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Do Nanomedicines Require Novel Safety Assessments to Ensure their Safety for Long-Term Human Use?

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Abstract

Nanomaterials have different chemical, physical and biological characteristics than larger materials of the same chemical composition. These differences give nanotechnology a double identity: their use implies novel and interesting medical and/or industrial applications but also potential danger for human and environmental health. Here, we briefly review the most important types of nanomaterials, the difficulties in assessing safety or toxicity, and describe existing test protocols used in nanomaterial safety evaluation. In general, the big challenge of nanotechnology, particularly for nanomedicine (nanobioengineering), is to understand which nano-specific characteristics interact with particular biological systems and functions in order to optimize the therapeutic potential and reduce the undesired responses. The evaluation of the safety of medicinal nanomaterials, especially for long-term application, is an important challenge for the near future. At present, it is still too early to predict, on the basis of the characteristics of the nanomaterial, a possible biological response because no reliable database exists. Therefore, a case-by-case approach for hazard identification is still required, so it is difficult to establish a risk assessment framework.

Nanotechnology can be defined as scientific and engineering developments in understanding and controlling matter <100 nm in size. This technology holds many promises in almost all fields of our daily life, and benefits could occur in terms of smarter electronics, improved health, advanced agriculture and energy, as well as cleaner environments. Nanotechnology is not only exploited because of the size of the materials, but it is also a tool to design (and manufacture) materials at the atomic and molecular scales for the development of active structures, devices and systems with features that cannot be achieved at a larger scale. This size-specific design has led to the belief that nanotechnology will be the start of a new technological revolution.[1,2]

Nanomaterials have chemical, physical and biological properties that differ from identical larger materials. These differences form the fundamentals of nanotechnology, which imply new functions and applications at the supra-molecular level. Bulk materials are scaled down (top down) and entirely new materials can be developed (bottom up). Nanotechnology has reached the stage at

which it can reasonably control the building of materials – atom by atom or molecule by molecule – and therefore help in the engineering of materials with specific, and selected, properties.[3]

New physicochemical properties also imply that nanoscaled material will interact with a biological entity in a 'nano'-specific way.[4] The biological effects can be desired or unintended. Unfortunately, our understanding of how nanoscaled materials interact with biological systems is incomplete. We are now at the point at which we can see the double identity of nanotechnology – on the one hand it can be of major value in medical and/or industrial applications but on the other it can be a potential danger to human and environmental health.[5,6]

Several reviews of the hazards and risks of nanomaterials have raised similar issues, but whether traditional risk assessment can ensure the safety of these materials is unclear.[1,7] In this paper, we briefly review the most important types of nanomaterials, and then focus on some difficulties in assessing the safety or toxicity of nanomaterials. Finally, we focus on existing test protocols used in nanomaterial safety evaluation.

1. Classes of Nanomaterials Important for Medical Sciences

In general, engineered nanoparticles comprise all materials and structures with a size (in at least one dimension) between 1 and 100 nm. This definition is somewhat unsatisfactory because defining nanomaterials on the basis of only size is impossible: larger materials may have nanostructures with size-specific properties.

Nanomaterials can be chemically 'simple' molecules such as metallic, ceramic or carbon nanoparticles (pure elements) or they can be more complex if composed from different types of materials, for example, core-shell nanoparticles. Other nanomaterials have organized structures, including systems such as nanoliposomes and nanoemulsions. Organic molecules such as dendrimers, polymers and peptides can also be defined as nanomaterials.[4] These materials can be merged into complex structures containing, for example, a metallic core covered with an organic molecule (e.g. an antibody) to increase specificity.[8]

Liposomes and emulsions are generally produced as microstructures, but they can also be produced as nanostructures, and have been used to improve the dispersion (and efficacy) of mainly lipophilic compounds.[9] Both types of structures are thought to pose no chronic adverse effects because these structures disintegrate easily into their constituents and thus no longer exist as nanomaterials.

