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# Toxicity of Nanoparticles

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**Abstract:** Nowadays more than thousands of different nanoparticles are known, though no well-defined guidelines to evaluate their potential toxicity and to control their exposure are fully provided. The way of entry of nanoparticles together with their specificities such as chemistry, chemical composition, size, shape or morphology, surface charge and area can influence their biological activities and effects. A specific property may give rise to either a safe particle or to a dangerous one. The small size allows nanoparticles to enter the body by crossing several barriers, to pass into the blood stream and lymphatic system from where they can reach organs and tissues and strictly interact with biological structures, thus damaging their normal functions in different ways. This review provides a summary of what is known on the toxicology related to the specificity of nanoparticles, both as technological tools or ambient pollutants. The aim is to highlight their potential hazard and to provide a balanced update on all the important questions and directions that should be focused in the near future.

**Keywords:** Nanoparticles, toxicity.

## INTRODUCTION

"Nano" prefix in the nanoparticle (NP) word comes from the ancient Greek language and means "dwarf", much smaller than most particles, indicating particles whose diameter is lower than 100 nanometers ($10^{-9}$ m), ranging from 1 to 100 nm [1-5].

A plethora of chemicals of different shapes and properties can be classified as nanomaterials, the most prominent ones being heavy metals as nickel, cadmium, manganese, zinc, titanium, gold, antimony, silicon and their metal oxides, carbon and others which can be engineered or incidentally released in the environment.

Engineered man-made NPs have found several applications mainly in biomedical fields for improving diagnostic tools and clinical treatments.

Incidental NPs can be produced by a number of sources. They derive from industrial activities and can be found in there and in the surrounding environments. They are mainly generated from power plants by coal, natural gas and oil.

Nano-scale particles can derive from incineration of solid waste, combustion of fossil fuels or traffic emissions. In these cases NPs can be a complex mixture of different chemical compositions.

A complex NPs mixture could also be formed in military shooting ranges as a result of the explosion of bombs developing very high temperatures; following this, all the surrounding materials may be pulverized, from the rocks to the soil, and easily transported as a fine suspension in air as well as in water.

Inorganic and metallic powders so produced are often insoluble and non-biodegradable particles: their small size allows them to be delivered in the whole environment, where they stick around forever.

In Fig. (**1**) human exposure to NPs, main anthropogenic sources, their release in the work place and environment and the subsequent occupational or public exposure through several ways of entry and translocation into human body are reported.

The focus on the NPs peculiarity stems from their nano-scale size that leads to a very high surface offered to all different reactions.

Despite the enthusiastic and promising applications in several fields of specific engineered NPs, several deleterious effects appeared that have become the topic of concern.

In fact, both engineered and incidentally, unintentionally released NPs may share several common adverse effects on human health.

In all cases, independently from their use and source, NPs may enter human body and accumulate in organs and tissues as foreign bodies. For that reason, recently, a new branch of science that has the aim to study the dangerous effects of nanomaterials on human health and environment has been named nanotoxicology [6,7].

This review tries to evaluate the unique peculiarities belonging to particles of nano-scale dimensions, which should be taken into account to cast light on their toxic effects. Here what is known on the toxicology of NPs will be summarized,

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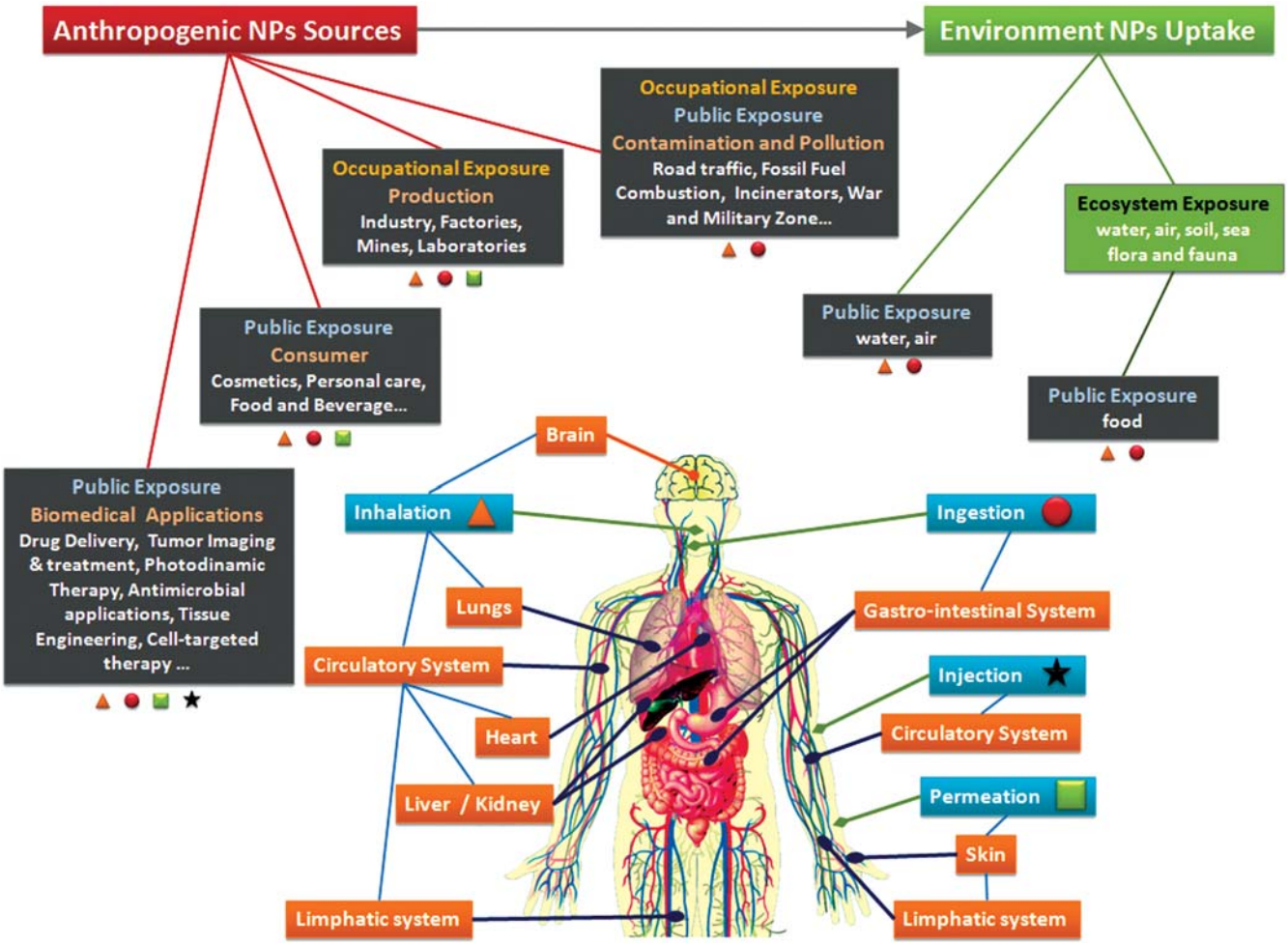


**Fig. (1).** Human exposure to NPs. Main anthropogenic sources of NPs, their release in the work place and environment, the subsequent occupational or public exposure through several ways of entry and translocation into human body.

both as technological tools or environmental pollutants, with the aim to describe their potential hazard and provide a balanced update on all the important topics related to their widespread uses.

