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EXPERT OPINION



# Advantages and risks of nanotechnologies in cancer patients and occupationally exposed workers

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***Introduction:*** In recent years, different nanotechnology platforms for drug delivery in the area of medical biology have gained remarkable attention.
***Areas covered:*** Nanoparticles (NPs) used as drug delivery vehicles consist of different materials such as natural or synthetic polymers, lipids or metals. They have an ultra-small size, large surface area-to-mass ratio and high reactivity. Although there are many data on the advantages in terms of both higher efficacy and less adverse effects of nanodrugs, several recent findings have reported unexpected toxicities giving origin to nanotoxicology.
***Expert opinion:*** Despite the great promise that NPs show, few studies have examined the human body's reaction due to NP exposure in both patients and workers. To perform this type of evaluation, it is necessary to define an adequate index of exposure, and the measure of this index is representative of what the worker is breathing. The properties of the nanomaterials used for designing NPs, such as in the case of poorly biocompatible materials (carbon nanotubes or heavy metals), and their chemical composition (as in the case of liposomes) largely contribute in determining potential side effects. Awareness of the levels of particles, which can cause health effects, is necessary for the workers and exposed patients.

**Keywords:** carbon nanomaterials, exposed workers, gold shell nanoparticles, liposomes, metallic particles, nanodrugs, nanotoxicology, polymers

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## 1. Introduction

According to the most recent statistics from the International Agency for Research on Cancer, about 14.1 million cancer cases and 8.2 million cancer deaths occurred in 2012 worldwide [1]. In general, cytotoxic drugs kill tumor cells, but also frequently display unwanted toxicities as they lack tumor cell selectivity. Moreover, drug resistance is often developed [2].

Many of the pharmacological properties of conventional ('free') drugs can be improved through the use of nanodrugs [3].

Nanotechnology has, in fact, opened a window for the development of diverse organic and inorganic drug carriers, known as nanoparticles (NPs). They are generally around 100 nm in at least one dimension and consist of different biodegradable materials, such as natural or synthetic polymers, lipids or metals such as phospholipids, lactic acid, chitosan, dextran, PEG, cholesterol, carbon and silica. Particle size, size distribution and zeta potential are the most important characteristics of NP systems. The zeta potential of a NP is commonly used for characterizing the surface charge property of NPs [4]. It reflects the electrical potential of particles and is

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**Article highlights.**

- Nanoparticles (NPs) used as drug delivery vehicles consist of different biodegradable materials such as natural or synthetic polymers, lipids or metals. They have an ultra-small size, large surface area-to-mass ratio and high reactivity.
- NPs are taken up by cells more efficiently than larger micromolecules and, therefore, they preferentially target tumor cells by the enhanced permeability and retention phenomenon exhibited by solid tumors compared with normal tissues.
- The toxic effects of NPs are generally linked to the low biocompatibility of the nanomaterial that is used for designing them.
- NPs with higher toxic potentials are carbon nanotubes (CNTs) that have shown to be carcinogenic for lung, but are also toxic for gastrointestinal tract (GIT), CNS and blood. Heavy metals can accumulate in liver and kidney and can again be toxic for CNS and GIT. Also, silicates are characterized by a prominent accumulation in liver and lung causing fibrosis and important side effects.
- Awareness of the levels of particles, which can cause health effects, is necessary for the workers and exposed patients.

This box summarizes key points contained in the article.

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influenced by the composition of the particle and the medium in which it is dispersed. These properties determine the *in vivo* distribution, biological fate, toxicity and the targeting ability of NP systems. In addition, they can also influence the drug loading, drug release and stability of NPs. Drug release is affected by particle size. Smaller particles have larger surface area, and therefore, most of the drug associated would be at or near the particle surface, leading to fast drug release, whereas, larger particles have large cores which allow more drug to be encapsulated and slowly diffuse out [4]. Smaller particles also have greater risk of aggregation of particles during storage and transportation of NP dispersion. It is always a challenge to formulate NPs with the smallest size possible but with maximum stability. When NPs are administered intravenously, they are easily recognized by the body immune systems and are then cleared by phagocytes from the circulation [4]. Apart from the size of NPs, their surface hydrophobicity determines the amount of adsorbed blood components, mainly proteins (opsonins). This, in turn, influences the *in vivo* fate of NPs.

Hence, to increase the likelihood of the success in drug targeting by NPs, it is necessary to minimize the opsonization and to prolong the circulation of NPs *in vivo*. This can be achieved by i) surface coating of NPs with hydrophilic polymers/surfactants and ii) formulation of NPs with biodegradable copolymers with hydrophilic segments such as PEG, polyethylene oxide, polyoxamer, poloxamine and polysorbate 80 (Tween 80). Drug loading and entrapment efficiency very much depend on the solid-state drug solubility in matrix material or polymer (solid dissolution or dispersion), which is related to the polymer composition, the molecular weight, the drug polymer interaction and the presence of end-functional groups (ester or carboxyl).

The PEG moiety has no or little effect on drug loading. The macromolecule or protein shows greatest loading efficiency when it is loaded at/or near its isoelectric point when it has minimum solubility and maximum adsorption [4]. In particular, NPs have an ultra-small size, large surface area-to-mass ratio and high reactivity, which are different from bulk materials (in microscale) of the same composition. In addition, NPs as therapeutic carriers have the ability to encapsulate and deliver poorly soluble drugs [5].

Although these characteristic are associated with highly desirable properties (e.g., mechanical, electrical, chemical) for specific medical uses, they could also be the main factors determining their potentially dangerous effects on human health [6].

Therefore, the different international scientific societies have stressed the importance of developing nanotoxicology, an important subdiscipline of nanotechnology, that studies the interactions of nanostructures with biological systems, with an emphasis on elucidating the relationships between the physical and chemical characteristics of nanostructures with the induction of toxic biological responses [6].

Nanodrugs can enter the body via the following main routes: intravenous, subcutaneous, lung, intraperitoneal and oral. Absorption can occur where the nanostructures for the first time interact with biological components, and afterward, they can be distributed to different organs in the body; here, nanomaterials may remain in the same wild-type structure, can be modified, or metabolized and they can enter the cells of the organ and reside in the cells for an unknown time before leaving to move to other organs or to be excreted. During all these stages the nanomaterials can cause toxicity through different mechanisms, such as inflammatory and pro-oxidant activity [6]. Interestingly, this risk does not exist only for patients in whom the drugs are administered by using nanotechnology, but also for workers who prepare the drugs to be administered such as researchers manufacturing the nanostructures or other healthy exposed workers [7]. In fact, although the production activities of nanodrugs follow the same safety procedures of standard anticancer drugs, the production of the nanocarriers such as carbon nanotubes (CNTs) can lead to a further specific risk to exposed personnel.

