Distribution and potential toxicity of engineered inorganic nanoparticles and carbon nanostructures in biological systems

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Biocompatibility, biodistribution, biodegradation, inflammation and interference with cells and normal functioning of organs, among other factors, will determine the toxicity of engineered inorganic nanoparticles and carbon nanostructures, and therefore the extent of their use. Recent examples in the literature show that engineered inorganic nanoparticles and carbon nanostructures, which may incidentally or intentionally enter into contact with living organisms, normally, at realistic doses, do not cause acute toxic effects. However, their prolonged interaction with living organisms may disrupt normal activity leading to malfunctioning and diseases. Indeed, observed nanoparticle-biological interactions, which can be used to detect and to manipulate biological states and therefore heal damaged organs, could also lead to environmental and human health hazards. In this scenario, how those nanostructures enter and are distributed inside the body is critical.

Keywords: Biodistribution; Carbon nanostructure; Disease; Environmental hazard; Health hazard; Inflammation; Interaction; Malfunctioning; Nanoparticle; Toxicity

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1. Introduction

Since society became aware of the use of nanomaterials in increasing quantities, in consumer products and their presence in the environment, interest in the impact of this emerging technology has grown. Besides their use in consumer products, nanoparticles (NPs) have also received enormous attention due to their potential applications in biology and medicine [1]. Recent developments in material physics and chemistry have allowed optical, magnetic and electrical detection of different states of biological systems and living organisms, where the characteristic biokinetic behavior of NPs is an attractive quality for applications in diagnosis and therapy (e.g., fluorescent labeling of cellular compartments [2], use of fluorescent or magnetic particles as contrast agents [2,3], magnetic separation [4] or targeted drug delivery [5]). NPs also serve as tools to investigate and to understand molecular processes in living cells [6]. NPs conjugated to biomolecules (e.g., elastin [7], antisenses [8], biotin-avidin [2], antigen-antibodies [9], peptides [10] and proteins [11]) are of great interest for biological detection, where the NPs can provide unique detection signatures. However, this unique biokinetic behavior of NPs, which makes them desirable for medical applications, may be associated with potential toxicity. The main concern is whether unknown risks of engineered NPs, in particular their impact on health and the environment, outweigh their potential benefits for society. For any application and future developments, a key issue is therefore accurate assessment of the potential toxicity of NPs.

It is known that foreign bodies, not only bacteria, viruses and parasites, but also minute inorganic matter, can cause various pathologies (e.g., silicosis, asbestosis or inflammatory reactions [12]), and, while there is a significant body of research on the effects of natural and fortuitous NPs (which occur as unintentional by-products of other processes, such as combustion), research on the health effects of engineered NPs is still in its infancy. Even if the same toxicological principles are likely to be applied to natural and engineered NPs, there are differences:

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(1) the polydisperse, chemical complex nature of natural NPs contrasts with the monodisperse, precise chemical characteristics of engineered NPs; and,
(2) the different particle morphology (often branched structures from combustion particles versus spherical forms in engineered NPs, even if other shapes, such as tubes, wires, rings and disks, are also manufactured).

Although humans have always been exposed to NPs of natural origin, from marine aerosols [13] to volcanoes or forest fires, or man-made NPs, starting with ancient cosmetics [14] or pigments (as the TiO_2 NPs found in the alveoli of a mummy over 5000 years old [15]), such exposure (not especially healthy [16]) has significantly increased in the past century due to anthropogenic sources, such as internal combustion engines, power plants, and many other sources of thermodegradation, which produce, among others, fullerenes (C_{60}) and carbon nanotubes (CNTs) [17]. This exposure may still dramatically grow in the years ahead when nanotechnology-based products will be common. It is also worth noting that biogenic NPs occur naturally in many species ranging from bacteria to protozoa to animals, as is the case for magnetosomes from magnetotactic bacteria, or ferritin, an iron-storage protein some 12 nm in size containing a core of 5–7 nm hydrous ferric oxide NP, also found in humans.

Despite the ongoing drive for interdisciplinary collaborations, the study of the biological interactions of nanotechnologically-designed objects still suffers from gaps between different disciplines. Chemists, physicists, and engineers create new advanced materials of sophisticated functionalities on a daily basis, but their understanding of biology is usually limited. This leads to studies where up-take of NPs by cells ignore if the incorporated particles are stuck in endosomal/lysosomal structures or free in the cytoplasm. However, in biological contexts, the up-take of NPs by cells is typically investigated with relatively-undefined NPs with large polydispersity, limited colloidal stability and unknown surface chemistry. Similarly, in the preparation of NPs, their surface functionalization, solvent, and state of agglomeration, for example, will modify the results for the same material [18]. Moreover, the optical activity of NPs and CNTs may interfere with readings in cell-viability tests [19]. Furthermore, finding information about nanotoxicology is complicated by the fact that NPs may be called ultrafine particles by toxicologists, Aitken-mode and nucleation-mode particles by atmospheric scientists, or engineered, nanostructured materials by materials scientists.

Finally, it is important to consider that the social burden of toxicity makes it difficult to reach conclusions at this early stage, and that results, even the more technical ones, may be biased towards toxicity or non-toxicity, depending on the context of the reporters (e.g., presenting a new medical device *versus* evaluating the toxicity of a particular material).

In this article, we summarize the observed environmental and bio-distribution of inorganic NPs and carbon nanostructures and their potential toxicity. We do not discuss organic NPs, polymer capsules or liposomes. Also, we do not discuss NPs used in some applications (e.g., as reinforcers, fire retardants or heterogeneous catalysts) in which the NPs are embedded in a solid matrix so that their accessibility is greatly reduced.