Polymers such as polysaccharide poliglusam (chitosan) nanoparticles or polymer-drug conjugates have been developed as drug delivery systems because they enhance permeability and retention.[10] Polymer-protein conjugates are made to improve protein stability and to prolong the plasma half-life.

Inorganic nanomaterials form a large and very diverse group and include metallic and ceramic nanomaterials. Several porous ceramic materials (e.g. silica, titania and alumina) are biocompatible and are under investigation for use as drug-specific transporters.[11,12] In addition, metallic oxides (e.g. iron oxide) can be used, but because they also have paramagnetic characteristics, they can be used as passive or active targeting agents in, for example, the treatment for cancer.[13]

Quantum dots are nanoparticles constructed of semiconductor materials and have fluorescent properties, and can therefore be used for imaging and histopathology. Quantum dots are covered with biocompatible coatings to prevent leakage of the toxic heavy metals and to improve dispersion.[14] Other imaging tools are produced by covering nanoparticles with a thin metallic shell of optically dense materials (e.g. gold shell).[15]

Carbonaceous nanomaterials are a specific group with typical structures made of carbon atoms. The two most important materials are fullerenes and carbon nanotubes (CNTs). Both materials can be used for tissue- or cell-specific drug delivery.[16] CNT preparations often contain traces of the catalytic metals used for their synthesis (most often iron or cobalt), which influence the biological properties of the material.[17]

Inorganic and carbonaceous nanomaterials can carry specific surface modifications, such as

antibodies, to improve tissue- and/or cell-specific delivery.

These complex organic and inorganic materials are a new class of materials with large potential but they probably also produce specific adverse effects.[18] So far, most of the toxicological and safety research has involved a limited group of engineered nanoparticles, mainly inorganic nanomaterials. The toxicity of nanoliposomes, dendrimers, polymers and emulsions has been studied to a much more limited degree; this situation is not surprising because there is a considerable database on the safety of similar submicron materials, which are already frequently used in healthcare products. Moreover, these nanomaterials are considered biodegradable and decay to their separate constituents, although this is not a specific nano-related problem.[19] For example, the polymer-protein conjugates and other complex structures containing antibodies can, in some instances, be considered biotechnology-derived products with a large molecular mass, which has repercussions for toxicity evaluation.[20]

2. Difficulties in Assessing Toxicity of Nanomaterials

2.1 Role of the Vehicle

One of the difficulties in testing the safety of nanomaterials is the preparation of a good dispersion. This issue has recently gained more attention, with respect to not only the aggregation and agglomeration of multiple particles but also the influence of the vehicle on the surface properties of the material.

Different approaches are used to form a stable suspension. Supplementation of the vehicle with serum or proteins is a common approach,[21-23] or dimethylsulfoxide (DMSO)[24-27] or a surfactant[28] have been used. Some surfactants or vehicles such as proteins (e.g. albumin and apolipoprotein-A1) and non-ionic surfactants (polysorbate 20 [Tween 20®] and poloxamer 103 [Pluronic P103®]) can bind to nanomaterials and hinder binding of a second ligand.[29,30] Studies of components of pulmonary surfactants (such as colfosceril palmitate [dipalmitoylphosphatidylcholine] or L-α-dipalmitoyllecithin) revealed that the presence of lung surfactant can modify the cytotoxicity of quartz,[31-33] an observation important for administration via the lung.

A few studies have evaluated how these vehicles interfere with cytotoxicity testing of nanomaterials.[30,34-36] On the basis of these observations, many discrepancies in the toxicity and biocompatibility data for CNTs may be due to differences in dispersion.[37,38]

2.2 Assay Interactions

In assays, routine endpoints can be disturbed by the presence of nanomaterials, which can interfere with the assay constituents; for example, because of the absorption of assay substrates (e.g. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay *in vitro* testing) or metabolites and/or cellular molecules (e.g. decreased cytokine levels).[22,23,35,39,40] Therefore, positive and negative controls must be included.

