Journal of
Ceramic Science
and Technology

J. Ceram. Sci. Tech., **04** [02] 123-130 (2013)
DOI: 10.4416/JCST2012-00038
available online at: http://www.ceramic-science.com

Nanomaterials and Ceramic Nanoparticles – Use without Side-Effects?

M. Roesslein[1], V. Richter*[2], P. Wick[1] , H. F. Krug[1]

[1]Empa, Swiss Federal Laboratories for Material Science and Technology, 9014 St Gallen, Switzerland
[2]IKTS, Fraunhofer Institute for Ceramic Technologies and Systems, 01277 Dresden, Germany

received September 28, 2012; received in revised form April 25, 2013; accepted May 17, 2013

Abstract

It is expected that ceramic nanoparticles will be more intensely used in various technical, medical and biological fields in the near future. But their interaction with biological objects has not yet been completely understood. Within the scientific community, NGOs and the public, there is considerable concern about possible side-effects on health and environment. In an investigation of the behaviour of nanoparticles in a biological environment or nano-toxicological effects, three crucial phenomena have to be analysed in detail: material properties, surface state/surface reactions and uptake/transport. Unfortunately, publications on the biological effects of nanoparticles often do not sufficiently describe the physical and chemical properties of the particles under consideration or are based on overdoses. Moreover, measurement uncertainties and methodological pitfalls contribute to the divergent opinions about the possible risks of nanomaterials. Of the numerous publications, only few deliver reliable data. To obtain comparable toxicological results as achieved previously in other fields of physics and chemistry, a worldwide effort is needed to standardize the measurement methods used in nanotoxicology, focussing especially on the characterization of nanoparticles and the specific effects of the particle nature on the measurement system.

Keywords: Ceramic nanoparticles, health risks, toxicology, comparability, standardization

I. Introduction

The Royal Society's report "Nanoscience and nanotechnologies: opportunities and uncertainties" published on 29 July 2004 initiated the debate on nanotechnology, suggesting "environmental, health and safety, ethical or societal implications or uncertainties may arise from the use of the technology, both current and future" [0]. The term "Nanotoxicology" also appeared for the first time in 2004 [1]. The relationship to the former research on ultrafine particles was highlighted one year later [2]. Today, the safety of nanomaterials is the subject of numerous international research groups, a substantial part of many funding programmes, international conferences, and regulatory activities worldwide. Whereas the number of publications regarding nanotoxicological issues has increased dramatically each year from that time (from around 150 in 2004 to nearly 1300 in 2009, compare Hristozov *et al* [3], 2012), clarity about possible risks has decreased.

Besides the fact that the number of products containing nanoparticles (NP) is steadily increasing, there are no general routines governing the testing of these products to ensure safety for the customer. Whereas most of the OECD guidelines can be used for testing nanomaterials, it has been demonstrated that not all biological screening methods are adapted for NP. Therefore, results are often false-positive or false-negative [4–6]. Moreover, experimental problems such as solvents ordetergents used to disperse the NPs are often neglected [7] and the appropriate controls as well as the required characterization of the materials tested are frequently lacking. In this review we focus on the need to obey nanotoxicological principles, which involves comprehensive characterization of nanomaterial, surface and uptake, the consideration and measurement of uncertainties concerning the biological effects of NPs in cell and animal tests (i.e. in *in-vitro* as well as in *in-vivo* studies). Furthermore, we make some recommendations on how to proceed with the development of adapted methods for risk assessment of nanomaterials.

II. Nanoparticles – Physical and Chemical Properties

The physical and chemical properties of particulate matter depend on the size of the particles, with the specific surface area being one of the most sensitive characteristics. For instance, the reaction rate in heterogeneous catalysis or the number of atoms and molecules that can be adsorbed increase significantly. In parallel, the portion of atoms or ions sitting close to the surface, possessing less nearest neighbours and being less well bonded, results in an increase in the energy content of the system. Particulate systems try to reduce the surface area (or the free energy or the free enthalpy, respectively) by means of rapid agglomeration, grain growth or adsorption of molecules. The melting point of the material may also fall. Brownian motion is another example of where particle size matters. In the case of very fine particles even the electron band

