production process. Because of their very small size, these particles offer a large contact surface per mass unit. It has been shown clearly that the degree of toxicity is linked to this surface and to the surface properties of these nanoparticles, rather than their mass.

Also bear in mind that the development of new materials in this field is being pursued intensively. For example, fullerene encapsulation in carbon or boron nitride nanotubes is in development and the effects have yet to be studied (Won Kang and Hwang, 2004). Toxicological tests will have to consider that nanoparticle 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). It has been clearly shown that surface coatings can radically alter the toxicological properties of nanoparticles.

The documented toxic effects on animals and the physicochemical characteristics of nanoparticles justify immediate application of all useful measures, based on the precautionary principle, to limit exposure and protect the health of potentially exposed individuals.

In such a 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.

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