2.3 Exposure, Uptake and Distribution in the Body

Nanoparticles, like other substances, can enter the human body through several portals. All of these routes have some importance for dosing in medical or healthcare applications. Our knowledge of the systemic uptake of nanomaterials is derived mainly from studies in other settings; inhalation has been extensively studied in the occupational setting, dermal exposure for materials such as sunscreens, oral exposure through food testing, and parenteral administration in medical applications.[41]

2.3.1 Inhalation Route

Inhaled ultrafine (or nano) particles can reach the alveoli, where they deposit as a result of diffusion. Particles deposited deep in the lung, in the alveoli, escape mucociliary clearance and are mainly cleared by alveolar macrophages. Compared with fine particles (100 nm to 2 μm), ultrafine particles have a longer retention time in the alveoli. This finding can be explained in part by their greater access to the interstitium, which

results in longer retention,[42] and by their greater surface area. Thus, Oberdörster et al.[42] showed in 1994 that surface area is positively correlated with impaired macrophage-mediated clearance. Moreover, when the lungs are continuously (or repeatedly) exposed to high concentrations of ultrafine particles, the high particle burden in the macrophages can hinder phagocytosis of other particles.

Ultrafine particles can translocate rapidly from the lungs into the systemic circulation, as has been shown in humans[43] and animals,[44-46] although this finding is still controversial. Despite focused research, the mechanisms involved in particulate translocation over cell layers and/or cell membranes have still not been elucidated. For example, Muhlfeld et al.[47] studied the pulmonary distribution of inhaled titanium dioxide nanoparticles (aerosol particle size 20 nm), but could conclude only that the nanoparticles did not move randomly from one pulmonary compartment to another and that a small fraction was thought to be released into the systemic circulation.

Inhaled particles can also translocate via neuronal axons. Studies of the nose revealed that the olfactory nerve and bulbs are portals of entry to the CNS. Inhalation of ultrafine carbon particles results in a significant increase of carbon particles in the olfactory bulb.[1] Ultrafine particles are connected with inflammatory and neurodegenerative changes in the olfactory mucosa, olfactory bulb, and cortical and subcortical brain structures.[1]

Recently, the therapeutic delivery of insulin (on particulate carriers) via inhalation has been withdrawn from the market for different reasons, one of the most important being the low economic benefit. Concerns surround the negative health effects reported from clinical studies, which revealed a small but significant decline in pulmonary function (forced expiratory volume in 1 second; FEV_1) with such treatment compared with subcutaneous insulin treatment.[48] This decrease in FEV_1 appeared during the first 3–6 months of use (in a 2-year study); pulmonary function improved as soon as treatment was stopped and returned to the level seen in patients receiving subcutaneous treatment within 2–4 weeks.

2.3.2 Dermal Route

Dermal exposure to nanomaterials can occur with the use of healthcare products such as dermal creams and lotions containing nanostructures such as zinc oxide, titanium dioxide or liposomes. The skin, a barrier protecting the body against environmental insult, consists of three layers: the epidermis, dermis and subcutaneous layer. The outer layer of the epidermis, the stratum corneum, contains only dead, strongly keratinized cells. For most chemicals, the stratum corneum is the rate-limiting barrier to penetration.

For particulate materials, the picture is not so clear. Nano-sized particles are considered to enter more deeply into the skin than larger particles. Kreilgaard[49] hypothesized that nanoparticles of titanium dioxide (5–20 nm) can penetrate the skin and enter the immune system (Langerhans cells) or reach the systemic circulation. Recently, Ryman-Rasmussen et al.[50] showed that quantum dots of different sizes and shapes (spherical and ellipsoid; 4.6 nm and 12×6 nm) and surface coatings (neutral, cationic, anionic) can penetrate intact porcine skin within 8 hours after exposure.