* Corresponding author: volkmar.richter@ikts.fraunhofer.de

structure may be modified significantly, which has a strong effect on electrical and magnetic properties. The colour of quantum dots, for instance, depends on their size. They usually consist of a semi-conducting material. Their particle size is typically below 10 nm. The smaller the size of the crystal, the greater is the energy gap between the highest valence band and the lowest conduction band and the higher is the energy released when an electron excited by UV light falls to the lower energy level. Small particles emit blue light whereas the light emitted by larger particles tends to be red [63]. Ferro- or ferrimagnetic materials like iron oxide may become super-paramagnetic if the particle size is reduced to a few nanometres. If there is no outer field the particulate material seems to possess no magnetisation and to be non-magnetic as the particles continuously change their direction of magnetisation owing to thermal excitation. If an outer field is applied the particles behave like a strong ferromagnetic material with all particles having the same direction of magnetisation [64].

From a technical and commercial point of view it was desirable to discriminate between these nanoscaled particles and bulk material and hence an internationally accepted definition of nanomaterials and nanoparticles has been found. ISO/TC 229 released corresponding documents in August 2008 and the same documents were published by CEN as CEN ISO/TS 2768719. According to this document the term "nanoscale" comprises a size range from approximately 1 nm to 100 nm. Nanoparticles are particles with all three external dimensions in the nanoscale range. Particles are, according to ISO 14644 – 6:2007 [8], minute pieces of matter with defined physical boundaries (see for instance [9]).

In several publications nanoparticles with dimensions < 100 nm are considered particularly risky owing to their reactivity, mobility and so on. But, these quantities change steadily with size and 100 nm is not a biological threshold value below which the interactions change dramatically. Different arguments are discussed by Lövestam and his colleagues [9]. Considering the difficulties in defining a biologically relevant particle size threshold and in measuring the size of nanoparticles, Maynard recommends with regard to legislation: "Don't define nanomaterials" [10]. Nevertheless, particle size must play more important role in future toxicological studies.

III. Fundamental Aspects and Principles of Nanotoxicology

(1) Entry ports for nanoparticles in healthy humans

The risk potential of any hazardous substance depends on the entry ports. Most of the recent research dealing with potential health hazards of NPs has focussed on cells and tissues likely to come into immediate contact with nanoparticles, such as the lung, skin or gastrointestinal tract. From an occupational perspective, it is not surprising that the lung was identified as the most sensitive entry port for loose nanomaterials. Instillation and inhalation studies in rodents demonstrated that certain micro- and nanoscaled particles such as crystalline silica or carbon nanotubes have the potential to cause fibrotic [11] or pulmonary granulomatous alternations [12] and that they have the potential to cross the airway-blood [3]. In contrast to the lung, healthy skin was described as an effective barrier against many nanomaterials. The uptake of particles, especially those used in cosmetics and in sunscreens, was hampered by the anatomic structure of the human skin [14]. However, particle surface coatings or functionalizations, which are often used to prevent agglomeration, may strongly affect the penetration [15–19]. The gastrointestinal tract with its impressive exchange area of about 2000 m^2 (incl. microvilli and other cellular sub-microstructures) is of minor significance as uptake seems to be hindered: 98 % of the NPs orally administered to the test animals were excreted. In case of a damaged gastrointestinal tract, the situation may change. Approximately 80 % of the intravenously applied material was observed to accumulate in the liver [2].

Nanotechnology will also result in designed uptake routes. New concepts for diagnostic or therapeutic medical applications, including for instance ceramic particles like iron oxides, have already been introduced onto the market or are on the verge of market introduction [20]. Common to these concepts is that the particles are injected either into the target tissue [21] or into the bloodstream [22] to achieve the desired effect. Natural barriers as described beforehand are bypassed and other types of barrier tissue such as blood-brain barrier [23–26] or placenta [27] become relevant.