This paper seeks to provide a comprehensive review of all articles published on nanomaterials and nanodrugs underlining their possible toxicity in both patients and occupationally exposed workers.

## 2. Overview of different classes of NPs

Since polyalkylcyanoacrylate NPs attached with anticancer drugs were described in the late 1970s [8], nanotechnology has developed different drug carriers that deliver drugs more specifically to tumor cells sparing the normal tissues

**Table 1. Nanodrug delivery systems*: in vivo* and *in vitro* studies.**

| Carrier | Drug encapsulated | *In vivo or in vitro* studies | Ref. |
|---|---|---|---|
| Liposome (PEG or not PEG-coated) | Zoledronate<br>DOX | ZOL-encapsulating liposomes were significantly highly effective in inducing *in vitro* and *in vivo* growth inhibition of prostate cancer and multiple myeloma cells.<br>Doxil, gives a stable drug delivery system with enhanced biocompatibility, efficacy and reduced cytotoxic effects. | [19,20] |
| CNTs | Epirubicin, DOX, cisplatin, methotrexate, quercetin and paclitaxel | CNTS coated with 10-hydroxyl camptothecin and amino group functionalized by carboxylic group or single-walled nanotubes conjugated with small interfering RNA and functionalized with DSPE-PEG-amine exhibited higher drug accumulation and bioavailability with little toxicity.<br>Europium-catalyzed single-walled CNTs are excellent cellular imaging probe for breast cancer, having excitation values with invisible ranges and 95 – 100% labeling efficiency. | [26,27] |
| Polymeric micelles | DOX and cisplatin | Biodegradable diblock amphiphilic copolymer (mPEG-b-p(LA-CO-MCG)) has carboxylate group for platinum chelation. The cytotoxicity of the drug–polymer conjugates toward breast cancer was lower than cisplatin but comparable to oxaliplatin. | [35] |
| Dendrimers | DNA,<br>DOX | *In vivo* studies on xenograft mice models showed that the G4 polyamidoamine dendrimer conjugated with ASODN has more efficiency in inhibiting tumor angiogenesis of breast cancer than naked ASODN.<br>DOX was conjugated with PPI as well as FA. The conjugated ligands DOX–PPI–FA and PPI–FA show less hemolytic activity and more stability. | [42,43] |
| Metallic NP | Daunorubicin | Chlorotoxin, a biocompatible iron oxide nanoprobe coated with PEG, is capable of specifically targeting glioma tumors via the surface-bound targeting peptide.<br>ZnO–NPs–DNR induced remarkable decrease in cytotoxicity of anticancer drug and considerable increase in the cancer cell targeting mediated by reactive oxygen species in human hepatocarcinoma cells (SMMC-7721 cells). | [55,57] |
| Gold NPs | No drug | Photothermal ablation | [65] |

ASODN: Antisense oligodeoxynucleotides; CNTs: Carbon nanotubes; DOX: Doxorubicin; DSPE: distearoyl-sn-glycero-3-phosphoethanolamine; FA: Folic acid; PPI: Polypropylene imine; ZOL: Zoledronic acid.

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(**Table 1**) [3]. The feasibility of selective and efficient delivery of anticancer therapeutics using nanocarriers has been demonstrated in numerous studies. There are two major mechanisms: passive targeting and active targeting.

In contrast to normal tissues, many solid tumors possess unique structural features of hyperpermeable vasculature and impaired lymphatic drainage [9]. As a result, tumor tissues are relatively permeable to macromolecules and nanocarriers [9]. Passive targeting, therefore, refers to the selective extravasation and retention of long-circulating nanocarriers at tumor sites due to the enhanced permeability and retention (EPR) effect. In contrast, active targeting is based on specific interactions between the nanocarrier and receptors on the target cell, which may also promote internalization of nanocarriers through receptor-mediated endocytosis. To take full advantage of the EPR effect, it is critical to incorporate several properties into the design of nanocarriers. A key consideration is the need for long circulation time in the bloodstream required for extravasation. It has been shown that the threshold size for extravasation in tumors is ~ 400 nm in diameter, and that nanocarriers with diameters of < 200 nm are preferred [9]. Surface charge of nanocarriers is another important parameter. Both highly positive and highly negative charged nanocarriers are susceptible to rapid clearance by the reticuloendothelial system (RES) [10]. Thus, it is important to design nanocarriers with either a neutral or a slightly negative zeta potential. In addition, a common method for reducing the recognition of nanocarriers by the RES is to coat their surfaces with PEG [11]. Due to steric effect of the hydrophilic PEG, the binding of nanocarriers to opsonins, which promotes RES clearance, is significantly reduced, resulting in prolonged circulation time and increased accumulation at the tumor sites via EPR. Examples of nanodevices delivering anticancer drugs include lipids (liposomes), CNTs, polymers (micelles, dendrimers or nanoemulsions), metallic NPs and gold nanoshells.

### 2.1 Liposomes

Liposomes and particularly nanoliposomes are the most used nanotechnology-based delivery systems for small molecules, peptides, small and long nucleic acids and proteins used for therapy of different cancers.

The carrier potential of phospholipid suspensions in medicine was predicted in 1935 and in 1965 Bangham described for the first time the possibility of using these materials to obtain vesicles (liposomes), at that time proposed as artificial membrane for partition experiments [12]. Based on net charge, liposomes are categorized into cationic, anionic and neutral NPs [13]. Since their inception,

**Table 2. Advantages and disadvantages of the different types of nanocarriers.**

| Types of carriers | Advantages | Disadvantages |
|---|---|---|
| Liposomes | Biocompatible<br>Longer duration of circulation<br>Amphiphilic | May trigger immune response |
| Carbon nanoparticles | Multiple functions<br>Chemical modification<br>Water soluble and biocompatible<br>Efficient loading | Toxicity |
| Polymeric micelles | Efficient carrier system for hydrophilic drug<br>Biodegradable, self-assembling and biocompatible<br>Potential targeting<br>Functional modification | Occasional cytotoxicity<br>Need of surface modifications |
| Dendrimers | Uniformity in size, shape and branch length<br>Tuned pharmacokinetics and biodistribution<br>Increased surface area, increased loading<br>Targeting is achieved | Complex synthetic route |
| Metallic nanoparticles<br>Gold nanoshells | Uniformity in size, shape and branch length<br>Tuned pharmacokinetics and biodistribution<br>Increased surface area, increased loading<br>Targeting is achieved | Toxicity |

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liposomes have been explored as carriers for delivering drugs and pharmaceuticals [13].