Therefore, skin penetration is a possible route for systemic exposure to 'small' nanomaterials from the site of local skin treatment, and testing of nanomaterials smaller than 20–30 nm applied to the skin should undoubtedly take this into account.

2.3.3 Oral Route

The uptake of particles after oral exposure probably depends on particle size and surface chemistry. Jani et al.[51] demonstrated that oral administration of polystyrene particles (50 nm to 3 μm) to rats resulted in their absorption from the gastrointestinal tract and their presence in the liver, spleen, blood and bone marrow. Particles >100 nm did not reach the bone marrow and no particles >300 nm reached the blood. Yamago et al.[52] showed that orally administered C_{60} fullerene in rats was not efficiently absorbed and was mainly (98%) excreted in the faeces within 48 hours after administration.

Some nano-carriers do not enter the systemic circulation but, rather, enhance the absorption of the pharmacologically active compound from the

gut into the circulation. For instance, amphiphilic poliglusam-based polymers (<20 kDa) form micelles of 10–30 nm. The drug incorporation by micelles is one order of magnitude higher than is seen with triblock co-polymers after oral administration.[53]

The pharmacokinetics of non-biodegradable (biopersistent) nanomaterials has not been studied in detail. Moreover, only a few studies have focused on the removal of nanomaterials from organisms. This issue is of major importance because these nanomaterials can potentially accumulate over a lifetime in an organism. Particulate material in general is expected to be removed from the circulation via phagocytic cells in the reticulo-endothelial system and, as a result, accumulate in the liver.[1,7,21,54,55]

One of the difficulties in the excretion of poorly degradable nanomaterials is the limited ability of large molecules to pass through the glomerular filter (fenestrae). The glomerules allow passage of low molecular-weight substances (<7 kDa) and intermediate-sized molecules (7–70 kDa), but prevent the filtration of larger molecules (e.g. proteins). For example, poly(amidoamine) [PAMAM] dendrimers with a diameter of 5 nm were found to be excreted by the mouse via urine but also accumulated in the kidney.[56]

3. Health Effects (Toxicity) of Engineered Nanoparticles with Long-Term Exposure

With repeated or long-term exposure to nanomaterials, the main questions are:

1. Which target organs show toxicity?
2. Is there a risk of tumour development?
3. Is there likely to be an immune response?

3.1 Formulation of the Nanomaterial

As discussed in section 2.1, the intake and body distribution of engineered nanoparticles depends strongly on the vehicle(s) used; consequently, if the health effects of only a specific nanomaterial are investigated rather than a specific medicinal formulation, the choice of the vehicle will significantly influence the results.[21,30,57-59]

3.2 Dosage and Route of Exposure

In inhalation studies, the daily delivery of material leads to changes in the clearance of particulate material, especially for materials of low solubility and particularly in the rat, in which pulmonary overload is common. The intraspecies differences, and thus the animal of choice, are therefore important and can lead to incorrect conclusions for exposure in humans. Administration via injection – whether intravenous, intraperitoneal, intramuscular or subcutaneous – can lead to local accumulation, because of the relatively slow distribution of material from the injection site to other tissues.

So far, no detailed reports exist of long-term oral administration of poorly soluble nanomaterials. However, although the gastrointestinal tract is regularly exposed to significant quantities of nano- and micromaterials – e.g. silicates (as natural environmental compounds or derived from nanomaterials) and titanium dioxide,[51,60] contained in food, toothpaste and atmospheric sources – no major effects have been observed. Factors affecting the transfer of nanoparticles from the gut lumen to the body include particle size, surface properties and cell surface ligands.[51,60,61]

The relevant dose for testing nanotoxicity is difficult to determine, in light of the previously mentioned findings concerning the route of exposure and the lack of information about the pharmaco- and toxicokinetics of nanomaterials. A case-specific toxicokinetic study is needed to define maximal doses for long-term exposure studies.