2. What is an engineered nanoparticle?

An NP can be considered as a small particle with at least one dimension less than 100 nm which presents novel properties that differ from the bulk material. This definition includes i) nanoclusters (amorphous/semicrystalline nanostructures with at least one dimension between 1–10 nm and a narrow size distribution), ii) nanopowders (an agglomeration of non-crystalline nanostructural sub-units with at least one dimension less than 100 nm) and iii) nanocrystals (nanomaterials with at least one dimension $\leqslant$100 nm and that are single crystalline), which includes metal, dielectric, and semiconductor materials (quantum dots), as well as hybrid structures (e.g., core-shell NPs). There is no a strict dividing line between NPs and non-NPs. The size at which materials display different properties to the bulk material is material-dependent and can certainly be claimed for materials larger in size than 100 nm. NPs are of great scientific interest as they are effectively a bridge between bulk and atomic or molecular structures. A bulk material should have constant physical properties regardless of its size, while at the nano-scale this is often not the case. The properties of materials change as the percentage of atoms at the surface of a material becomes significant. Size-dependent properties are also observed, such as quantum confinement in semiconductor NPs, surface plasmon resonance in some metal NPs and superparamagnetism in magnetic materials. Many of these nanomaterials are prepared as independent nanometric units but they rapidly aggregate losing their appealing nanoscale properties. If NPs need to be kept separate, surface engineering is needed in order to provide them with repulsion forces to prevent aggregation, either by electrostatic or steric means (Fig. 1).

In these terms, potential cytotoxicity of inorganic NPs and carbon nanostructures (CNs) can be attributed to the nature of NPs themselves and the special features that make them unique. In fact, their potential toxicity has been attributed to their small size [20], shape (e.g.: needlelike CNTs [21]), chemical composition (e.g.: reactive [22] and heavy [23] metals), their large and

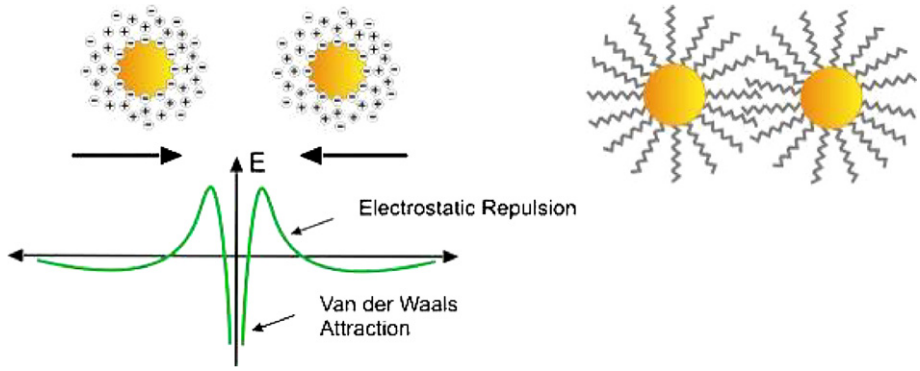

Figure 1. Stabilization of colloidal nanoparticles: (a) electrostatic; and, (b) steric. Modifications of the surface state may lead to irreversible aggregation that will strongly determine dispersion and distribution.

accessible inorganic surfaces (e.g.: TiO_2 NPs versus microparticles [24]), their interactions with cells (e.g.: CdSe/ZnS particles [25]) or some critical size (e.g.: Au_{55} attaching to DNA [26]). However, here we will not discuss the intrinsic reactivity of NPs; this and others related subjects can be found in reference [27].

2.1. Dispersion of nanoparticles in the environment

The dispersability and persistence of NPs in the environment is a key parameter for risk assessment since it will determine how susceptible living organisms are to contaminants. The dispersion in the environment will strongly depend on the ability of NPs to remain independent avoiding sedimentation, agglomeration or disintegration. Inorganic NPs are not very common in nature due to their instability. Thus, NPs' fate is either to aggregate with other materials, change nature (as iron oxides [28]) or disintegrate into atomic and molecular species (as CdSe [22]), which in turn will be, or transformed into stable species, or incorporated into other materials, in any case resulting in deactivation of the NPs. Of course, the aggregate or disintegrated species can be toxic, as micrometric SiO_2 particles (cause of Silicosis) or the Cd cations released from CdSe NPs [22]. Therefore, in some cases, NP should be considered as a pro-toxin rather than a toxin itself.

Before deactivation (aggregation or disintegration), NPs may be active in the environment. To date, environmental studies have been carried out in only a few species that have been accepted by regulatory agencies as models to define ecotoxicological effects. Tests with uncoated, water-soluble, colloidal C_{60} demonstrated that the average lethal concentration (LC50) in *Daphnia magna* after 48 hours is 800 ppb [29]. Lipid peroxidation in the brain and glutathione depletion in the gill were observed in largemouth bass (*Micropterus salmoides*) after exposure to 0.5 ppm of C_{60} for 48 hours [30]. However, mortality was not detected. Other studies showed toxicity of Al_2O_3 NPs in crops because the NPs perturbed the microbial substrate around the roots [31], reducing root growth. However, scientists have also found ways of using nanomaterials for environmental remediation. Recently, iron oxide NPs were proposed as useful materials to develop a low-cost technology for cleaning arsenic from drinking water [32]. Although many of these are still in testing stages, dozens of sites have already been injected with various nanomaterials [33]. However, as noted by Lecoanet et al, nanosized materials may not migrate through soils at rates that are rapid enough to be valuable in remediation [34]. Another interesting case reported recently, showed a significant potential for dispersion of nanostructures in aqueous environments, especially when natural organic molecules were present: multi-walled carbon nanotubes (MWCNTs) mixed with natural organic matter present in water from a non-polluted mountain river remained suspended for more than a month. Besides, the addition of MWCNTs to organic-free water makes the water become completely transparent in less than one hour. However, if the addition is to a sodium dodecyl sulphate (SDS) solution, the CNTs immediately turn the water dark and cloudy, and some MWCNTs remain suspended for more than a month, mirroring natural conditions [35]. This process - unspecific coating of NPs by organic molecules present in the environment- increases NP biocompatibility which facilitates its mobility in the environment.