## PROPERTIES OF PARTICLES RELATED TO THEIR ACTIVITY

It is now generally recognized that the mechanisms underlying NPs uptake, translocation, biological and toxicological effects, depend strongly on their physical properties and that, first, their characterization as size, shape, surface charge and surface area, hydrophilicity, agglomerate and aggregate formation, together with solubility, chemical and geometrical properties is an indispensable and critical step to obtain reliable studies.

The size, which influences the surface area, is important in determining the reactivity. In fact, it can influence the area involved in the specific effect, by enhancing or diminishing it: the smaller the particles the greater the surface to volume ratio and usually, the more pronounced will be their reactivity.

Many examples show evidence for that. For instance, the tissue distribution of gold nanoparticles has been demonstrated to be size dependent: the 10 nm sized were widespread distributed in several organs whereas the larger, from 50 to 250 nm, were found only in liver, spleen and blood, after intravenous administration in rats. [8]

The carcinogenic activity of nickel particulates has been consistently related, together with the water solubility, to their dimension. Particles smaller than 200 nm are likely to enter the epithelial cells, but larger are phagocytized by macrophages. Also the surface charge determines the fate of these particles: amorphous positively charged surface particles do not enter cells; on the contrary, crystalline nickel sulfide (NiS) and sub sulfide ($Ni_3S_2$) particles which are negatively surface charged enter cells by phagocytosis [9] (Fig. **2**).

After that they can be dissolved by the acidic pH of endocytic vacuoles. In this way a continuous source of $Ni^{2+}$ ions is provided which can reach the cellular nuclear components and then cause multiple types of cellular nuclear damages *via* direct or indirect mechanisms.

They include epigenetic effects in chromatin, especially effects on histone acetylation and methylation, promutagenic DNA damage, impairment of mechanism of DNA repairing. All these ways can be part of the machinery responsible for the recognized carcinogenic activity of Ni(II) particles [10-25].

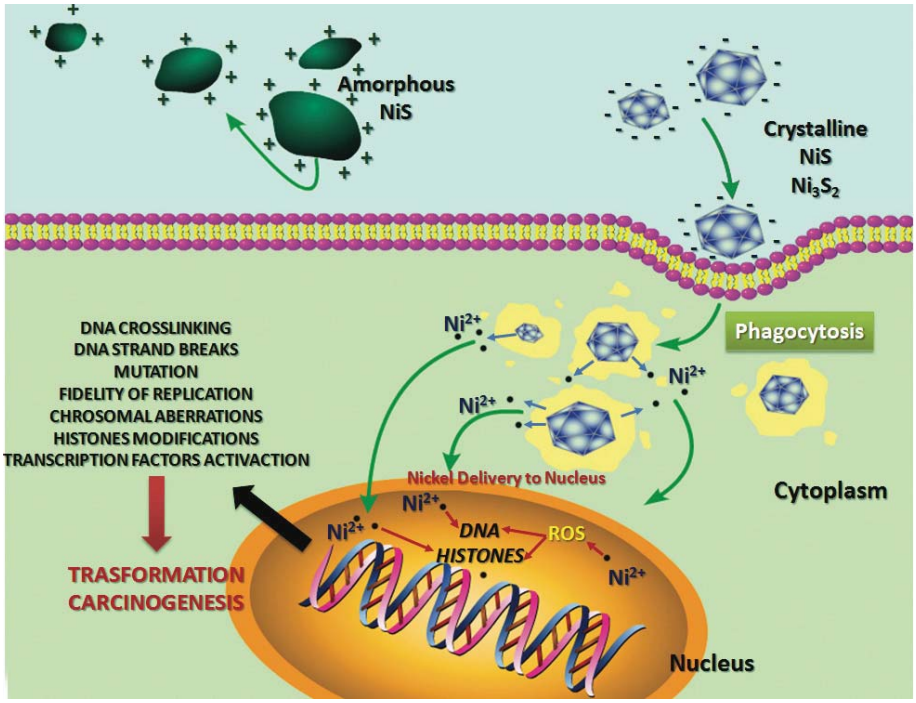


**Fig. (2).** Model of phagocytosis mechanism and intracellular dissolution of nickel sulphide particles into cells and the potential cellular nuclear damages. Crystalline nickel sulphide particles were selectively phagocytized by the cells, while amorphous NiS particles were not. Afterwards, phagocytized nickel sulfide particles were dissolved in the cytoplasm by the acidification of cellular vacuoles and the nickel ions released produced selective damage in heterochromatin. Adapted from [16] with permission from Elsevier.

Also, the charged gold NPs of 1.5 nm size lead to cell death by apoptosis, while neutral gold NPs cause necrosis in HaCaT cell lines [26].

Thus, it appears that also the particle surface may play an important role concerning toxicity in that it determines the first direct contact with biological materials and cell surface and components.

The DNA damaging ability of metal and metal oxide engineered NPs has been reviewed.

NPs can cross the nuclear envelope and then have an important role in inducing genotoxicity.

Oxidative DNA mechanisms together with impairments of gene expression and strand breaks have been reported based on *in vitro* experiments [27].

Together with intercalation processes, ROS formation is believed to play an important role in the DNA damages, though in this case NPs may not reach the cellular nucleus [28-31].

The most common biological effects related to the physical and chemical properties of nanoparticles are reported in the following Table **1** from [32]:

The structural and geometrical shapes that make NPs appear as belts, tubes, wires, spheres, fibers, (Fig. **3**), are very important in determining the toxic bioactivity and it seems that the shape of greater concern is the long wire or fiber.

The alveolar macrophages, which should be responsible for the removal of inhaled materials, are unable to remove the fibers by the usual clearing processes. Then, for example, though 200 nm sized $TiO_2$ NPs are considered relatively biological inert material *in vivo* and *in vitro*, nevertheless $TiO_2$ nano-belts longer than 15 μm are highly toxic particles which are not able to be sequestered inside the cells into a lysosome; so they become persistent in the lung where they can induce inflammatory response and release of inflammatory cytokines [56] with potential detrimental consequences.

The same effect occurs with different forms of asbestos and silica and it has been reported that also some types of carbon nanotubes can induce asbestos-like pathologies in mice [57].

Thus, the length and shape of the material rather than the chemical composition seem to be the determinant factor in causing inflammation *in vivo*. Serious diseases, as lung fibrosis, can be determined by long-term exposure with manufactured wires resembling asbestos fibers.

## WAYS OF ENTRY AND TRANSLOCATION

The ability of particles to enter the body across specific pathways and the propensity to be retained or, otherwise, to be transported to different organs or tissues through the body is affected by both their physical and chemical properties.