Liposomes are generally composed of one or more bilayers of an aliphatic lipid molecule arranged to form a vesicle that encloses an interior aqueous space. Liposomes with a defined and uniform size can be produced by different methods such as sonication or extrusion through polycarbonate filter membranes [14]. Stability of the membrane bilayer as well as retention of incorporated drugs thereby depends on lipid composition and cholesterol content of the liposomal membranes [13]. Liposomes have several advantages (**Table 2**) over many other nanodelivery systems by being less toxic and having a high therapeutic index; in fact, liposomal carriers have a protective effect on incorporated drugs by preventing their enzymatic degradation. Moreover, concentrations of lipophilic drugs in aqueous media can be increased considerably using liposomal formulations. Liposomal carriers have a protective effect on incorporated drugs by preventing their enzymatic degradation.

The head groups of aliphatic lipid molecule can be modified to introduce functional groups, which can facilitate conjugation to antibodies or other ligands, and/or polymerizable moieties to generate stable liposomes. Liposome preparations have also included PEGylated lipids to bypass the RES and promote accumulation in tumors and to mitigate or suppress immune response [13,14].

In fact, liposomes extravasate through the gaps between the endothelial cells of the tumor vasculature (passive targeting) and collect in the interstitial space, where they are retained due to the lack of lymphatic clearance; this process is known as the EPR effect [13,15]. Liposomes can also be actively targeted to tumor tissues by recognizing specific tumor epitopes or receptors, which is achieved by coupling tumor-specific ligands or antibodies onto the surface of the liposomes or by means of stimulus-sensitive drug carriers such as acid-triggered release or enzyme-triggered drug release [13-15].

Liposomes' direct toxicity can be primarily caused by their composition, particle size or charge. For example, cationic liposomes can interact with serum proteins, lipoproteins and the extracellular matrix, leading to aggregation or release of agents that are loaded before reaching the target cells leading to systemic toxicity [13].

At doses significantly higher than those used (multiple injections at a dose ≥ 100 mg/kg lipid), liposomes have been shown to result in an impairment of RES function, hepatomegaly, granulomas and splenomegaly [16]. In addition, an increasing lipid dose has been shown to deplete plasma of various proteins. Although the identity and significance of all the depleted proteins are unclear, it is possible that their loss will result in a disruption of normal homeostasis [16].

It is worthy to note that toxicology of a delivery system cannot be associated with that type of carrier, but it strictly depends on the nanocarrier composition. A pivotal example in this sense is given by the encapsulation of zoledronic acid (ZOL) in PEGylated liposomes with different compositions. ZOL encapsulation in liposomes modified with folic acid (FA) resulted in $IC_{50}$ values on folate receptor-expressing colon tumor cells to be > 100-fold lower than those of free ZOL [17]. The same group demonstrated that these liposomes unluckily caused detrimental side and toxic effects in an animal model. Toxicity was noncumulative and appeared to

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involve macrophage/monocyte activation and release of cytokines [18]. On the other hand, our group recently demonstrated that the use of liposomes with a different lipid composition can significantly reduce the uptake of ZOL by macrophages and their consequent activation and induction of severe side effects. In fact, it was reported that ZOL-encapsulating liposomes was significantly highly effective in inducing *in vitro* and *in vivo* growth inhibition of prostate cancer and multiple myeloma cells without inducing animal death or necrotic effects in normal tissues collected from animals or biochemical evidences of liver, bone and kidney toxicity [19].

Doxil, a liposomal-based formulation, which consists of cholesterol and high phase-transition temperature phospholipids hydrogenated soy phosphatidylcholine, gives a stable drug delivery system with enhanced biocompatibility, efficacy and reduced cytotoxic effects. Anthracycline doxorubicin (DOX), an active cytotoxic agent, when encapsulated inside the aqueous core of the liposome, significantly shows decrease in the cardiotoxicity [20]. Hence, higher dose of the chemotherapeutic agents can be given to the patient as in the form of liposomal drug delivery system, which can transfer significant amount of the anticancer drug to the desired targeted site.

### 2.2 Carbon nanomaterials

CNTs are allotropes of carbon with a cylindrical nano-shaped structure, similar to the rolled sheets of graphene rings. CNTs are very dynamic and are used potentially not only in cancer cell imaging but also for drug delivery system (**Table 2**). In fact, their biological and chemical properties allow a passive diffusion of CNTs across the lipid bilayer, or attachment to the cell surface with subsequent endocytosis [21]. CNTs can be single-walled CNTs (SWCNTs) or multi-walled CNTs (MWCNTs). SWCNTs consist of one layer of graphene sheet with diameter of 1 – 2nm, whereas MWCNTs are multiple layers of SWCNTs that are coaxially arranged with size variation of 5 – 100nm [21]. Confocal microscopy imaging showed that SWCNTs and MWCNTs have different mechanisms of cellular uptake due to the size of the CNT; therefore, SWCNTs show localized effect in cell and prolonged distribution compared to MWCNTs [22,23].

CNTs have been first used as additives to various structural materials for electronics, optics, plastics and other materials in nanotechnology fields. At the beginning of the twenty-first century, they have been introduced in pharmacy and medicine for drug delivery system in therapeutics [24]. Many anticancer drugs have been conjugated with functionalized CNTs and successfully evaluated both *in vitro* and *in vivo*, such as epirubicin, DOX, cisplatin, methotrexate, quercetin and paclitaxel [25]. Drugs can either be loaded into the CNTs or be attached to the surface of the CNTs. Several studies show that the chemical functionalization of single wall nanotubes (SWNTs) or MWCNTs makes carrier systems more effective. CNTs coated with 10-hydroxyl camptothecin and amino group functionalized by carboxylic group or SWNTs conjugated to small interfering RNA and functionalized with distearoyl-sn-glycero-3-phosphoethanolamine (DSPE)–PEG–amine exhibited higher drug accumulation and bioavailability with little toxicity [26]. Another study revealed that europium-catalyzed SWCNTs (Eu-SWCNTs) are an excellent cellular imaging probe for breast cancer, having excitation values with invisible ranges and 95 – 100% labeling efficiency [27].

On the other hand, many publications found in the literature suggested that pristine CNTs could be the source of occupational lung diseases in workers of CNT industries such as asbestos [28]. On a dose per mass basis the nanotubes were more toxic than quartz particles that are, in turn, well known for their lung toxicity.

Several studies using intratracheal instillation of high doses of nanotubes in rodents demonstrated chronic lung inflammation, including foreign-body granuloma formation and interstitial fibrosis [28].