2.2. Biodistribution and presence of inorganic NPs in tissues, organs and cells

Once NPs have been released in the environment and humans are exposed to them, the response of the body may be diverse. The biological activity and biokinetics of NPs depend on different parameters: size, shape, chemistry, crystallinity, surface properties (area, porosity, charge, surface modifications, coating), agglomeration state, biopersistence, and dose. These parameters are likely to modify biological responses, such as translocation across epithelia to other organs, induction of oxidative stress, binding to proteins and receptors, and localization in cellular organelles as mitochondria. In

general, translocation rates are largely unknown; they are probably very low but likely to change in a compromised/diseased state [36]. Most of the toxicity research on NPs *in vivo* has been carried out in mammalian systems, focused on respiratory system (RS) exposures. Other exposure routes, such as the Gastro Intestinal (GI) tract and the skin have also been studied.

Several defense mechanisms exist throughout the RS and GI tract keeping the mucosal surfaces free from cell debris and particles deposited by inhalation [37,38]. However, physiological barriers (the pulmonary and gastro-intestinal mucosa) may be ineffective in the exclusion of nano-sized particles from entering the organisms.

In addition, the presence of particulate-non-nanometric-inorganic matter (such as crystalline silica dust or asbestos fibers) in diseased tissues and internal organs [39]. For example, traditionally, detectable amounts of fine titanium dioxide powder, thought not to be easily absorbed, have been found in the blood, brain and glands with the highest concentrations being in the lymph nodes and lungs. Also, aluminum has been found to accumulate in the cells of the nervous system (and it has been found in the brain cells of Alzheimer's disease patients in high levels), and several reports also suggest that a high intake of aluminum may have adverse effects in the metabolism of phosphorous and calcium in the human body inducing or intensifying skeletal abnormalities such as osteoporosis. Finally, long, regular consumption of silver and gold can lead to kidney damage and a blue-grey discoloration of the eyes, nose and nasal septum, throat and skin. Of course, one needs to know where this inorganic matter comes from, how did it enter inside body and how did it change since then. In this regard, nanotechnology may have a lot to propose.

2.2.1. NPs and the respiratory system. Regarding nanotoxicity, one of the major concerns is of course inhaled NPs, and how deep they can enter during the breathing process, or persist in the air, depending on their size. In a simple model, the smaller the particle is, the further it may travel inside the body. However, as reactivity increases when size decreases [27], the traveling will depend also on the surrounding conditions. In addition, it has been observed that when airborne NPs pass through a specific channel (as the respiratory track), the smaller ones impact more often with the channel walls and get retained in a more efficient way because the Brownian motion increases as particles become smaller (Fig. 3). This effect will finally depend on the ratio between Brownian and translation motions. According to that, the fractional deposition of inhaled particles under conditions of nose breathing in different regions of the human respiratory track has been estimated by a predictive mathematical model [40].

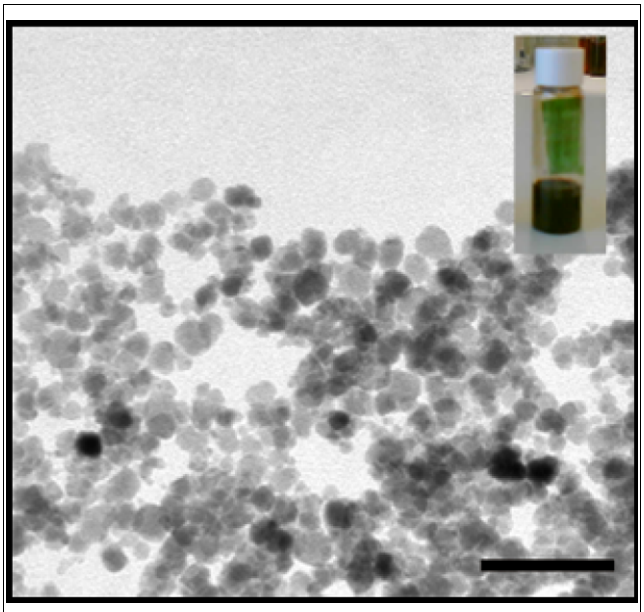

Figure 2. Iron-oxide nanoparticles (NPs): 80-kV transmission electron microscopy image of 7-nm iron-oxide NPs obtained by treating a solution of chloride (II) and (III) with an alkali, and subsequent oxidation of the precipitate. These NPs were obtained following processes (alkali co-precipitation) similar to those employed to obtain food additive E-172.

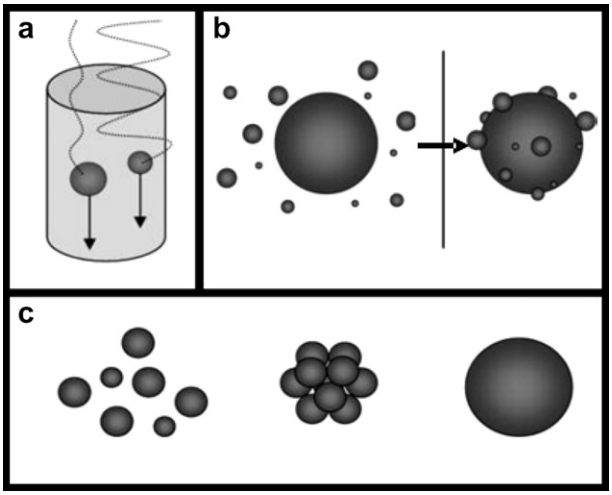

Figure 3. Traveling of airborne nanoparticles (NPs) of different sizes: (a) the ratio between the translation motion and the Brownian motion will determine how far NPs can reach inside a channel, in such a way that the smallest NP travels shorter distances in the respiratory track than medium-sized NPs; (b) in the case of polydisperse collections of airborne NPs, the large ones tend to scan the air collecting the smaller ones, so filtration of the larger ones helps the small ones to persist in the air and to travel farther; and, (c) effective size is important, as agglomerated NPs behave as a large particle (polycrystalline in nature).