There are numerous ways (Fig. **4**) whereby NPs may enter the body: the main routes are by inhalation through the respiratory tract, by permeation through the skin and by ingestion through the digestive tract.

## INHALATION

Inhalation is believed to be one of the most common routes of human exposure. To what extent dusts and airborne particles enter, deposit or eventually move to other sites seems to be first determined by the size of particles.

**Table 1. Toxicokinetic findings and biological effects due to physicochemical properties of NPs. Reprinted with permission from Elsevier [32].**

| Physicochemical Property | | Toxicokinetic Findings | Biological Effects | Ref. |
|---|---|---|---|---|
| **Size** | 15 nm gold nanoparticles (NPs) | Most widespread organ distribution including blood, liver, lung, spleen, kidney, brain, heart, stomach in mice | Biodistribution of the nanoparticles | [33] |
| | 15 and 50 nm gold NPs | Pass blood–brain barrier (BBB) in mice | Blood Brain Barrier (BBB) permeability | [33] |
| | 40–50 nm gold NPs | Activation of membrane receptors in SK-BR-3 cells | | [34] |
| | 50 nm gold NPs | Maximum uptake by Hela cells | | [35] |
| | 50 nm quantum dots | Efficient receptor-mediated endocytosis in Hela cells | | [36] |
| | 1–10 nm silver NPs | | Exclusively attach to HIV-1 | [37] |
| | 1–10 nm silver NPs | Penetrate inside the bacteria | | [38] |
| **Shape** | Open-ended Single-walled carbon nanotubes (SWNTs) | Efficient blocking of ion channels in CHO cells | Spherical shaped close-ended SWNTs are comparatively less reactive | [39] |
| | Spherical gold NPs | Higher uptake by Hela cells | Rod-shaped gold NPs showed less uptake | [35] |
| | Carbon particles, except C60CS | Stimulated human platelet aggregation *in vitro* and accelerated the rate of vascular thrombosis in rat carotid arteries | Biological reactivity: mixed carbon nanoparticles (MCNs) ≥ single-walled carbon nanotubes (SWNTs) > multi-walled carbon nanotubes (MWNTs) | [40] |
| | Filomicelles (Filamentous micelles) | | More efficient for drug delivery than their spherical counterparts in rats and mice | [41] |
| **Surface area/volume ratio** | $TiO_2$ (300 $cm^2$ surface area) | Increased lymph-node burdens and Inflammation | More reactive in rats as compared to $BaSO_4$ (200 $cm^2$ surface area) | [42] |
| | $TiO_2$ and $BaSO_4$ with same surface area | Inflammatory effects were similar | Inflammation | [42] |
| | Ultrafine carbon black particles (270 $m^2$/g surface area) | Cause greater pulmonary toxicity in rats | Increased reactivity in comparison with larger-sized carbon black particles (22 $m^2$/g surface area) | [43, 44] |
| **Chemical composition** | Incorporation of 1% (w/w) manganese doping into titania particles | Increase in UVA absorption and reduction in free radical generation *via* surface reactions | | [45] |
| | Carbon nanomaterials | Different geometric structures exhibit quite different cytotoxicity *in vitro* | The cytotoxicity follows a sequence order: SWNTs > MWNTs > quartz > $C_{60}$ on alveolar macrophages isolated from guinea pigs | [46] |
| | Metal traces associated with the commercial carbon nanotubes | A dose- and time-dependent increase of intracellular reactive oxygen species and a decrease of the mitochondrial membrane potential in rat macrophages (NR8383) and human A549 lung cells | More reactive as compared to purified carbon nanotubes | [47] |

**(Table 1) contd….**

| Physicochemical Property | | Toxicokinetic Findings | Biological Effects | Ref. |
|---|---|---|---|---|
| | Quantum dots core metalloid complexes of Cadmium, Cd | Can cross the blood–brain barrier and placenta, and are systemically distributed to all bodily tissues, with liver and kidney being target organs of toxicity | A probable carcinogen | [48] |
| | Quantum dots core metalloid complexes of Selenium, Se | | A marked impact on the local ecosystem resulted from elevated environmental concentrations of Se | [48] |
| | Ag, $MoO_3$, $Fe_3O_4$, Al, $MnO_2$ and W (Tungsten) | Ag was highly toxic whereas, $MoO_3$ moderately toxic and $Fe_3O_4$, Al, $MnO_2$ and W (Tungsten) displayed less or no toxicity at the doses tested on *in vitro* rat liver derived cell line (BRL 3A) | Reduced cell proliferation and death | [49] |
| | $La_{0.7}Sr_{0.3}MnO_3$ (LSMO) nanoparticles doped with cerium ($La_{0.7-x}Ce_xSr_{0.3}MnO_3$ where $0 \leq x \leq 0.7$) and $La_{1-y}Sr_yMnO_3$ nanoparticles with different values of y (La/Sr ratio) | Low cytotoxicity in Ce-doped samples as well as in samples with reduced La/Sr ratio as revealed by *in vitro* studies on HT-1080 (human fibrosarcoma) and A431 (human skin/carcinoma) cells | Improved cell proliferation upon Ce doping | [50] |
| **Surface charge** | Neutral NPs and low concentration anionic NPs | | Drug delivery applications to brain in rats | [51] |
| | Cationic NPs | | Toxic effect at the blood brain barrier in rats | [51] |
| | Anionic NPs at lower concentrations | | Superior uptake rates as compared to neutral or cationic NPs at the same concentrations in rats | [51] |
| | Positive surface charged poly(amidoamine) dendrimers | Deposition into tissues is higher than neutral surface dendrimers in B16 melanoma and DU145 human prostate cancer mouse tumor model | Higher deposition in tissues | [52] |
| | Coating of respirable quartz surface with aluminum lactate or polyvinyl-pyridine-*N*-oxide (PVNO) | Inhibits DNA strand breakage and formation of 8-hydroxy-deoxyguanosine in human lung epithelial cells | Reduction in toxicity | [53] |
| **Aggregation state** | Rope-like agglomerates of carbon nanotubes | Induced more pronounced cytotoxic effects than well dispersed carbon nanotubes in human MSTO-211H cells | Cytotoxicity | [54] |

Usually, the main particles are deposited in the nose, mouth and larynx, whereas the finest grains can reach the bronchial tree where they can be retained and exert their dangerous effects or from where they translocate to other different sites.

A correlation between exposure and incidence of cancer to respiratory tract (lung and nasal cancer) has been demonstrated from epidemiological studies carried out on miners and refinery workers exposed to metal particles [58]. The propensity of nickel workers, exposed to nickel-containing dusts, to develop cancers of the nasal cavities was first described in 1933.

The pattern of adverse effects on the retention sites appears specific to a specific particle. For example, chrysalite asbestos fibers deposit mainly on airways bifurcations and lead to fibrosis "spots" after acute exposure; they appear as emphysema in "coal workers", as fibrotic nodules in chronic beryllium disease through lymphatic route along airways [59-61].