(2) Three particular mechanisms or principles of nanotoxicology

Despite the lack of evidence for any severe hazard of insoluble globular nanoparticles caused only by the small size of the particles [61], these particles may follow different uptake pathways or exhibit alternative modes of action compared to micrometre particles or ions. Careful assessment of NPs regarding their availability, dose, biodistribution, acute and chronic effects is necessary [28]. Mechanisms of interaction that are characteristic to nanoparticles are fundamental subjects of nanotoxicology. These "principles of nanotoxicology" (Krug and Wick 2011) are briefly explained below.

a) *Cellular uptake and transport principle*

There are different ways for particles to get into a cell. For particles of micrometre size it was shown by previous particle toxicity studies that phagocytosis is the key process [29]. Phagocytosis corresponds to a cellular process of the cell membrane engulfing solid particles. But for NPs it is not the only possibility for uptake. Other mechanisms such as uptake by vesicles coated with specific proteins like clathrin or caveolin [30] may also occur. After uptake these particles are kept in vesicle-like structures. Pinocytosis, a non-specific process in which a cell takes in surrounding fluids, including all solutes present, may be relevant too. In excess such NPs may also be taken up by means of receptor mediated transport [31–33] or even adhesive interaction [13, 34, 35]. A small fraction of NPs were identified even in erythrocytes (red blood cells) [34]. Several *in-vitro* experiments indicate that particles below a certain size are able to cross biological membranes. Bio-distribution analysis