These predictions apply to particles that are inhaled as individual particles of a given size. In all cases, significant amounts of a certain size of NPs (1–100 nm) are

deposited in the three regions of the respiratory tract (nasopharyngeal, tracheobronchial and alveolar). In the case of 1 nm NPs, 90% are deposited in the nasopharyngeal compartment, only approximately 10% in the tracheobronchial region, and essentially none in the alveolar region. For particles of 5 nm, the distribution is equal (approximately 30%) in the three regions. The highest deposition efficiency in the alveolar region (~50%) is obtained with 20-nm particles. In the case of larger particles, alveolar deposition progressively decreases while tracheobronchial and nasopharyngeal increase [40]. Related to that, it has also been observed that filtering a polydisperse collection of airborne NPs sequesters the larger particles leaving the smaller ones to pass. In this situation, the smaller ones become more persistent and travel further. In the case of non-filtered air, the larger ones serve to collect the smaller ones. Therefore, in the design of purification systems it would be necessary to let the larger ones collect the smaller ones, as a first step, and then pass the mixture through the filter.

Once the NPs have been breathed, a number of diverse processes involving physical translocation of inhaled particles, some of them showing significant particle-size dependence, have been observed. The most prevalent mechanism for solid-particle clearance in the alveolar region is mediated by alveolar macrophages, through phagocytosis of deposited particles. This process takes place within few hours. Essentially, all the particles are phagocytized by alveolar macrophages 6–12 h after deposition, and subsequently cleared by the slow alveolar clearance mechanism [41]. The retention half-time of solid particles in the alveolar region based on this mechanism is about 70 days in rats and up to 700 days in humans [36]. Another proposed mechanism involves transcytosis across epithelia of the respiratory tract into the interstitium and access to the blood circulation directly or via the lymphatic system, resulting in distribution throughout the body [36]. Berry et al. [42] described translocation of NPs across the alveolar epithelium using intratracheal instillations of 30-nm gold particles in rats already in 1977. They found large amounts of these particles in platelets of pulmonary capillaries after 30 min. of exposure. Researchers suggested that this is an elimination pathway for inhaled particles, transporting the smallest air-pollutant particles, including those from tobacco smoke, to distant organs. Since then, a number of studies with different particle types have confirmed the existence of this translocation pathway. Collectively, these studies indicate that particle size and surface chemistry (coating) govern translocation across epithelial and endothelial cell layers. In particular, regarding translocation of NPs in the lungs, the studies summarized by Mehta et al. [43] and those performed by Heckel et al. [44] using intravenous administration of albumin-coated gold NPs in rodents demonstrated receptor-mediated transcytosis (albumin-binding proteins). Similarly polystyrene particles of 240 nm translocated across the alveolus-capillary barrier when coated with lecithin, whereas uncoated particles did not translocate [45]. The presence of both, albumin (the most abundant protein in plasma and interstitium) and phospholipids, in alveolar epithelial lining fluid might be important to facilitate epithelial cells to up-take NPs after their deposition in the alveolar space. The small size may also facilitate up-take into cells and translocation across epithelial and endothelial cells into the blood and lymph circulation. Thus, from the respiratory track, NPs may travel to other places and interact with tissues prolonging their residence in the body (sometimes in unexpected places). Those particles could thus reach potentially sensitive target sites such as the heart. This translocation to the blood circulation could provide a mechanism for the deleterious observed effect on the cardiovascular system induced by inhaled ambient ultrafine particles. However, it has been also suggested that the harm observed in the heart is produced by the inflammation in the lungs caused by the presence of foreign matter [46], not that the foreign matter physically reaches the heart. Results on direct deleterious effects of ultrafine particles have been reported from epidemiologic and controlled clinical studies in humans, rodents, or *in vitro* cell culture systems [47].

Besides, distribution of NPs in the liver, kidney, and immune-modulating organs (spleen, bone marrow) has been commonly reported (Fig. 4) (*vide infra*).

An intriguing case is the translocation of solid particles in the respiratory tract involving neuronal axons. This pathway was described more than 60 years ago, when studies revealed that the olfactory nerve and olfactory bulbs were, indeed, portal entries to the central nervous system (CNS) for intranasal instilled nanosized polio virus particles. Therefore, the olfactory nerve pathway should also be considered as a potential entry for NPs. However, this has been tested in mice, and there are important differences between rodents and humans (one can argue that the olfactory route may only be an important transfer route in animals with a well developed olfactory system).

2.2.2. NPs and the gastrointestinal (GI) track. If NPs are not translocated in the lungs during the breathing process, they can be cleared from the respiratory tract via the mucociliary escalator and then ingested through the GI tract. Alternatively, nanomaterials can be ingested directly, for example, from food, water, cosmetics, drugs or drug-delivery devices. Another way to ingest and disperse inorganic fine particulate matter are food additives, where inorganic materials corresponding to some of the most common NPs are listed, such as Au (E175), Fe_3O_4 (E172), TiO_2 (E171), Al_2O_3 (E173), etc, (Table 1) having submicrometer or smaller