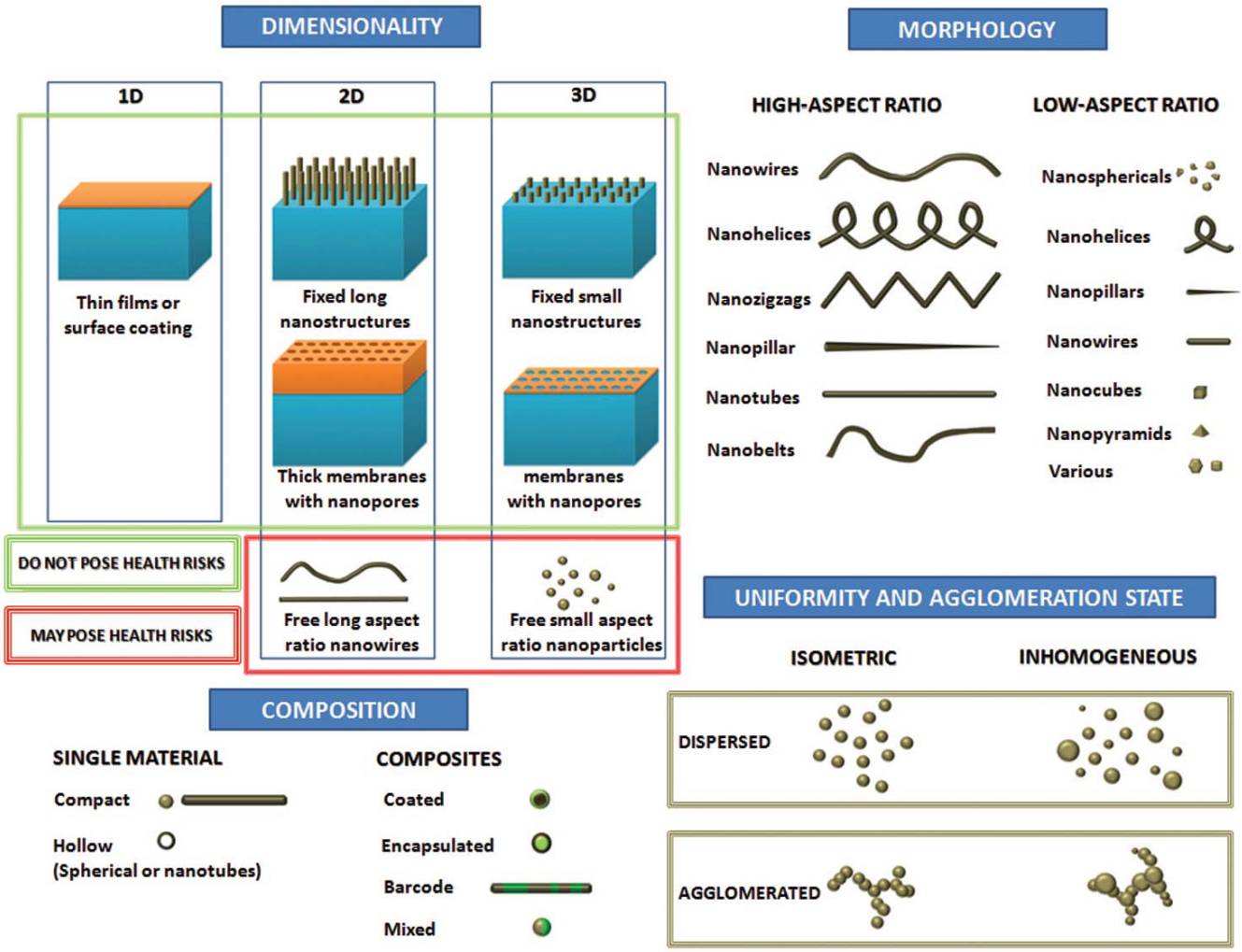


**Fig. (3).** The classification of NPs based on shape or morphology, size, composition and agglomeration state. Adapted from [55] with the permission from Springer Science.

In any cases, inflammatory and adverse effects on cellular targets and biochemical activities can be found in the sites of retention, as summarized in Table **2** [62].

Following inhalation route the smallest, especially ultrafine particles, can translocate to other organs and tissues and several researchers have reported that NPs from ultra-fine dusts and aerosols carry the major hazard after inhalation exposure [89-92].

The translocation is reported to be mainly through endocytosis of alveolar epithelial cells [93].

Translocation to different organs of ultra-fine particles such as manganese, manganese oxide, carbon, zinc, iridium and others has been extensively studied [89,90,94-98].

Evidence of short-term translocation of metal nanoparticles from lungs into the blood stream and to target organs has been proven by animal studies. For example, following 1 h of inhalation exposure in rats, ultra-fine radiolabeled iridium particles (of about 20 nm in size) have been found, one week after exposure, in many extra-pulmonary organs, mainly in kidneys, liver, spleen, heart and brain [99].

Within only 30 minutes after exposure, large quantities of intratracheally instilled gold nanoparticles (of about 30 nm in size) and $TiO_2$ (of about 20 nm in size) have been found in platelets inside of pulmonary capillaries of rats [20]. This fact leads to hypothesize that nanoparticles produce platelets aggregation and then blood clots formation.

20 nm nanoparticles of titanium dioxide showed a longer retention time in the lungs and an increased translocation to the interstitial sites than larger nanoparticles (250 nm) having the same crystalline structure, after inhalation for 12 weeks in rats [100].

By using scanning electron, environmental scanning electron microscopy and X-ray-microanalyzed with an energy dispersive system techniques on organs and blood of patients with metallic orthopedic implants [101, 102] or worn dental prostheses [103], micro and nanoparticle debris was detected.

Concerning the chemistry of particles, it is noteworthy to point out that there is a remarkable difference between the metallic and non-metallic species. In fact, after inhalation or