*In vitro* incubation of keratinocytes and bronchial epithelial cells with high doses of SWCNTs results in reactive oxygen species (ROS) generation, lipid peroxidation, oxidative stress, mitochondrial dysfunction and changes in cell morphology [29]. Therefore, the results of these studies indicate that caution should be used to limit human exposures to CNT that now are regulated as respirable particulates not otherwise regulated with permissible exposure limit of 5 mg/m$^3$, data too high in relation to *in vivo* and *in vitro* studies.

### 2.3 Polymeric micelles

Micelles are generally colloidal particles having tunable size and surface functionality, high monodispersity and excellent stability [30]. The polymers used for micelles range from simple natural polymers to complex synthetic copolymers, which have generally a hydrophobic tail and mostly hydrophilic head. Polymer selection plays an important role in the formation of micelles, and the selection for micelle formation is based on the characteristics of both hydrophobic and hydrophilic block polymers. By arranging these block polymers, different patterns of micelles are formed; hence, these polymers are called diblock copolymer (A-B type copolymers), triblock (TR) copolymer (A-B-C type copolymer), and grafted polymers [31]. Hydrophilic outer shell of the micelles gives steric stability and prevents rapid uptake of the formulation by RES and provides longer duration of circulation time inside the body. Recently the behavior of core-shell poly(ethylene oxide)-poly(epsilon-caprolactone) micelles derived from copolymers with linear TR and 4-arm star-diblock (ST) architectures for the delivery of docetaxel (DTX) was investigated. Both free DTX and DTX-loaded TR micelles displayed a significantly lower cytotoxic activity in G(2)/M phase synchronized cells, whereas cytotoxicity of DTX-loaded ST micelles did not change. Cytotoxicity was related to micelle stability, uptake and release rate in cell culture media [32]. Moreover, the same authors have reported that amphiphilic block copolymers of poly($\varepsilon$-caprolactone) and poly(ethylene

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oxide) can be assembled in core-shell NPs by a melting sonication technique. These NPs also have reduced side effects in an animal model, if compared to free DTX [33].

There are two typical routes to load drug by using this amphiphilic micelle structure: drug conjugation and drug encapsulation. Drug conjugation uses a non-water-soluble drug as a hydrophobic core of micelles, which are conjugated to the hydrophilic polymer backbone. For drug release, biodegradable chemical linkers are usually selected for conjugating the drug to the main chain. For example, Duncan *et al.* studied PEG–DOX conjugates with peptide linkers. Their study covered several factors for drug delivery, for example, drug release profiles, *in vitro* cytotoxicity and biodistribution, in regard to PEG–DOX polymers of linear or branched architecture (molecular weight: 5000 – 20,000 g/mol) and with different peptidyl linkers [34]. Xue *et al.* developed biodegradable diblock amphiphilic copolymer (mPEG-b-p(LA-CO-MCG) having carboxylate group for platinum chelation. The cytotoxicity of the drug–polymer conjugates toward breast cancer was lower than cisplatin but comparable to oxaliplatin. This polymer conjugate showed the potential use as a targeted carrier vehicle due to its reduced side effect [35]. The second route is drug encapsulation, that is, emulsions of the drug with readymade amphiphilic copolymers form drug-loading micelles. In this case, drugs are physically entrapped into the hydrophobic core of micelles. Poly(lactic-*co*-glycolic acid) (PLGA) is one of most popular hydrophobic polymers used as a core part for drug encapsulation. PLGA has ester bonds that are destroyed in the body, resulting in sustained drug release. Consequently, many researchers presented natural polymer-PLGA as biodegradable amphiphilic copolymer, for example, hyaluronan-PLGA, dextran-PLGA, heparin-PLGA and chitosan-PLGA [36]. The physicochemical properties of amphiphilic polymers for drug-encapsulated micelles determine the factors that influence the drug delivery features in a similar manner as chemical linkers do in drug-conjugated micelles.

Polymer-based imaging with near-infrared (NIR) fluorophores provides efficient advantages for tumor imaging, such as improved plasma half-lives, large surface area, less toxicity, stability and improved targeting [37]. Kim *et al.* have developed NIR Cy5.5-labeled hydrophobically modified glycol chitosan NPs (HGC-Cy5.5) with molecular weight ranging from 20 to 250kDa. *In vivo* biodistribution studies revealed that low molecular weight HGC-Cy5.5 showed faster clearance from the body in comparison to high molecular weight HGC-Cy5.5, whereas high molecular weight HGC-Cy5.5 had high tumor targeting capacity than low molecular weight HGC-Cy5.5. These probes provide promising imaging agents, which are used for detecting solid tumor [38].

### 2.3.1 Dendrimers

Dendrimers are nanosized branched structures. Uniformity in size, branching length, shape and increased surface area can be achieved with various changes in dendrimer structures. Dendrimers can be optimal carrier system for anticancer drug [39] because of their high biocompatibility and pharmacokinetic parameters. Dendrimers can be grown toward outward direction from the central core; this process is known as divergent method designed by Tomalia *et al.* [40], or it may be formulated by the Frechet's method, in which the dendrimers are made toward inside direction [41]. Based on the branching unit, dendrimers can be classified; for example, dendrimers with central branch core molecule is considered as generation 0 (G0) and with each successive addition of increased branching point they may be considered as G1, G2 and so forth [41]. Dendrimers and dendrons are monodispersed and usually highly symmetric, spherical compounds. They can be used as carrier systems for the treatment of diseases such as AIDS, cancer, malaria and so forth.

Wang *et al.* had synthesized G4 polyamidoamine dendrimer conjugated to antisense oligodeoxynucleotides (ASODN). The conjugates showed more stability, less toxicity and increased bioavailability. *In vivo* studies on xenograft mice models showed that the conjugate has more efficiency in inhibiting tumor angiogenesis of breast cancer than naked ASODN [42]. Gupta *et al.* had conjugated DOX to polypropylene imine (PPI) as well as FA to fifth-generation PPI. The conjugated ligands DOX–PPI–FA and PPI–FA show less hemolytic activity and more stability [43]. Fluorescence studies showed higher cellular uptake by tumor cells of the formulated conjugate ligand. Results of the study revealed that FA-conjugated PPI dendrimers could be a better choice for anticancer drug targeting in the future.

Samuelson *et al.* have developed translocator protein (TSPO) dendrimer imaging agent with significantly increased targeting and imaging characteristics. The reported study revealed that TSPO can be used as an imaging agent in brain, breast, and ovarian cancers as well as in prostate carcinoma [44].