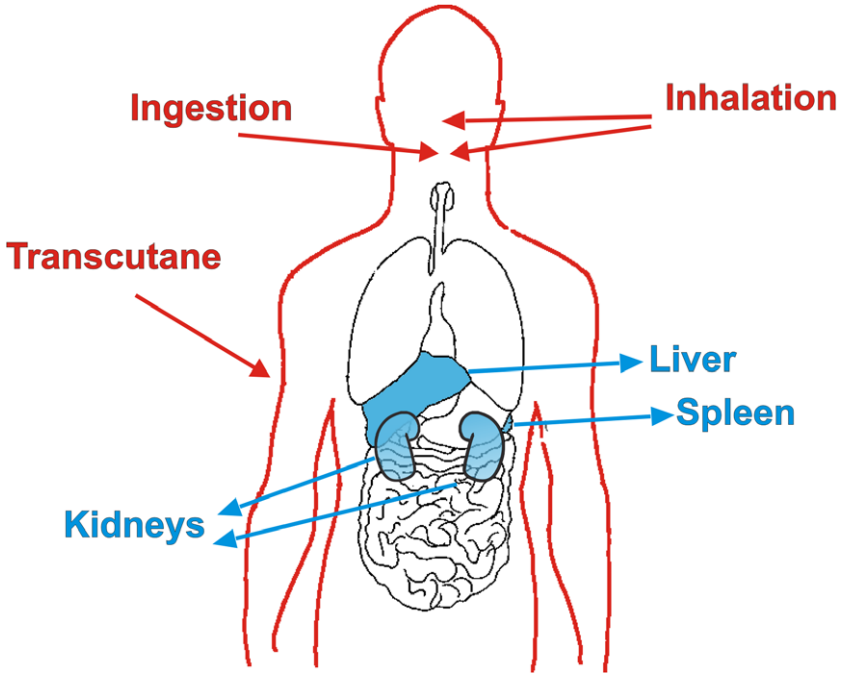

Figure 4. In-body biodistribution of nanoparticles (NPs): the portals where NPs enter the body (other than intravenous or muscular injection) are the dermis, the respiratory track and the gastro-intestinal track. Studies have repeatedly found accumulation of NPs in organs with high phagocytic activity, mainly in the liver (up to 90%), then the kidneys and the spleen, among others.

sizes. For example, iron oxides and hydroxides usually manufactured by treatment of ferrous sulphate or chloride solutions in alkaline conditions, and the subsequent oxidation of the precipitate in hot air yield 7-nm NPs (Fig. 2). Fortunately, since the iron present in these oxides is mainly in the ferric form, it is not very available to body tissues. However, those additives are banned in some countries, such as Australia or Germany.

Only few works have studied the uptake and disposition of nanomaterials by the GI tract, and in most of them NPs passed through the GI tract and were rapidly eliminated [36]. In rats treated orally with radiolabeled C_{60}, water solubilized using PEG and albumin, 98% were cleared in the feces within 48 hr, whereas the rest was eliminated via urine, indicating some up-take into the blood circulation [48]. In contrast, when administered intravenously, 90% of these radiolabeled fullerenes were

Table 1. The use of nanoparticles as food additives

	Substance	Food Additives	Effects
E-171	Ultrafine Titanium Dioxide Power	• Tables and Capsules	• Detectable amounts in the blood, brain and glands
		• Cottage and Mozzarella cheses • horseradish cream • sauces, lemon curt and sweets	• High concentration in the lymph nodes and lungs
E-172	Iron oxides and Dioxides	• Cake, dessert mixes • Meat paste, salmon and shrimp paste	
E-173	Aluminium	• External decoration in cakes	• Cells of nervous system; brain cells accumulated in neurofibrillary tangles and nueritic plaques.
		• Antacid treatments • Tap watter drinking supply	• Skeletal abnormalities: osteoporosis
E-174	Silver	• External Decoration	• Kidney damage, blue-grey dislocation of eyes
E-175	Gold	• External Decoration	

retained one week, mostly in the liver (80%, depending on time course). Other studies using ultrafine ^{192}Ir did not show significant up-take in the GI tract [41,49], whereas earlier studies with larger TiO_2 particles (150–500 nm) found up-take into the blood and movement to the liver [50,51]. Apparently, there are differences in GI tract up-take also depending on both particle surface chemistry and particle size. Indeed, Jani et al. [51] found a particle–size dependence in the up-take of polystyrene particles (ranging from 50–3000 nm) by the GI, mucosa after oral doses in rats. This up-take (6.6% of the 50-nm particles administered, 5.8% of the 100-nm NPs, 0.8% of 1-μm particles, and 0% for 3-μm particles) was mainly via the Peyer's patches with translocation into the mesenteric lymph and then to the systemic organs (i.e., liver, spleen, blood, bone marrow, and kidney).

2.2.3. Cutaneous exposure of NPs. Another important up-take route is through dermal exposure. The dermis has a rich supply of blood and tissue macrophages, lymph vessels, dendritic cells, and five different types of sensory nerve endings. An increased inflammatory activity and epithelial translocation of manmade 20-nm and 30-nm solid particles were observed already 20 years ago [52]. Broken skin represents a readily available entry even for larger (0.5–7 μm) particles, as evidenced by reports about accumulation of large amounts of soil particles in inguinal lymph nodes from people who often run or walk barefoot [36]. However reports show that broken skin is not necessary for uptake of NPs. Tinkle et al. [53] showed that skin when flexed—as in wrist movements—can make the epidermis more permeable to NPs, and then favor uptake into the lymphatic system and regional lymph nodes. In those studies, a solution of buckyball-containing amino acids was placed on small sections of pig skin. In some of the experiments, the skin was held still, and in others it was flexed for either an hour or an hour-and-a-half. Measurements were taken eight hours after exposure and 24 hours after exposure. The more the skin was flexed, the more buckyballs were taken up and they penetrated deeper. Penetration was also found to be deeper after 24 hours than just after eight hours. Similarly, it has been shown that repetitive movement can speed up the up-take of NPs through the skin [54], as happens for conventional anti-inflammatory gels, where massaging the affected area translocates the gel to the swollen tissue.

Studies by Kim et al. [55] in mice and pigs with intradermal injected near-infrared quantum dots confirmed that NPs, once having crossed the dermis, will be localized in regional lymph nodes then being useful for *in vivo* imaging. The transport mechanisms to the lymph nodes, are via skin macrophages and dendritic (Langerhans) cells [56]. From the lymph nodes, they may reach the liver and the kidneys by venal translocation. However, the potential of sensory skin nerves to take up and translocate NPs has to be considered. This mechanism has been demonstrated for the nasal region [47], so, it will be interesting to know if this occurs in the dermis with its dense supply of different types of sensory nerves.