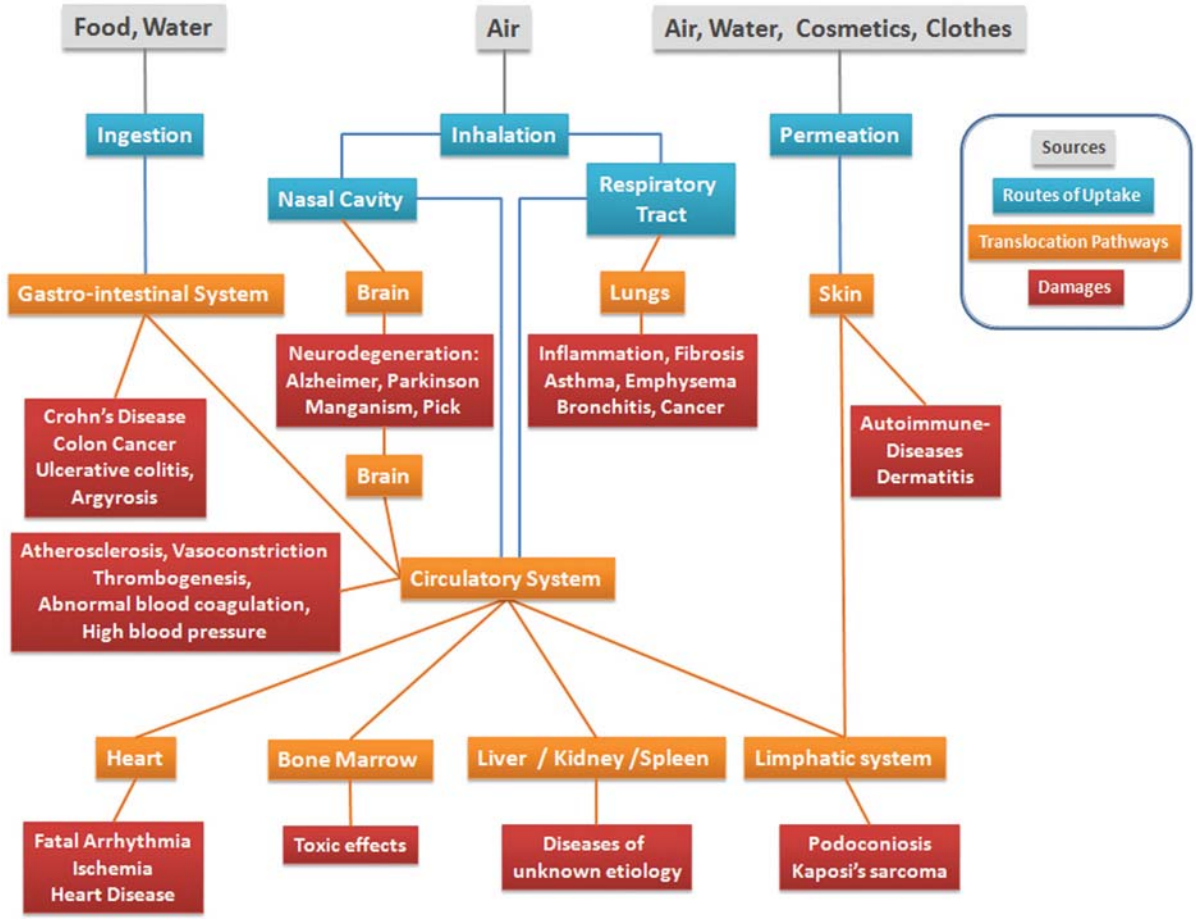


**Fig. (4).** The predominant routes of NPs exposure, uptake, translocation and potential risks. The gray boxes indicate the source of NPs exposure, blue boxes indicate the primary routes of uptake, the orange boxes the potential translocation pathways and the red boxes the potential damage.

instillation experiments carried out on healthy animals, metallic nanoparticles smaller than 30 nm rapidly move into the blood circulatory system [104-106]; on the contrary, non-metallic nanoparticles sized between 4 and 200 nm move very little or not at all [107,108].

Particularly, much attention has been recently devoted to the mechanism involved in the transport of NPs from pulmonary site to the most vulnerable organ, the brain, after inhalation exposure.

Among others, manganese mining, welding and manufacturing workers, welders in stainless steel facilities exposed to fumes and dusts are considered professional groups at risk of occupational exposure to metal NPs. In these cases it has been found that manganese NPs were transported through the axons of olfactory neurons from the nasal epithelium to the olfactory bulb of the brain and a progressive damage of functioning in the central nervous system has been evidenced [89,90, 91-98, 109-117].

Oberdorster *et al.* reported that, after inhalation for 7 days of exposure in rats, 30 nm sized $MnO_2$ NPs have been translocated from lungs to the olfactory organ [104] from where they can enter into the brain.

Inhalation of $MnO_2$ NPs results in the formation of reactive oxygen species that cause oxidative stress in the brain [6,89]. The possible involvement of ambient air NPs in neurological diseases is also reported in studies carried out on biopsies from city dwellers [118].

It is noteworthy that the effects related to fine dusts of biopersistent $TiO_2$ nanoparticles, though bulk $TiO_2$ is considered inert, seem to be similar to the chronic processes of inflammation involved in inhaled asbestos fibers [119].

$TiO_2$ is largely used to provide opaqueness or brilliance to a large number of products as paints, colorants, plastics, papers, foods, toothpastes and many others. $TiO_2$ NPs are also used in cosmetic, skin care products and sunblocks in order to enhance protection against UV rays.

An interesting review reporting a summary for risk assessment on the carcinogenic potential of nanoproducts has been recently published [120].

Even though controversial results and no sufficiently thorough and conclusive epidemiological studies concerning carcinogenesis are afforded, however some data give evidence on the potential ability of nanoparticles to induce tumors: carbon nanotubes (CNTs) of different forms as well as fine (<2.5 μm) and ultrafine (<100 nm) $TiO_2$ particles at high concentrations cause cancer on the respiratory tract in sensitive animal models [121-126]. For that reason, the International Agency for Research on Cancer has recently

**Table 2. Summary of *in vitro* and *in vivo* evaluations of NPs toxicity focused on experimental lung, dermal, liver and brain targets. Reprinted with permission from Elsevier [61].**

| Target | NPs | Concentration (time/size)/route of administration | Cellular target | Animal target | Major outcomes | Ref. |
|---|---|---|---|---|---|---|
| **Lung** | **SWCNT** | 1.56-800 μg/mL (24 h) | A549 human lung cancer cells | | Low acute cytotoxicity was further reduced by dispersion of SWCNTs in serum. | [63] |
| | **SWCNT** | 47 mg on days 0 and 7 (follow-up: 4 months) Intravenous infusion | | Nude mice | No significant inflammatory changes were observed, however, particle deposition in liver macrophages was observed. | [64] |
| | **MWCNT** | 0.5, 2 or 5 mg/animal (3 and 15 days) Intratracheal instillation | | Sprague-Dawley rats | Dose-dependent increase in inflammatory markers post-BAL. Dose-dependent fibrotic change and interstitial granuloma formation. | [65] |
| | **Silica NP** | 10-100 μg/mL (24 h, 48 h and 72 h) | A549 human lung cancer cells | | Dose- and time-dependent decrease in cell viability: up to 50% reduction at highest dosage after 72 h. Oxidative stress indicated as mechanism of cytotoxicity. | [66] |
| | **Silica NP** | 25 μg/mL (24 h) | A549 human lung cancer cells HepG2 cells RPMI 2650 human nasal septal epithelial cells N2a mouse neuroblast cells | | Nuclear protein aggregation and subsequent interference with gene expression resulting in inhibition of replication, transcription and cell proliferation. | [67] |
| | **Silica NP** | 33-47 μg/cm$^2$ (small NP), 89-254 μg/cm$^2$ (larger NP) (24 h) | EAHY926 endothelial cells | | Size-dependent reduction in viability with smaller particles in the nanoscale exhibiting higher toxicity compared to particles >100 nm. | [68] |
| | **Silica NP** | 20 mg/animal (1 or 2 months) Intratracheal instillation | | Wistar rats | Nano-sized silica particles produced relatively lower pulmonary fibrosis compared to micro-sized silica particles. This is thought to be due to the translocation of ultrafine nanosilica away from the lung parenchyma. | [69] |
| | **Silver NP** | 515 μg/m$^3$ (6 h/day, 5 days/week for 13 weeks) Inhalation | | Sprague-Dawley rats | Dose- and time-dependent increase in blood Ag nanoparticle concentration was observed along with correlating increases in alveolar inflammation and small granulomatous lesions. | [70] |
| **Dermal** | **Silver NP** | 0.76-50 μg/mL (24 h) | A431 (human skin carcinoma) | | No evidence for cellular damage up to a concentration of 6.25 μg/mL. Morphological changes at concentrations between 6.25 and 50 μg/mL with concomitant rise in GSH, SOD and lipid peroxidation. DNA fragmentation suggests cell death by apoptosis. | [71] |
| | **Silver NP** | 0-1.7 μg/mL (24 h) | HEK cells | | Significant dose-dependent decrease in cell viability at a critical concentration of 1.7 μg/mL with concomitant rise in inflammatory cytokines (IL-1β, IL-6, IL-8, and TNF-α). | [72] |
| | **Silver NP** | Silver-coated wound dressing 'Acticoat' (1 week) | | Human burns patient | Reversible hepatotoxicity and argyria-like discoloration of treated area of skin, elevated plasma and urine silver concentrations and increased liver enzymes. | [73] |