3.3 Genotoxicity and Carcinogenicity

The guidelines for genotoxicity testing[62] suggest the following biphasic approach:

(1) Assessment of mutagenicity in a bacterial reverse-mutation test.

(2) Evaluation in mammalian cells *in vitro* and/or *in vivo* with use of rodent haematopoietic cells (either micronucleus or chromosomal aberration testing) and another mammalian tissue.

Direct DNA damage can take place only if the material comes in contact with the DNA. As

shown in several studies, nanomaterials can enter into the cell and, in some instances, into the nucleus.[63,64] How these materials pass through cellular membranes and what influence physicochemical characteristics, such as size, coating, surface charge and specific chemical functions, might have on this process is largely undiscovered. Better insight is needed from pharmacological (drug delivery) and toxicological points of view.

In relation to this issue, attention should be paid to the accumulation of nanomaterials in cells and/or the nucleus, because this may lead to disturbance of normal gene expression even in the absence of DNA damage. For example, cadmium telluride quantum dots in human breast carcinoma cells induced genomic and epigenetic responses, including general hypo-acetylation and activation of p53 expression.[65] In a human cohort, environmental particle exposure was negatively associated with methylation in both Alu repetitive elements and long interspersed nucleotide element (LINE)-1, which likely reflects long-term particulate effects.[66] These epigenetic changes may have long-term effects on gene expression programming, even some time after the initial signal (exposure) has been removed. As such, subtle lesions often remain undetected and can lead in the long term to undesired effects.

Besides a direct effect of contact of nanomaterials with DNA, nanomaterials can affect DNA indirectly, as reviewed by Schins and Knaapen.[63] The authors pointed out that tumourigenesis of poorly soluble particles also involves a mechanism of secondary genotoxicity, which implies damage resulting from oxidative DNA attack by reactive oxygen/nitrogen species. The mechanism of induction of the inflammatory response may be orchestrated via the initial oxidative stress and calcium ion signalling.[67] However, how this inflammation affects responses to DNA damage, such as mutagenesis and/or carcinogenesis because of cell cycle arrest, DNA repair errors, increased proliferation and apoptosis, is unclear.[68]

Moreover, CNTs were recently found to induce foreign body responses 7 days after their injection in the peritoneal area in mice – responses that were comparable to those induced by asbestos.[69] Although intratracheal administration of CNTs seemed not to damage DNA directly, they significantly increased numbers of micronuclei in pulmonary epithelial cells after a single exposure in rats.[70] *In vitro*, in MCF-7 cells, CNTs induced both centromere-positive and -negative micronuclei. Therefore, CNTs were considered to induce clastogenic as well as aneugenic effects.[70]

In view of these observations, whether bacterial mutagenicity tests are useful is questionable; eukaryotic cells and *in vivo* models will be essential to study the complex interaction between nanomaterials and proliferating, or dividing, cells.

3.4 Toxicity Related to Development

The development of an organism is characterized by the sequential expression of a unique repertoire of both structural and functional molecules. Nanomaterials (as with all substances) can possibly interact with this process. Recently, nanomaterials were found to cross the placenta in rats, which suggests that they pose a risk for the developing fetus.[71,72]

No large-scale studies of the developmental effects of nanomaterials have been published, but some important observations were recently made. In culture media, CNTs can interfere with different inflammatory metabolites, such as adenylate kinase and pro-inflammatory cytokines, thereby giving false-negative results, apparently due to the absence of inflammation in the presence of CNTs;[30,73] therefore, through the binding of biological molecules, nanomaterials can disturb homeostasis. In addition, signalling molecules – cytokines such as interleukin (IL)-6 and IL-8 – can bind to inorganic nanomaterials.[74,75] The adsorbed molecules can presumably be released at an inappropriate time or to another target. Both low concentrations and inappropriate delivery of signalling molecules (e.g. hormones) can result in developmental effects.