### 2.3.2 Emulsions

Nanoemulsions are oil-in-water (o/w) emulsions with mean droplet sizes ranging from 50 to 1000 nm. The NPs can exist as o/w and water-in-oil forms, where the core of the particle is either oil or water, respectively. These emulsions are easily produced in large quantities by mixing a water-immiscible oil phase with an aqueous phase under high shear stress or mechanical extrusion process that is worldwide available [45].

The capacity of nanoemulsions to dissolve large quantities of hydrophobics, along with their mutual compatibility and ability to protect the drugs from hydrolysis and enzymatic degradation, makes them ideal vehicles for drug delivery.

Nanoemulsions are part of a broad class of multiphase colloidal dispersions. Although some lyotropic liquid crystalline phases, also known as 'micellar phases', 'mesophases' and 'microemulsions', appear to be similar to nanoemulsions in composition and nanoscale structure, such phases are actually quite different [45]. Lyotropic liquid crystals are equilibrium structures, comprising liquids and surfactant, such as lamellar sheets, hexagonally packed columns, and wormlike

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micellar phases, that form spontaneously through thermodynamic self-assembly. By contrast, nanoemulsions do not form spontaneously; an external shear must be applied to rupture larger droplets into smaller ones. Compared to microemulsion phases, relatively little is known about creating and controlling nanoemulsions. High shear, well beyond the reach of ordinary mixing devices, must be applied to overcome the effects of surface tension to rupture the droplets into the nanoscale regime [45]. This is one of the most common and effective routes of drug administration usually adopted for active drugs with low bioavailability and narrow therapeutic index. Major clinical and preclinical trials have hence been carried out with nanoemulsion-based carriers. Chlorambucil, a lipophilic anticancer agent, has been used against breast and ovarian cancers. Its pharmacokinetics and anticancer activity have been studied by loading it in parenteral emulsions prepared by high-energy ultrasonication method. Treatment of colon adenocarcinoma in the mouse with this nanoemulsion leads to higher tumor suppression rate compared to plain drug solution treatment concluding that the drug-loaded emulsion could be an effective carrier for its delivery in cancer treatment [46].

### 2.4 Metallic particles

Hollow inorganic NPs represent a unique structure for drug carriers. To produce a cavity in the NP, removable templates are introduced such as polymeric or rather soft inorganic NPs. Hollow silica NPs, extensively used as drug carriers, have been reported using various templates, including poly(styrene-β-2-vinyl pyridine-β-ethylene oxide) (PS-PVP-PEO) block copolymer [47], $Fe_3O_4$ clusters [48] and so forth. After silica coating of the templates, the following steps are required for proper removal of the template: dissolution using apt solvents or calcination for organic templates and acidic etching for soft inorganic templates. Yang *et al.* reported high drug-loading efficiency and sustained release kinetics of a model drug (DOX) using hollow silica NPs with $Fe_3O_4$ clusters as template [48]. Venkatesan *et al.* investigated chitosan-modified hydroxyapatite nanocarriers loaded with celecoxib, which is a potential anticancer drug against most carcinomas, especially in patients with familial adenomatous polyposis and precancerous disease of the colon. NPs exhibited small, narrow hydrodynamic size distributions, hemocompatibility, high entrapment efficiencies and sustained release profiles [49]. Similarly, Wang *et al.* fabricated flower-like nanostructured hydroxyapatite hollow spheres as carriers for the cellular delivery of anticancer drug mitoxantrone [50].

Iron (III) oxide ($Fe_2O_3$) is a reddish brown, inorganic compound that is paramagnetic in nature and also one of the three main oxides of iron, whereas other two being FeO and $Fe_3O_4$. The $Fe_3O_4$, which also occurs naturally as the mineral magnetite, is also superparamagnetic in nature. Due to their ultrafine size, magnetic properties and biocompatibility, superparamagnetic iron oxide NPs have emerged as promising candidates for various biomedical applications, such as enhanced resolution contrast agents for MRI, targeted drug delivery and imaging [51]. All these biomedical applications require that the NPs have high magnetization values so as to provide high-resolution MR images. In general, the superparamagnetic NPs resemble excellent imaging probes to be used as MRI contrast agents since the MR signal intensity is significantly modulated without any compromise in its *in vivo* stability [52].

Converging advances in the understanding of the molecular biology of various diseases recommended the need of homogeneous and targeted imaging probes along with a narrow size distribution between 10 and 250 nm in diameter. Developing magnetic NPs in this diameter range is a complex process and various chemical routes for their synthesis have been proposed. These methods include microemulsions, sol–gel syntheses, sonochemical reactions, hydrothermal reactions, hydrolysis and thermolysis of precursors, flow injection syntheses and electrospray syntheses. However, the most common method for the production of magnetite NPs is the chemical coprecipitation technique of iron salts [53]. The main advantage of the coprecipitation process is that a large amount of NPs can be synthesized but with limited control on size distribution. In order to improve the cellular uptake, these particles can be modified with a peculiar surface coating so that they can be easily conjugated to drugs, proteins, enzymes, antibodies or nucleotides and can be directed to an organ, tissue or tumor. Whereas traditional contrast agents distribute rather nonspecifically, targeted molecular imaging probes based on iron oxide NPs have been developed that specifically target body tissue or cells [54]. For instance, Sun *et al.* developed (chlorotoxin) a biocompatible iron oxide nanoprobe coated with PEG, which is capable of specifically targeting glioma tumors via the surface-bound targeting peptide [55]. Moreover, MRI studies showed the preferential accumulation of the nanoprobe within gliomas. In another study, Apopa *et al.* engineered iron oxide NPs that can induce an increase in cell permeability through the production of ROS and the stabilization of microtubules [56]. These are the few applications of iron oxide NPs in biomedical imaging.

Zinc oxide (ZnO) NPs also provide a promising approach for imaging and drug delivery system in cancer therapy. ZnO NPs are self-organizing nanomaterials that can be grown on any substance with high quality of crystalline and amorphous properties. This provides ZnO NPs with large surface area–to-volume ratio and higher efficiency of photoimaging. Generally, white light is being observed in photonic device and is potentially used in photodynamic therapy. Photosensitizers are being taken by cancer cell in photodynamic therapy for cancer followed by exposure to white light. Zhang *et al.* had fabricated ZnO NPs as a drug carrier for the anticancer drug daunorubicin (DNR) in photodynamic therapy, by using simple one-step solid-state reaction at a normal room temperature in the air. The investigation revealed that the combination of ZnO–NPs–DNR has induced remarkable

decrease in cytotoxicity of anticancer drug and considerable increase in the cancer cell targeting mediated by ROS in human hepatocarcinoma cells (SMMC-7721 cells) [57].