**(Table 2) contd….**

| Target | NPs | Concentration (time/size)/route of administration | Cellular target | Animal target | Major outcomes | Ref. |
|---|---|---|---|---|---|---|
| | **$TiO_2$ NP** | 15 μg/$cm^2$ (24 h) | HaCaT (keratinocyte cell line), human dermal fibroblasts, human immortalized sebaceous gland cell line (SZ95) | | Cytotoxicity was observed affecting cellular functions such as cell proliferation, differentiation and mobility resulting in apoptosis. | [74] |
| | **$TiO_2$ NP** | NP containing sunscreen | | Human volunteers | Increased skin permeation of NP when sunscreen was applied at hairy skin of human volunteered. | [75] |
| | **Silica NP** | 70, 300 and 1000 nm in size | XS52 (murine Langerhans cells) | | Size-related toxicity with faster cellular uptake of smaller particles and concomitant higher toxicity. | [76] |
| | **Silica NP** | 30-300 μg/mL (48 h) | CHK (human keratinocytes) | | Reduced cell viability. | [77] |
| | **Gold NP** | 95, 142 and 190 μg/mL (13 nm) 13, 20 and 26 μg/mL (45 nm) (3 or 6 days) | CF-31 (human dermal fibroblasts) | | Cytotoxicity was size- and dose-dependent. Larger particles (45 nm) exhibited greater toxicity at smaller doses (10 μg/mL) compared to smaller ones (13 nm) which only exhibited cytotoxicity at a concentration of 75 μg/mL. | [78] |
| | **Gold NP** | 0.8-15 nm in size (48 h) | SK-Mel-28 (melanoma cells), L929 mouse fibroblasts | | Maximum cytotoxicity with smaller NP (1.4 nm) characterized by apoptosis and necrosis. | [79] |
| **Liver** | **Gold NP** | 8 mg/kg/week (3-100 nm in size) (4 weeks) Intraperitoneal | | BALB/C mice | Naked NP: severe adverse effects with resultant death with particles ranging from 8 to 37 nm in diameter. Microscopically, Kupffer cell activation in the liver and lung parenchymal destruction was observed. Surface modified NP: elicited increased host immune response and improved cytocompatibility. | [80] |
| | **Gold NP** | 0.17, 0.85 and 4.26 mg/kg body weight (13 nm in size) (30 min after injection for 7 days) Intravenous | | BALB/C mice | NPs were found to accumulate in liver and spleen. Significant upregulation of inflammatory cytokines (IL-1, 6, 10 and TNF-α) with subsequent apoptosis of hepatocytes at highest concentrations (4.26 mg/kg). No significant changes in the liver at lower doses. | [81] |
| | **Silica NP** | 50 mg/kg (50, 100 or 200 nm in size) (12, 24, 48 and 72 h, 7 days) Intravenous | | BALB/C mice | Size-dependent hepatic toxicity with inflammatory cell infiltrates. Macrophage-mediated frustrated phagocytosis of larger NP (100 and 200 nm) resulted in release of pro-inflammatory cytokines and cell infiltrates within hepatic parenchyma. | [82] |
| | **Silica NP** | 2 mg/kg (20-25 nm in size) (24 h) Intravenous | | Nude mice | Greatest accumulation of NP in liver, spleen and intestines but no pathological changes were observed with small NP (<25 nm). Near-total excretion of NP via the hepatobiliary system. | [83] |
| | **CdSe QD** | 62.5, 250 and 1000 μg/mL (24 h) | Primary rat hepatocytes | | Cytotoxicity was thought to be due to the release of free cadmium ions which could not be fully eliminated by ZnS coating of the OD core. | [84] |

**(Table 2) contd….**

| Target | NPs | Concentration (time/size)/route of administration | Cellular target | Animal target | Major outcomes | Ref. |
|---|---|---|---|---|---|---|
| | **CdSe QD** | 62.5, 100 and 250 μg/mL (24, 48 or 72 h) | HepG2 cells | | Dose-dependent cytotoxicity. In extreme conditions (250 μg/mL for 72 h) a reduction in cell viability of almost 40% was observed which correlated with an increase in free cadmium ion concentration of 1.51 ppm. | [85] |
| **Brain** | **Gold NP** | (12.5 nm in size) (40, 200 or 400 μg/kg/day for 8 days) Intraperitoneal | | C57/BL6 mice | Small amounts of NP were able to cross the BBB but did not induce evident neurotoxicity. | [86] |
| | **Silver NP** | 30, 300 or 1000 mg/kg/day for 28 days (60 nm in size) Per oral | | Sprague-Dawley rats | Dose-dependent accumulation of NP was observed in the brain and other organs suggesting systemic distribution after oral administration. ALP and cholesterol increased significantly in high-dose group (1000 mg/kg/day) indicating hepatotoxicity. | [87] |
| | **CdSe QD** | 0.68 mg containing 50 nmol Cd (13.5 nm in size) (6 h) Intraperitoneal | | ICR mice | Relatively high amounts of Cd ions found in brain tissue but no signs of inflammation or parenchymal damage were observed. | [88] |

reclassified $TiO_2$ as "possibly carcinogenic to humans" in the group 2B.

*In vitro* studies on the effects on the host response of several NPs of different source and size provide evidence for clot formation and inflammatory cells activation [127].

Polymerization of fibrin and its cross-linking and aggregation of platelets have been promoted also by nickel, antimony and silver NPs. Macrophage phenotypes secreting higher levels of tumor necrosis factor α, a member of a group of cytokines that stimulate the acute phase reaction, involved in systemic inflammation, have been found to be stimulated by cobalt, titanium and iron NPs.