In addition to these changes in cell signalling with nanomaterials, interactions with the cellular skeleton have been reported.[76-78] Single-walled carbon nanotubes induce changes in cell

proliferation, cell activity, cytoskeletal organization, apoptosis and cell adhesion, owing to interactions with the skeleton *in vitro*. The consequences of such interactions *in vivo*, or in developing fetuses, are not known.

3.5 Immunological Responses

Immunotoxicological analysis of new substances is one of the most complex health safety areas, in part as a result of the broad range of tissues involved and the diversity of possible effects. Because of this complexity, no clear-cut international guidelines exist, and safety evaluation must be considered on a case-by-case basis.[79]

The decision to perform additional immunotoxicity testing is based on findings of routine toxicity testing, such as changes in cellular composition or the weight of lymphoid organs and/or histopathological observations.

In the EU, the initial immune function test results are generally derived from subchronic toxicity studies in rodents.[80] In the US, the FDA recommends immunological testing only when results from preceding tests indicate it; this is also the approximate strategy in Japan.

From a practical point of view, a clear distinction must be made between nanomaterials produced from inorganic material and those derived from biological molecules (biotechnology-derived materials) in terms of the immune response.[20] Nanomaterials can, as shown in several recent papers discussed in this section, interact with the immune system in different organs or tissues and at different functional levels. A nanomaterial can stimulate or suppress the immune system; it can modulate the innate and/or adaptive immune system. A specific antibody response (or antigenicity) for biotechnology-derived materials is a well-known phenomenon; nanomaterials (both organic and inorganic) composed mainly of a large biological molecule linked to a nanotechnology-derived component, such as an antibody, can interact with the immune system in a similar way. The generation of specific antibodies against inorganic nanoparticles is less likely, but perhaps not for C_{60} fullerenes because some studies found C_{60}-specific antibodies, whereas others did not.[81,82]

Nanomaterials can stimulate the immune system in an indirect way and act, for example, as an adjuvant, as was shown a decade ago for environmental particles and more recently for engineered particles.[83,84] The mechanism behind this nonspecific stimulation of the immune system is not well understood; nanoparticles could enhance antigen uptake and/or stimulate antigen-presenting cells.[85,86]

In general, positively charged (cationic) particles are more likely to induce acute inflammatory reactions (innate reaction) than negatively charged (anionic) particles.[87] Two parameters – size and surface charge – play a central role in these responses. The phagocytic activity of macrophages in the lung has also been linked to particle size; although micrometre-sized particles stimulate phagocytosis, smaller nanometre-sized materials often do not, or even reduce the capacity of the macrophages. Moreover, particles >1 μm were recently shown to induce a T helper-1 cell response, whereas smaller particles (<500 nm) were associated with T helper-2 cells.[86,88]

Besides charge and size, the chemical composition or, especially, the chemical impurities in nanomaterials can be pivotal in the inflammatory response. For example, inflammatory reactions induced by CNTs could be linked to their impurities, because purified CNTs did not induce an inflammatory response.[89]

Immunosuppression, or the down-regulation of the immune response, is a major concern in drug design and is associated with undesired responses such as a reduction in the immune capacity to fight infections and recognize cancer cells. Several examples of immunosuppressive effects can be found in the literature:

- Fullerenes and some fullerene derivatives are known free radical scavengers and can quench the production of nitric oxide produced by macrophages both *in vitro* and *in vivo* in rat lungs.[90]
- Nanoparticles reduce the phagocytic activity and mobility of macrophages *in vitro*.[91,92]
- Conjugates of glucosamine on PAMAM dendrimers (3.5 generation) inhibit the induction

and release of inflammatory cytokines and chemokines from human macrophages and dendritic cells upon exposure to bacterial endotoxin *in vitro*.[93]

- Other PAMAM dendrimers, modified with 2-hydrohexyl groups, suppress inflammatory cytokine secretion both *in vitro* and *in vivo* in mice. These dendrimers might reduce the endotoxin concentration (absorption) and therefore protect against endotoxin-induced sepsis.[94]
- CNTs reduce the capacity to fight infections after pulmonary exposure in mice.[95]

These different effects can be desirable in the development of medical applications of nanomaterials, but they can also be undesirable. Tests for undesirable immunosuppression should be an important part of preclinical nanoparticle evaluation.