### 2.5 Gold shell NPs

Inorganic NPs, such as gold NPs (AuNPs), can be attractive carriers to deliver drugs, genes and proteins because they can provide unique drug release strategies using internal or external stimuli, such as glutathione, pH, heat and light [58].

AuNPs show tunable parameters, such as particle size, surface properties and biocompatibility with low toxicity [59]. Drugs are usually loaded on the surface of inorganic NPs by conjugation, charge interaction or hydrophobic interaction [60].

In addition to drug delivery, AuNPs, due to their special physicochemical properties, are widely used for imaging, biosensing and photothermal therapy. Inert and nontoxic nature of AuNPs makes them a suitable nanomedicines carrier system applicable in biomedical field [61]. The single AuNP and aggregated AuNPs follow different cellular uptake patterns and during their uptake these particles interact with the compartments of cellular membrane [61]. Eghtedari *et al.* had functionalized AuNPs for *in vivo* targeting to breast cancer. Herceptin was used to functionalize the AuNPs by molecular recognition of breast cancer cells along with PEG. Eghtedari *et al.* revealed the *in vitro* stability of these herceptin–PEG–AuNPs in blood. To prolong the circulation time, AuNPs can escape the RES, and PEG coating has shown a promising effect protecting AuNPs from the uptake by the RES of liver and spleen [62].

Connor *et al.* had studied the cytotoxic effect of AuNPs under suitable experimental conditions. Small size of AuNPs makes them potentially useful for drug delivery and gene therapy, because of their lower cytotoxicity toward normal cell and increased chemotherapeutic efficiency toward abnormal cancer cell [63]. Xiao *et al.* had developed multifunctional water-soluble AuNPs as a nanocarrier for anticancer drugs. The pH-sensitive behavior of these AuNPs causes the release of drug, by minimizing the cytotoxic nonspecific systemic distribution of anticancer drug and increasing the efficiency of anticancer drug to targeting tumor [64]. Wang *et al.* have developed multifunctional NPs of gold and pearls consisting of single amine-modified AuNPs, and $Fe_3O_4$ 'pearls' were used to give final touch with the help of carboxyl group. Reported study demonstrated the effectiveness of the AuNPs in breast cancer photothermal ablation and dual-mode imaging of breast cancer [65].

## 3. Organ toxicity of medical NPs

### 3.1 Respiratory system

One of the most important doors and organ target for NPs is the respiratory system and one of the most widespread routes of human exposure to airborne NPs is inhalation in the workplace and the environment (**Figure 1**). The deposition of NPs in the respiratory tract is determined essentially by the particle aerodynamic or thermodynamic diameter with high probability of NPs reaching the alveoli peaked at a size of approximately 20 nm [66].

The administration of NPs as aerosol for the treatment of lung cancer is becoming possible for oncologist since a number of drugs delivered in aerosolized NPs have been already investigated *in vitro*, in animal models and in human trials [66].

Respiratory exposure to NPs can cause important adverse respiratory effects, such as multifocal granulomas, peribronchial inflammation, progressive interstitial fibrosis, chronic inflammatory responses, collagen deposition, pleural lesions and gene mutations, at least in experimental animal studies [67].

One of the suggested mechanisms is the significant correlation between the surface area of NPs and the induced inflammation via increased oxidative stress. For example, cationic liposomes have been shown to cause cellular influx and inflammation of lungs through ROS induction [68]. Interestingly, various types of NPs can induce different inflammatory reactions [67,69]. The greater toxicity to the respiratory tract has been demonstrated for CNTs [69].

Although most of the studies for this type of NPs were performed in animal models using intratracheal instillation technique, which is not the usual way of exposure, most of the literature agrees in describing that chronic inflammation and oxidative stress observed during and after exposure to CNT can induce adverse health effects, such as fibrosis, genotoxicity and cancer, that is secondarily caused by fibers [69]. In fact, Wang *et al*. demonstrated that chronic exposure to CNT can produce malignant transformation of human lung small airway epithelial cells [70]. Sargent *et al.* showed that inhaled CNTs are strong promoters of pulmonary adenomas and adenocarcinomas in B6C3F1 mice [71]. Results from these studies suggest that caution should be taken during use, production and processing of CNT to limit human inhalation exposures.

### 3.2 Blood and vascular system

Besides the blood penetration directly via intravenous injection, nanodrugs can pass through epithelia of the respiratory tract into the interstitium and access the bloodstream directly or via lymphatic pathways (**Figure 1**).

Nemmar *et al.* found that inhaled (99 m)Tc-labelled carbon particles (100 nm) pass to the blood circulation 1 min after exposure [72]. Once the nanodrugs reach the bloodstream, it could induce adverse biological effects. For example cationic liposomes can interact with serum proteins, lipoproteins and the extracellular matrix, leading to aggregation or release of agents that are loaded before reaching the target cells leading to systemic toxicity [73]. Moreover, cationic liposomes can also directly induce macrophage-mediated toxicity [73].

As previously described, NPs such as liposomes and CNTs can deplete plasma of various proteins. CNTs instilled into the blood has been reported to induce platelet aggregation