In addition, translocation of these particles, observed by SEM analysis, appeared to produce adverse effects on vital organs, in particular on cardiovascular system.

Engineered nanoparticles are designed to have very specific properties and recent progress in development of strategies to improve out their activity in medical and industrial field has been performed.

Carbon and gold NPs for inhaled and oral drugs delivery, often as chemotherapeutic agents; zinc and titanium oxide for creams and sunscreens preparations; quantum dots, semiconductor nanocrystals, for *in vivo* imaging and diagnostics and cell labeling; superparamagnetic iron oxides as guided fluorescent labels in MRI (magnetic resonance imaging); silver nanoparticles for their antimicrobial activity in surgical instruments, bone prostheses, contraceptive devices.

Recently, despite all such instances, researches to test the possible engineered NPs adverse effects have been continuously carrying out and several concerns related to their use have been raised.

In Table **2** are summarized the *in vivo* and *in vitro* data available after evaluation of engineered NPs effects. The major outcomes have been collected depending on the target organs (lung, liver, derma, brain), the NPs concentration, size, time and route of administration, as well as on the cellular, animal targets (mice, rats) or human volunteers.

Inconsistencies between the data obtained from the *in vivo* experiments and those *in vitro* are evident and it appears clear that to extrapolate the knowledge from studies carried out on cell cultures to human can led to misinterpretation and sometimes to unnecessary alarm.

It is also clear that, in order to have more realistic data, it is crucial first to adopt a unifying protocol. In addition, extreme care must be taken in the dose and in the route of administration of NPs and especially long-term exposure should be evaluated in order to extrapolate data on human.

Indeed, among others, from *in vivo* studies, particularly with rat and mouse models, 30 nm sized gold nanoparticles after interstitial instillation are delivered and localized in the alveolar epithelium in rats; 10 nm sized quantum dots were found in liver, lymph and bone marrow in experimental mouse. When deposited in the alveolar tract they have been found to trigger inflammatory response and they need two months in rats and about two years in humans to be cleared.

Considerable amount of silver could be detected in rat brain after inhalation of ultrafine silver NPs [128-131].

Following rat inhalation experiments, 4-10 nm sized silver nanoparticles enter into the circulatory system within 30 minutes and after a day they have been found in the liver, kidney and heart. From these organs they have been subsequently cleared after a week [105]. From the liver they have been cleared *via* the biliary way into the small intestine.

An interesting case under study is represented by some novel materials as cerium and cerium oxides ($CeO_2$) that are especially used as diesel fuel additive in order to reduce fuel consumption, $CO_2$ emission and to catalyze the burning of particulates.

First considered as a safe material, recently controversial results have been published.

Indeed, it has been reported that the use of nano sized cerium oxide is unlikely to lead to health damages because only limited absorption has been determined in the lung of workers occupationally exposed to cerium [132, 133].

However, in a study on the potential effect of nano sized $CeO_2$ particles on the lung injury, after exposure of Male Sprague Dawley rats by intracheal instillation, it has been reported that they induced cytotoxicity and alveolar macrophages apoptosis that may cause lung fibrosis after inflammation and pulmonary stress. Aggregates of fine granules or fine needles of cerium from 30 to 60 nm in length, with longer needles following four weeks of exposure, appeared in lung tissue [134].

## PERMEATION

A theme under scientific discussion and concern is related to the risk of NPs dermal exposure and the conclusions related to this are still controversial.

Most of the research regarding uptake, delivery and distribution of NPs in and through the skin reports that the related toxicity seems to be minimal; normally, it is reported that the skin behaves as a defensive barrier and NPs through the skin can only reach the upper layers of the epidermis and eventually permeate the zone close to the hair follicles.

Nevertheless, the skin is porous to nanomaterials and holes are offered on the skin surface by the hair follicles and glands that can behave as channels of entry, especially suitable for ultra-fine materials.

Indeed, it has been demonstrated that several NPs were able to cross the derma and reach through lymph nodes the systemic vascular system [104].

A lot of personal care products and cosmetic containing nanoparticles are used daily by millions of people: shampoo, toothpaste, deodorant, soap, sunscreen, cream, foundation, face powder, perfume, eye shadow, just to name a few of them. Thus, it has been reported that in the US 33 million people use daily sunscreen products and much more occasionally [135] and an ever-increasing number of people are daily exposed.

Toxic effects on epidermal keratinocytes and fibroblasts have been detected by using cultured human skin cells; single (SWCNT) and multiwall carbon nanotubes (MWCNT), as well as nanoscale titanium and quantum dots have been shown to be capable of modifying protein and gene expression. SWCNT and MWCNT are reported to activate the production of cytokines and lymphokines and also to provide decreased viability and increased oxidative stress after crossing the cellular membrane [136-144].

Skin alterations may enhance NPs uptake in that they could behave as ways of entry for finer as well as for larger particles [145].

In a clinical report, silver NPs released from a nanosilver coated medication used for acute burn treatment has been related with liver malfunctioning. The silver levels in plasma (107 μg/kg) and urine (28 μg/kg) were clearly elevated, as well as the liver enzymes [73].

Several studies supported the cytoxicity of these products [146,147], though they have been approved for clinical applications [148-150].

ZnO NPs are largely used in industry so that workers and consumers exposed to this material are continuously increasing. In fact, ZnO NPs are used in several applications such as sunscreens, dyes, electronics, personal care products and additives in foods.

Spray sunscreens containing ZnO NPs are becoming more popular due to the more convenient and quick application.

These peculiar NPs, often added with $TiO_2$, are able to provide a barrier, long lasting and non-irritating, against UV rays. In order to obtain better dispersion quality and a higher UV protection level, ZnO particles sized less than 100 nm are used.

Though, normally, they are not able to cross the stratum cornea layers, inhalation of sunscreen spray can constitute an important way of exposure in that, unlike applications to the skin, spray preparations may find a way to translocate to the vital organs.

Airway exposure to ZnO NPs can be an important risk and expression of genes involved in oxidative stress and apoptosis increased in BEAS-2B cells exposed to 20 nm sized ZnO NPs.

Many studies regarding the toxicity of ZnO NPs have paid attention to the damage induced on cells, including oxidative damage, cytotoxicity and genotoxicity. Much attention, in these cases, has been devoted to the solubility of ZnO materials that are able to release free $Zn^{2+}$ ions inside the cells, which could interact with different binding sites in proteins and enzymes thus strongly affecting their functions [151].

It has also been reported that human epidermal cells exposed to 0.8 μg/ml of 30 nm sized ZnO NPs had DNA damages [152-154].

## INGESTION

Studies concerning toxicity of NPs after their ingestion are not as numerous as for the other routes of entry into the body.

The digestive tract can be reached by NPs from the respiratory way through the nose or directly by water, food or drugs containing NPs.

Regarding ingestion, the main area of interest and alarm is especially related to engineered NPs that are designed to be incorporated into different products as foods or drugs, in order to perform specific properties.