2.2.4. NPs inside the body beyond the portal of entry. In general, on exposure to the body, particles of different surface characteristics, size and morphology attract different arrays of serum proteins and opsonins. An opsonin is a substance present in the body which binds to the surface of foreign particles and microorganisms making them more susceptible to the action of phagocytes of the immune system. A second step consists of the endocytosis/phagocytosis of the particles, generally by the circulating monocytes or the fixed macrophages, leading to their elimination from circulation and their simultaneous concentration in organs with high phagocytic activity. Thus, after translocation form the RS, the GI, the skin or intravenous administration, generally, NPs are cleared within minutes from the bloodstream and their typical final biodistribution is in the liver (over 90%) and the kidneys (up to 9%). In general, the larger ones are retained first in the liver and the ones that pass this filter end up in the kidneys. From there, NPs are expelled with the feces or the urine. Once the particles are secreted from the body, they will be either dissolved, agglomerated or absorbed onto sediment and soil particles and finally immobilized because of their high surface-to-volume ratio [6]. However, the ingestion of those sediments could introduce the nanomaterials back into the food chain.

The liver is the major receptor site via Kupffer cells, followed by the kidney, the spleen and other organs of the reticulum endothelial system. Surface modifications, such as coating with polyethylene glycol (PEG), may prevent hepatic and spleen localization, opening the possibility of other organs to be targeted [6]. These NP collectors are regenerated within days (weeks) and expelled, and then the NPs disappear from the body. However, recently, it was hypothesized that NPs could be the nucleation sites for kidney stones [57]. As an example, researchers have determined that CNTs injected directly into the bloodstream of research lab animals cause no immediate adverse health effects and circulate for hours before being removed by the liver [58]. Similarly, Nemmar and co-workers showed that an aerosol consisting mainly of 100-nm carbon particles radiolabeled with ^{99m}Technetium (*Technegass*) passed through the lung barrier in 60 sec and in 1 hour reached the liver [59]. They measured the distribution of radioactivity in five healthy volunteers after the inhalation. Radioactivity was detected in blood already after 1 min. reaching a maximum 10–20 min., and remained at this level up to 60 min. Afterwards, the NPs were accumulated in the liver prior to their elimination. In a similar

work, Brown and colleagues observed the same facts [60].

2.2.5. NPs entering the cells. Once NPs have entered the body, and they have been distributed, the next step is to investigate their penetration into cells crossing the cytoplasm membrane. The cytoplasm membrane controls entries into the cell and it has a crucial role in development, uptake of nutrients, the immune response, neurotransmission, intercellular communication, signal transduction, and cellular and organism homeostasis. Particles can enter cells through various up-take mechanisms, depending mostly on their size. Essential small molecules, such as amino acids, sugars and ions, can pass through the plasma membrane by the action of integral membrane protein pumps or channels, while larger molecular entities must be carried into the cell via membrane-bound vesicles formed by invagination and pinching-off pieces from the plasma membrane in a process termed endocytosis [61]. This cell penetration mechanism (endocytosis) can be performed actively (receptor mediated) or passively (Fig. 6). These include the up-take of large particles (0.25–10 μm) by phagocytosis, performed by specialized cells, such as macrophages and neutrophils, and a variety of other endocytic processes at a smaller scale. Receptor-mediated routes require recognition of some ligand (surface molecule or epitope) by a specific biological receptor and involve a vesicle of a defined size of 100 nm. However, the receptor-mediated routes of up-take do not account for all uptake of material into cells, and other mechanisms, which include macropinocytosis, micropinocytosis, clathrin-mediated endocytosis, caveolae-mediated endocytosis, and clathrin- and caveolae-independent endocytosis, are also present [61]. All these up-take endocytic routes involve delivery of material into a sub-cellular compartment, the endosome, which is still separated from the cytoplasm of the cell by a membrane. Most of these endocytic routes also end up in a degradative compartment of the cell, the lysosome, where materials are exposed to high concentrations of a wide variety of hydrolytic enzymes active on proteins, polysaccharides and nucleic acids. Active internalization mechanisms have been observed mainly when the NPs were coated with vectors that activate specific cell-membrane receptors; otherwise NPs stick to the cell membrane from where they are passively internalized. During this process, cell activity or viability may be compromised. In fact, intense uptake may cause cell activation by itself, and internalized particles may also cause cellular stress,