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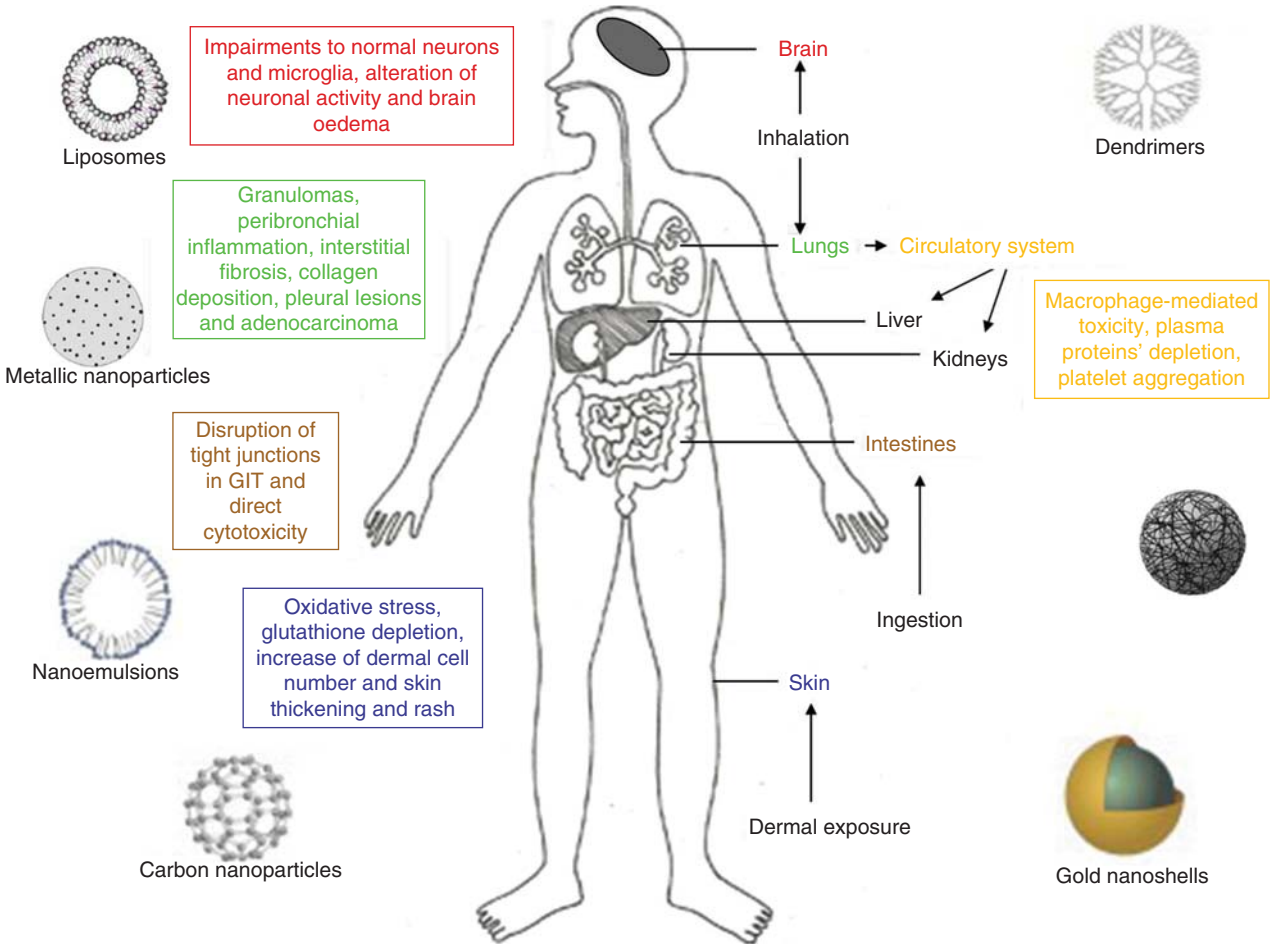


**Figure 1. Illustration of the pathways of exposure to several types of nanoparticles (NPs) and associated adverse effects.** Liposomes, polymeric micelles, metallic NPs, nanoemulsions, dendrimers, gold nanoshells and carbon NPs can be internalized in cells by inhalation, ingestion or dermal exposure. Inhaled nanodrugs can pass through epithelia of the respiratory tract into the interstitium and access the bloodstream directly or via lymphatic pathways. Successively, the bloodstream transport nanodrugs to the CNS, liver, kidneys and other organs. Moreover, they can be directly ingested or alternatively, inhaled NPs can also arrive in gastrointestinal tract. Once NPs are internalized in cells, they can induce organ-specific toxicity.

in the hepatic microvasculature of healthy mice in association with prothrombotic changes on the endothelial surface of the hepatic microvessels. In addition, they accelerate the rate of vascular thrombosis in rat carotid artery [74].

In fact, for these nanomaterials, a proinflammatory action on endothelial cells, inhibition of cell growth and reduction of endothelial nitric oxide synthase were proven [75].

### 3.3 CNS

Drug transport from the bloodstream to the CNS is hindered by the presence of an endothelium characterized by a low permeability, namely the blood–brain barrier (BBB), whose cells are linked by tight junctions hindering the passage of most drugs. NPs may produce potential toxicity on human neural cells because of their ability to pass through biological membranes (**Figure 1**) [76]. Effects from the presence (or even accumulation) of metallic NPs in the brain and through the BBB have not yet been fully studied. Small-sized particles have better mobility and NP's transport across the BBB is possible either by passive diffusion or by carrier-mediated endocytosis [77]. In addition, NPs may be uptaken directly into the brain by transsynaptic transport [77]. For example, silver NPs (Ag NPs) can enter via the BBB [78] and accumulate in different regions of the brain, and this may be useful for drug delivery, but may also represent a risk for the patient [79]. We have also demonstrated that PEGylated liposomes encapsulating ZOL can be useful in the treatment of neuropathic pain in an animal model and that they can accumulate in CNS modifying microglia phenotype thus demonstrating their ability to cross BBB [80]. In this case, the use of PEGylated liposomes did not hamper the restoration of microglia architecture, thus suggesting absence of acute toxicity. It has also been reported that NP exposure can induce impairments to normal neurons [81], microglia and even aggravate the

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process of brain pathology [82]. Voltage-gated sodium current is responsible for neuronal cells excitability and neuronal activity and function in the CNS. Therefore, metallic NPs could modulate the current by leading to alterations in functionality. Some reports have shown that NPs can impair cell function and even induce certain cell death [83]. In recent studies on the neurotoxicity of metallic NPs, a neuroendocrine cell line (PC-12 cells) was exposed to NPs such as Ag ($5 \times 10^{-5}$ g ml$^{-1}$), which reduced the level of dopamine. These findings suggest that metallic NPs might have significant pathological consequences on the brain of mammalians, while enhancing or inhibiting some particular functionality [84].

In a separate study, up to 30 µg ml$^{-}$SWCNTs significantly decreased the overall DNA content in chicken embryonic spinal cord or dorsal root ganglia [85]. According to a study by Sharma *et al.* [82], which focused on the effects of NPs on the BBB, administration of metallic NPs showed disrupted BBB function and induced brain edema formation.

### 3.4 Gastrointestinal tract

Another door for NP's entrance in the body is the gastrointestinal tract (GIT). The GIT is a selective mucosal barrier that represents a considerable surface area, estimated at 300 m$^2$ in the adult human, for potential interaction with NPs [86]. They can be directly ingested; alternatively, inhaled NPs can also arrive in GIT once they are cleared by respiratory tract (**Figure 1**) [87].

It is important to emphasize that the absorption of the NPs at the level of GIT depends not only on particle size but also by their chemicophysical characteristics [88]. For example, metallic NPs and the CNTs have greater absorption if they are smaller in size; on the other hand, anionic polyamidoamine larger dendrimers deposit at higher levels due to adherence to negatively charged cell membranes of the gut epithelium [89].

Based on literature, data have not been reported on acute or severe toxic effects of ingested NPs at typical levels of exposure [8], even if these data require a more careful evaluation in experiments *in vitro* and *in vivo*.