NPs are increasingly used as food additives and in numerous food processing industries. For that reason much attention should be addressed also to the possibility that

through the digestive tract they may reach other target organs and the blood circulation system causing several injuries.

Though many studies reported that ingested NPs are fast eliminated from the intestinal tract due to the continuous regeneration of the epithelium, many others, however, give clear evidence of the translocation of certain NPs to target organs.

In fact, it has been found that nanoparticles can translocate to the blood stream and then distribute all over the body after uptake through the gastro intestinal route [155].

Actually, when administered orally certain NPs have been found in many organs: besides in the small intestine and in the stomach, also in the liver, lymph nodes, bone marrow, lungs, kidneys and brain.

As well as for the other tracts, the size and the morphological properties of NPs influence their absorption from the gastrointestinal area; the absorption is greater for the smaller than for the larger and for the negatively charged particles which are spread trough the negatively charged mucus layer, whereas the positively charged are there trapped.

The absorption from intestinal tracts has been reported for 150 to 500 nm sized titanium particles into the liver, spleen and lymph and in a more pronounced way than those normally used in sunscreen creams [155,156].

Following oral exposure in adult animals, cerium compounds are found to be absorbed only poorly, while suckling animals showed a higher absorption and retention of cerium in the gastrointestinal tissues. Following oral gavage of $CeCl_3$ in rats, although cerium seems to be absorbed only poorly from the gastrointestinal area, the highest cerium levels have been found in the bones and liver [157]. Following oral exposure to 20 or 200 ppm of $CeCl_3$ significantly elevated concentration of cerium has been found in the lung, kidney, liver and spleen of male Imprinting Control Region mouse [158]. The highest concentrations of cerium have been found in the lung and spleen.

By ingestion of radiolabeled metal NPs, they have been found to be delivered to several target organs [159].

Recently, it has been determined that Cu NPs orally ingested can induce adverse effects and heavy injuries in the kidney, liver and spleen of experimental mice, compared to Cu μ-particles [148].

We have also to mention that, while absent in healthy people, NPs of different chemical compositions have been constantly found in colon tissue of people affected by cancer or Crohn's disease or ulcerative colitis. Among others, carbon, stainless steel, zirconium, silver, silicon nanoparticles [160] have been seen by using microscopic and energy dispersive spectroscopy analysis on colon mucosa. The debris particles, from 50 nm to 100 μm in size, were identified at the interface between the healthy tissue and the cancerous ones. It was proposed that the barrier offered by the gastrointestinal tract is not effective enough for particles that are smaller than 20 μm [103].

## CONCLUSION

Nanotechnology is an emerging branch of science that is growing at an exponential rate and whose potentiality is still under debate; indeed, a new branch of science has been named nanotoxicology with the specific aim to study the possible adverse health effects of materials of nano dimensions.

Recently, many researches have been continuously carrying out to test the possible NPs activities and several concerns related to their use have been raised.

What is evident is that the physical and chemical properties of NPs must be taken into close consideration before interpreting the results and drawing conclusions. In fact, it appears clear that, first, characterization of the specific nanoparticle through its size, shape, surface charge and surface area, agglomerate and aggregate formation, chemistry, hydrophilicity and solubility properties, is an indispensable and critical step to obtain reliable studies. In addition more regard should also be devoted to the possibility that crystal structure after interaction with water or other liquids or biological structures could be modified giving different properties. Thus, much attention should be addressed to the relationship between the exact NPs property and toxicity.

Following all the matters raised above it appears that a multitude of data are available in the recent literature, regarding both the beneficial and exciting properties of NPs as well as their potential deleterious outcomes.

Several adverse properties have been unveiled for nanomaterials that were previously regarded as safe in their bulk form. A few for all, silver that is known for its antibacterial activity, has cytotoxic effect higher than that of asbestos when in the nano form and it is now reported to be toxic to animal cells as well as to humans [160,161]; $TiO_2$, that is quite inert as a bulk material, becomes extremely dangerous when designed in a long wire shape and as a fiber that is longer than a lung macrophage is capable of sequestering. In addition, it is noteworthy that low doses (10 $mg/m^3$) of 20 nm diameter sized of inhaled $TiO_2$ nanoparticles resulted in a greater lung tumor incidence than exposure to higher doses (250 $mg/m^3$) of 300 nm diameter sized particles. $TiO_2$ nanoparticles with diameter larger than 100 nm are considered inert material both in humans and animals.

Thus far, taken together all the available data highlight that the crucial key to appreciate the activity of nanoparticles comes from their infinitesimal size, smaller than cellular subunits and cells, permitting them to permeate the biological structures, hence disturbing or disrupting the usual functions. In general, smaller particles exhibit a higher toxicity than larger particles of the same chemistry, composition and crystalline structure; smaller particles are less efficiently removed than larger particles by the clearance systems.

The contradictory reports sometimes found in the literature concerning the toxicity of a specific NP may be associated with the multitude of shapes, surfaces and different physical properties that have been used from time to time; then, they may be due primarly to the insufficient characterization of the NPs under study together with the different animal models or different cell lines, different doses, sizes and experimental routes undertaken in the investigation study.

Actually, although several inconsistent outcomes related to their toxicity are reported, it is nevertheless clear that,

regardless of their origin, man made or unintentionally released NPs may share several common adverse effects on health.

Considering that the exposure to nano-sized particles has enlarged dramatically over the last century, accurate information about safety or potential risk is urgently necessary and the evaluation of new findings and health hazard should be kept under strict control.

For that reason, it is noteworthy that the National Institute for Occupational Safety and Health started a very important initiative that was the creation of an online library giving information concerning nanoparticles. The library is constantly updated taking into account the different compositions of NPs as well as the known health effects [152,153]. In fact, it is becoming really pressing to inform about the current knowledge on nanoparticles toxicology and production, bearing in mind that nanotechnology as well as nanotoxicology fields are developing so rapidly.

Toxicity of nanoparticles has been reviewed in several reports and the most severe problem is related to the carcinogenic potential of NPs that has been associated both to the chemistry as well as to the physical properties. In particular, chemistry was considered to be relevant for the oxidative DNA damage and the formation of radical oxygen species (ROS) involved *via* direct mechanisms, whereas size, morphology and surface seem to be more important in all the other indirect mechanisms that underlie of cancer.

Nevertheless, the majority of studies on genotoxicity of NPs have been so far performed on cell cultures, thus in order to obtain data more relevant to the knowledge on human exposure, also animal and long-term exposure experiments should be carried out.

In conclusion, it is hoped that an increased awareness and understanding can derive from the increased ongoing studies on nanotechnology and nanotoxicology; thus, more caution and attention should be paid in the use and also in the manipulation and manufacturing of engineered as well as in the unintentionally released NPs, by taking into account all the intriguing and complex aspects of materials of nano size that underlie their interactions with biological materials in order to figure out a more correct risk assessment.

Ultimately, several disciplines (chemistry, medicine, biology) should interact together to shade light on all the complex molecular events involved in the toxicology belonging to the peculiar world of the nanoparticles.

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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