3.6 Thrombosis and Atherosclerosis

Epidemiological studies have shown a strong link between exposure to environmental particulate matter and cardiovascular morbidity and mortality.[96-98] Baccarelli et al.[99] investigated the association between air pollution levels and changes in coagulation in 1218 healthy subjects in Italy. The authors revealed that increased concentrations of particulate matter were associated with changes in the global coagulation function (shortened prothrombin time), which suggests a tendency towards hypercoagulability after exposure to particulate matter. *In vivo* and *in vitro* experiments revealed that nanoparticles enhance thrombosis and platelet activity.[44,100,101] Unfortunately, no reliable animal models exist to study the genesis of plaques and atherosclerosis.[102]

4. Conclusions

The properties of nanomaterials are unique. At this size, materials show specific (quantum) characteristics, which influence their optical, electrical and magnetic behaviour. In brief, the unusual properties of nanomaterials are due to their small size, chemical composition (e.g. crystallinity, purity), surface structure (e.g. surface reactivity, surface groups, inorganic or organic coatings), solubility, shape and aggregation.[3,68] These new features of nanomaterials have already led to new, commercially available, products. Although the benefits of nanotechnology are promising, concerns have been raised about the adverse effects of consumer and industrial products.[3,41]

The most important questions to be answered are related to the distribution and accumulation of nanomaterials in the body and, linked to that, foreign body responses, immune responses, genotoxicity and, possibly, developmental and atherosclerotic effects.[1,2] These challenges are large but do not necessarily imply new regulation.

When setting up a long-term toxicity (or safety) test for a nanomaterial, the route of administration and the use of a vehicle need to be well evaluated and should correspond to the expected usage in humans. This also applies to the species chosen, because overload conditions, particularly in the lung, are undesirable, and immune responses to biological molecules should be comparable to that in humans.[40,103] The test design and test outcomes must be supervised with great caution.[104]

In general, the big challenge of nanotechnology, and in particular for nanomedicine (nanobioengineering), is to understand which nano-specific characteristics interact with particular biological systems and functions in order to optimize the therapeutic potential and reduce the undesired responses. At present, it is still too early to predict, on the basis of the characteristics of the nanomaterial, a possible biological response because no reliable database exists. Therefore, a case-by-case approach for hazard identification is still required, and it is difficult to establish a risk assessment framework.

The general finding of the legislators is that the current regulatory environment is adequate and flexible enough to cope with the already existing nanomaterials. Nevertheless, we have to be aware of the rapid development of more complex nanomedical products, with possibly greater risk potential. It is also likely that these new generation products span the regulatory boundaries between medical products, devices and therapies, so their classification and evaluation may have to be revised.[104,105]

The European commission recognizes the need for specialized expertise for these toxicological, technical and regulatory difficulties. The European Medicines Agency[106] has created the Innovation Task Force to evaluate emerging therapies and technologies, including nanomedicine, and to evaluate the need for revision of the guidelines. The purpose of the Innovation Task Force is also to establish an early dialogue with the applicants to give guidance at the development stage. In the US, the FDA with its Nanotechnology Task Force has come to comparable conclusions.[107,108] It is a good opportunity to establish a more permanent cooperation between the EU and the US for standardization and regulation along the lines of the International Conference on Harmonization.[109]

Laboratory tests are essential for nanomedical products, as for all compounds, but are too limited to reveal the long-term human effects of these products. Postmarketing studies, conducted by the manufacturer, are mostly not obligatory and are restricted in duration. On the other hand, the general use of post-approval registries can be helpful to detect the unprecedented limitations of innovative technologies like nanotechnologies.[110,111]

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