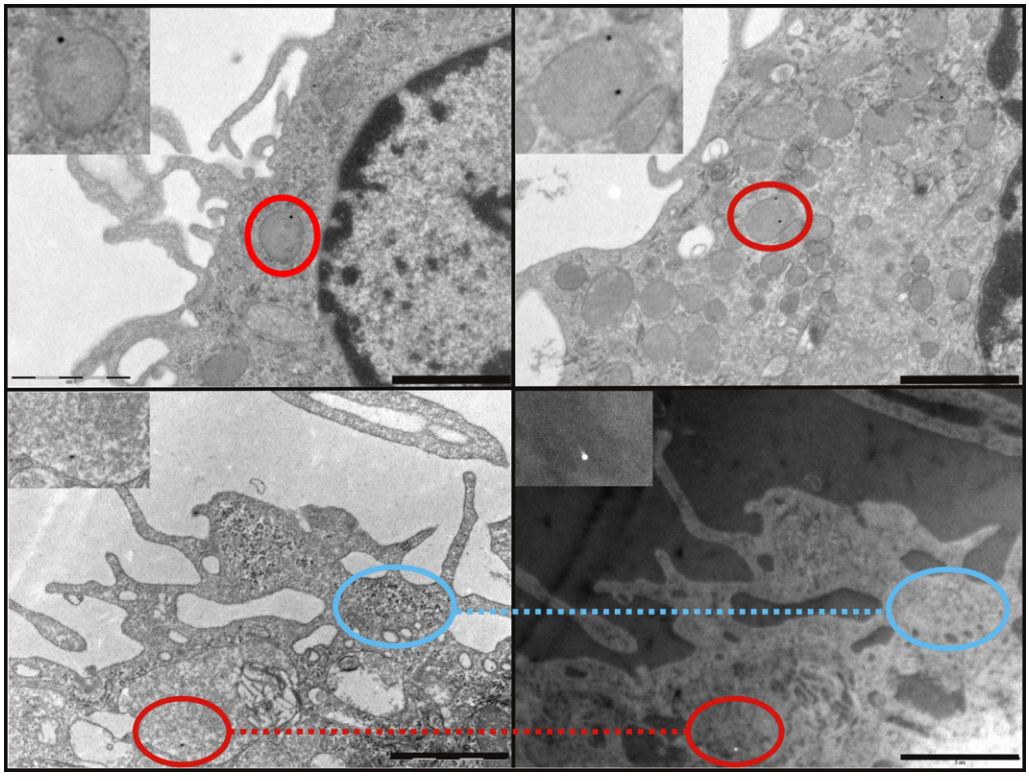

Figure 5. Gold nanoparticles (AuNPs) phagocytized by the macrophages of the immune system. 80-kV transmission electron microscopy images of murine bone-marrow macrophages: (top) resin-tomography images revealing internalization of peptide-coated AuNP. Peptide sequence: CLPDFF; (bottom) dark-field imaging and high contrast of the AuNPs allowed to them to be easily distinguished (red circles) against ribosome and other high-contrast organelles (blue circles). Particles are 10 nm in diameter. Insets are details of the images. Scale bar is 1 μm.

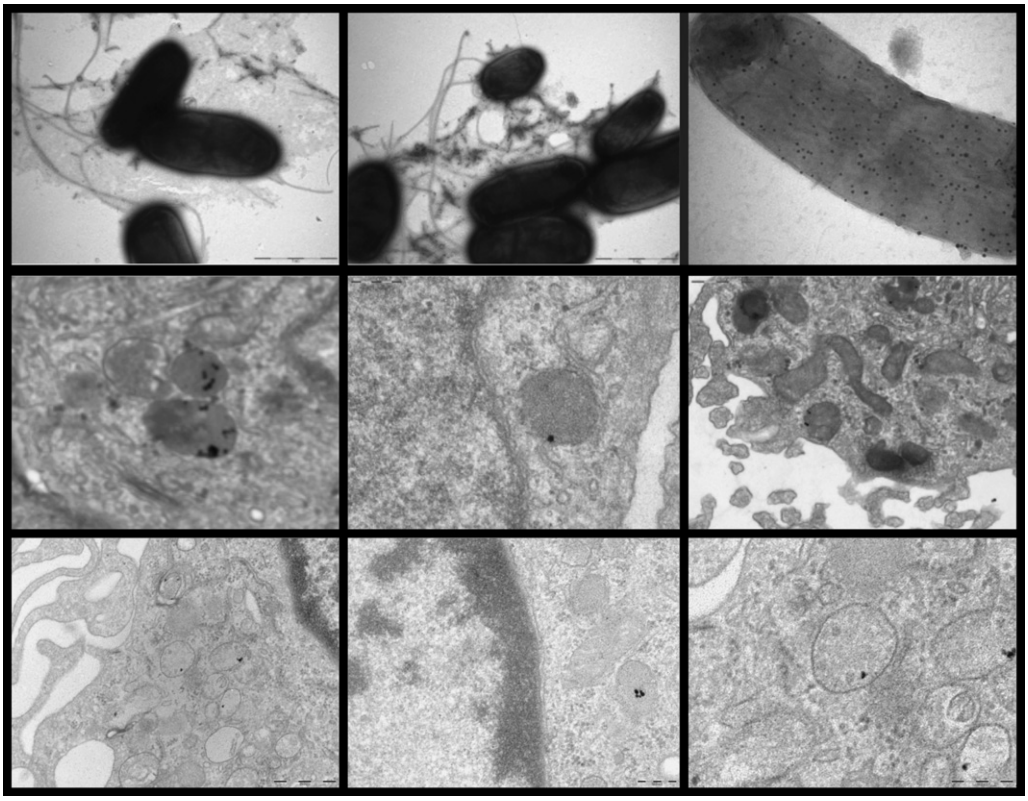

Figure 6. Transmission electron microscopy images of cells and internalized nanoparticles (NPs): (top) Bacillus + β-Amyloid fibers + gold nanoparticles (AuNPs); (middle) AuNP + Hela cells; and, (bottom) Macrophages + AuNP. In the two first cases, NPs have been passively internalized via cell-membrane recycling, and the third actively mediated by membrane receptors. To distinguish active from passive mechanisms both the NP-internalization and macrophage-activation time, and the temperature dependence of the endocytosis, serve to discriminate between the active and the passive mechanisms. AuNPs are 10 nm in diameter. (Middle pictures in collaboration with Silvia Pujals and Ernest Giralt (PCB-UB)).

resulting in stress-related signal transduction and further cell activation. It is worth mentioning that cells are highly sensitive to signals from the environment and easy to stress. For example, cellular reproduction is regulated by (complex) external signals and synchronized with neighboring cells. Under stress conditions (a non-expected modification of the environment), the cell halts temporarily its reproduction cycle until it readjusts to a new situation. If this process does not function properly, erroneous cellular duplication may be induced and may generate into tumoral cells.

It is believed that NPs do not remain for a long time in the endosome before they are expelled again (in an exosome), all without ''entering'' the cell, i.e., without reaching the cytoplasm or the nucleus. Release from endosomes and reaching the cytoplasm is difficult. Some of the strategies performed to obtain cytoplasmatic release from the endosomes are isotonic shock or use of disrupting peptides [62,63].

If NPs stay inside the cell, their fate will be bonded to that of the cell. Since cell recycling keeps the body continuously regenerating, their permanence in the body will be limited and they will disperse in time. The cells turnover rate varies from weeks in tissues, such as the skin and gastro intestinal track, to years in bone or neurons. There are a few substances, such as heavy metals, which escape from these recycling activities, forming organic species (such as methylmercury in the case of Hg^{+}), which are liposoluble and tend to accumulate, resulting in them being toxic. Another case of extended permanence of inorganic matter in the body is granulomatosis (such as silicosis, asbestosis, etc.), where macrophages are not able to internalize and degrade micrometric particles because of their large size and non biodegradability, leading to chronic inflammation (and cancer). These mechanisms are not likely to apply in the case of inorganic NPs or CNs, since they are much smaller and can be easily internalized by macrophages (Fig. 5), and at the same time they are not likely to be inserted into the lipidic layers.