Carbon nanomaterials had little adverse effects on GIT; in fact, SWCNT-COOH appears to inhibit efflux pump activity in Caco-2 cells or co-cultures through interaction with the P-glycoprotein efflux system, with increased cellular accumulation of the pump substrate, rhodamine-123. SWCNT-COOH also modulated the tight junctions through perturbation of zonulin-1 distribution, a tight junction marker protein [90].

These findings were viewed as evidence that CNTs could enhance paracellular permeability via disruption of tight junctions in GIT.

### 3.5 Skin

The effects of nanocarriers used in cancer therapy on skin appear to be more limited (**Figure 1**). It has been found *in vitro* that CNTs can induce proinflammatory responses in human keratinocyte cells in skin [91]. In particular, exposure of non-purified CNTs to mice skin seems to cause oxidative stress, glutathione depletion, increase of dermal cell number and skin thickening probably due to the presence of metals, particularly iron [92,93]. *In vivo* studies demonstrating the possible toxic effects on the skin by anticancer nanodrugs are rare too.

In the clinical practice, toxic effect on skin frequently appears when systemically administering nanodrug. Typical dose-limiting toxicity of PEGylated liposome containing DOX (Doxil/Caelix) consists of palmar-plantar erythrodysesthesia (also known as acral erythema or hand-foot syndrome) and mucositis [94] A relatively high incidence of skin rash was noted in patients who were given NP albumin-bound paclitaxel and cisplatin. The authors hypothesized that albumin component of nab-paclitaxel might be the cause of the skin disorder [95]. However, these effects are expected to be due to drug delivered in peripheral tissues rather than to the nanocarrier.

However, more research is needed to investigate the toxic cellular effects of NPs on skin *in vitro* and *in vivo*.

## 4. Conclusion

The use of nanotechnology in medicine and more specifically drug delivery is expected to spread rapidly. For decades pharmaceutical sciences have been using NPs to reduce toxicity and side effects of drugs. Till recently it was not realized that these carrier systems could impose risks on the patient. In fact, nanovectors are not completely inert materials and can be endowed with intrinsic cytotoxicity that causes, sometimes, potential deleterious effects in normal tissues. The development of novel NPs for pharmacology must proceed together with assessment of any toxicological and environmental side effects of these particles both for patients, to whom these drugs are administered and for the personnel involved in the development, production and administration. Therefore, for efficacy and safety evaluation of nanodrugs, a method based on an approved animal model or an appropriate primary normal cell culture is assertively recommended. This so important task should be paid proper attention to completely fulfill the criteria to prove safety of nanodrugs, whose mechanisms and doses of toxicity are still very limited.

## 5. Expert opinion

The use of nanotechnology has already found wide space in the delivery of anticancer drugs. This strategy offers many opportunities such as the delivery of the drug against specific cells or tissues, reducing the side effects and maximizing the therapeutic effect and the possibility of overcoming biological barriers of different nature. This aim is mainly achieved by the small size of these particles, which can penetrate across different barriers through small capillaries into individual cells. In addition, NPs can be prepared to entrap, encapsulate or

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bind molecules improving the solubility, stability and absorption of several drugs, as well as avoiding the RES, thus protecting the drug from premature inactivation during its transport. Among the carriers of greater use, liposomes are certainly the most studied systems in the clinical setting. Their success is due to the biocompatibility and biodegradability of the materials used, also demonstrated by the presence of numerous products based on liposomes present on the market [96]. The promising results obtained by use of nanotechnology have led the research to develop new nanosystems based on biocompatible materials and suitable to be prepared immediately before use. [97-99].

The delivery of drugs through targeted nanocarriers that are internalized by cells provides an alternative route to diffusion of drugs into cells. This approach may allow targeted carriers to bypass the activity of integral membrane proteins, known as MDR transporters, which transport a variety of anticancer drugs out of the cancer cell and produce resistance against chemotherapy [100]. When targeting cell surface markers present a significant challenge, as in the case for solid tumors, targeting tumor vasculature or the extracellular matrix surrounding the tumor microenvironment may be necessary. In the case of circulating cancer cells, as in leukemia and lymphoma, a therapy that targets surface antigens with high affinity and includes a carrier with a long circulating half-life may be the most efficacious. Similar to combination drug strategies that may be personalized to optimize treatment regimens, oncologists in the near future may be presented with the ability to choose specific nanocarrier/targeting molecule combinations which could lead to improved therapeutic outcomes.

Recently, considerable attention has been given to the toxicity of NPs, but the importance of their genotoxic potential on workers' health has been largely overlooked. The toxic effects of NPs are generally linked to the low biocompatibility of the nanomaterial that is used for designing them. In fact, NPs with higher toxic potentials are CNTs that have shown to be carcinogenic for lung, but are also toxic for GIT, CNS and blood. Heavy metals can accumulate in liver and kidney and can again be toxic for CNS and GIT. Also, silicates are characterized by a prominent accumulation in liver and lung causing fibrosis and important side effects. Side effects for the more biocompatible and mostly clinically used liposomes cannot be excluded since effects on serum proteins, lipoproteins and the extracellular matrix of liver and kidney have been also reported.

In the literature, there is increasing evidence to suggest that NPs are potentially hazardous to humans and that strict industrial hygiene measures should be taken to limit exposure during their manipulation. New approaches are urgently needed to evaluate potential hazards posed by NP exposure. At present, gene expression profiling provides information on the potential modes of action of NPs and their human relevance. Recent work has identified ways that these methods may be used to promote workers' health and safety, which was an important step toward ultimately recognizing significant biomarkers to gauge health risks in the workplace.

Engineered NPs appear in a variety of consumer products, including clothes, sportswear, paints, even self-cleaning windows and, of course, in a number of industrial applications About 1.5 million workers are exposed to NPs today, and by 2015 the number has been estimated to be 3.5 million. Despite the great promise that NPs show, especially for future industrial and biomedical applications, few studies have examined the human body's reaction to NP exposure. Likewise, few studies have explored the possible reactions that uncontrolled uptake of NPs could have on workers' health. Hence, there is an obvious need to promote research in this area. There is an urgent need to explore the effects and mechanisms of these particles in humans and the environment (inflammation, effects on different organs and tissues and cells, DNA-interaction), its distribution in the environment and effects of it and monitoring methods to assess exposure to NPs. As NPs are a diverse group of molecules and have different properties and effects, even sometimes in the same materials and standard sizes, this task will be complex. Overall, industrial hygiene controls worker exposure by comparing pollutant concentrations in the breathing zone of the worker with a limited value. To perform this type of evaluation, it is necessary to define an index of exposure adequately, and the measure of this index is representative of what the worker is breathing. Awareness of the levels of particles which can cause health effects is necessary.

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## Declaration of interest

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