2.2.6. Long-term effects. If NPs stay for long periods of time inside the cells or inside the body, even at non-toxic concentrations they may have deleterious effects. Thus, long-term chronic and repetitive exposure should be considered. It should be carefully investigated how cells

respond to treatments with nanomaterials below the doses causing high percentages of cell death. Even small changes may cause profound effects on the integrity and viability of the cells over multiple cellular divisions. In this context, it is important to take into account the special ability of NPs to interact with DNA, in the nucleus, or in the cytoplasm during mitosis, inducing gene damage or blocking gene reparation, which ultimately may lead to cancer. Recently, high-throughput gene expression testing of cells exposed to modified CdSe NPs showed no significant genetic damage [64]. Researchers examined the impact of the treatment of both human lung and skin epithelial cells with poly(ethylene glycol) silanized quantum dots (PEG-silane-Qdots). Human skin (HSF-42) and lung fibroblasts (IMR-90) were selected because the skin and the respiratory track are the most common routes of human exposure to NPs. Two dosages were selected, one reported to be non-toxic and a 10-fold higher dosage. Their results indicated that both high and low doses of PEG-silane-Qdots presented a similar average response from the cells. They did not observe any adverse effect in lung epithelial cells, while, in the case of skin epithelial cells, PEG-silane-Qdot treatment exerted a slight repression of gene regulation in cell-cycle progression. However, fewer than 50 genes out of more than 22,000 tested (equivalent to 0.2% of total genes) showed significant changes in the expression level due to the presence of the PEG-silane-Qdots. Detailed analysis allowed the classification of these genes into functional categories, and promoter analysis revealed the affected regulatory pathways [64].

2.2.7. Future perspectives. Apart from industrial uses in materials and commodities, which can be delayed until full risk assessment, nanomedical devices are also under development due to the special interactions of inorganic engineered NPs with biology, where the major impact of the new nanometric revolution is expected. Therefore, if scientists start putting nanomaterials into the living machinery for diagnosis and therapeutics, their particular interaction with biological systems, and the associated mechanisms, has to be carefully studied, taking into account that interactions with living organisms are difficult and often unpredictable.

It has to be understood that a major environmental, or health and safety problem—real or not—with a product or application that is labeled 'nanotechnology'—whether it actually is nanotechnology or not—could dampen public confidence and investment in nanotechnology, and could even lead to unwise regulation. At this point, adequate governmental regulation is difficult due to the lack of accurate data on engineered NPs. Respective efforts have been initiated by the American National Standards Institute (ANSI), the International Council on Nanotechnology (ICON, a coalition of academic, industrial, governmental and civil society organizations), as well as the International Organization for Standardization (ISO, Geneva, Switzerland), which collect data to aid understand and regulate nanotechnology. In parallel, international non-governmental ecological, environmental, labor and *biocentric* associations have also recently teamed up to issue a list of recommendations to avoid nanotechnology poisoning [65]. Their concerns are not only about consumer and worker health and safety but also about the social and ethical implications regarding economical issues (as related to weak economies in developing countries and patents). It is hoped that, in the near future, governmental and non-governmental associations will work closely together to assess risks and introduce nanotechnology into society. It is also expected that these concerns regarding health and environment will be extended to anticipate responsible and rational use, and sustainability, of any other human activity, not just nanotechnology.

The balance of positive and negative consequences of the interaction of NPs with humans can be illustrated in this final example: iron oxide is proposed to be useful as hyperthermia agent for cancer treatment in places as the brain, where surgery fails, as a magnetic resonance imaging (MRI) contrast agent, as a drug delivery device and as a drug for iron administration in cases of anemia. However, iron-oxide NPs were recently found concomitant with Alzheimer disease [66] with size and composition that excluded a ferritine origin. In addition, it was reported that iron-dextran NPs administrated to mice were dissolved in the liver and transformed into other iron deposits showing the *in vivo* metabolization of iron-oxide NPs [28]. In fact, the toxicity of iron oxide NPs has been also studied for a long time [67] but still the reports of potential medical benefits appear constantly, together with those about its toxicity. Thus, while some find a new promise for nerve cell regeneration [68], another detects the toxic effect of this material to neuronal cells [69] and so on. In the end, both, the potential toxicity and benefits will have to coexist.

3. Conclusions

Nowadays, many people believe nanotechnology will be the key to solve many of the world's most pressing medical problems, while others believe it could lead to a potential disaster. Because of that, opinions addressing this situation are now profusely issued, also in the most prestigious scientific journals [70], where calls for a rational and broad approach based on common sense are made. What is clear is that the approach to risk assessment has to be comprehensive: dose, time, memory, cell damage, immunotoxicity, genotoxicity, etc. The most probable scenario is that, as with electricity and other chemicals, we can handle it. Fortunately, inorganic NPs possess signatures that allow easy monitoring in or-

ganic/biological environments. Thus, it is likely that at the same time that NPs and CNS start being used in controlled medical applications, restrictive regulations will appear to control the dispersion of these *new* chemicals in the environment.

In general, it has been observed that when NPs enter the body, either inhaled, through the GI track, the skin, or intravenously, they are rapidly found in the liver (up to 90%), then the kidneys and in organs with a high phagocytic activity, such as the bone marrow or the spleen. After a period of time (from hours to months), NPs are expelled from the body through feces and urine. Localization of inorganic NPs in other tissues has also been reported and often related to diseased organs, however, at a much lower quantity than in the previous cases and without a clear knowledge about where this inorganic matter came from, how it entered the body and how it changed since then. In this regard, nanotechnology, as an analytical tool, may have a lot to offer.

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