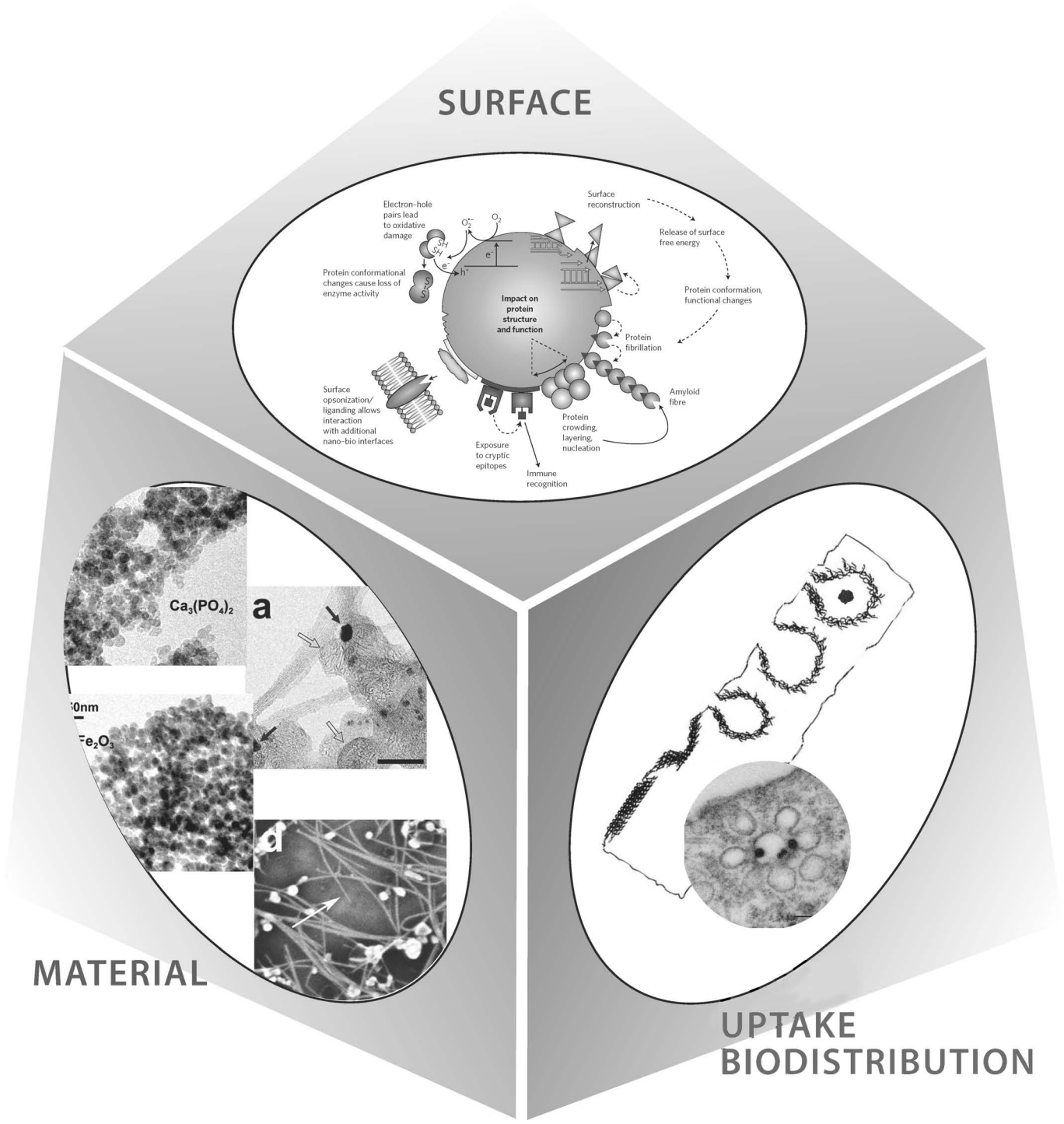

Fig. 1: The three principles of nanotoxicology: "uptake and transport", "surface" and "material" (the scheme of surface is adapted to Nel *et al* 2009 *Nat. Mater.*).

of NPs performed in animal studies showed clearly that they are distributed within the whole organism [36–38], indicating that these particles are indeed capable of crossing biological barriers. Cellular uptake and transport is mainly controlled by size, shape and surface state (functionalization, charge) of the particle with nanoscaled particles being able to reach cells or tissues that cannot be reached by larger particles. But the degree of agglomeration and the surface state are modified by the biological environment. These phenomena have to be taken in consideration in the assessment or development of toxicological test routines for NPs.

b) ***Surface principle***

The volume-specific surface area of particulate matter plays an important role in physical-chemical processes. Beside reaction or dissolution rates, the agglomeration rate of nanoparticles in aqueous media is also a function of the volume-specific surface area [64]. This also holds in a biological environment.

After uptake, especially biopersistent NPs may accumulate in cells or tissues and interact with cellular com-

ponents. Owing to their high surface area, nanoparticles may bind cellular components or initiate unspecific reactions producing, for instance, free oxygen radicals. Nel *et al.* (2009) summarized these surface-dependent effects and their impact on chemical and biological processes very clearly [39].

Several inhalation studies in mice and rats demonstrated a direct correlation between inflammatory potency in the lung of the animals and the applied TiO_2 nanoparticle dose expressed as specific surface concentration [40]. Similar findings were observed with combustion-derived NP [41] or nickel particles [42]. Even in the case that, owing to minimal changes in the exposure media, rapid agglomeration of nanoparticles takes place, the agglomeration does not prevent uptake into cells and the expression of toxicity [66].

As the rate of physical-chemical reactions including solids is usually controlled by the volume specific surface area and surface state, an adequate characterization of the surface of the NP must be described in any toxicological test protocol.

c) *Material principle*

The small size of nanoparticles does not mean that nanoparticles are in general more toxic than ions or larger particles of the same material. In the end, specific toxic reactions are often correlated with reactions on the atomic or molecular level. That's why material matters at nanoscale level too. And the biological effects of NPs may not only depend on chemical composition but also on the lattice structure. Carbon is an excellent example to underline this principle. Although no adverse effects have so far been observed for nanosized diamonds [43, 44], carbon black – at least at a high dose – exhibits some impact [45, 46]. Also solvent-free fullerenes seem to remain without any effect [7, 47]. Furthermore, differences in shape may become important. Carbon nanotubes (CNT), particularly the long and rigid ones that follow the fibre paradigm [48–50] or highly agglomerated CNT [51], can trigger health effects. Therefore, careful and comprehensive material characterization is required prior to any toxicological assessment [28, 52].

Of course these three principles are interlinked with each other and their combination triggers the specificity of the biological mode of action of the given nanomaterial (Fig. 1).

IV. Nanotoxicology Studies: What is Measured? – What can be Compared?

In 2010 Haynes [53] published in an editorial including a figure showing the number of publications with the main subject of "nanotoxicology" since 1980. Especially for the last 10 years it shows almost exponential growth in numbers, the most recent number for 2011 confirming this trend. Unfortunately, a detailed analysis of the publications revealed that different publications on the same nanomaterial came to contradicting "toxic" and "non-toxic" findings. Such controversy causes great uncertainty in the general public and hence anxiety about the safe use of nanomaterials.

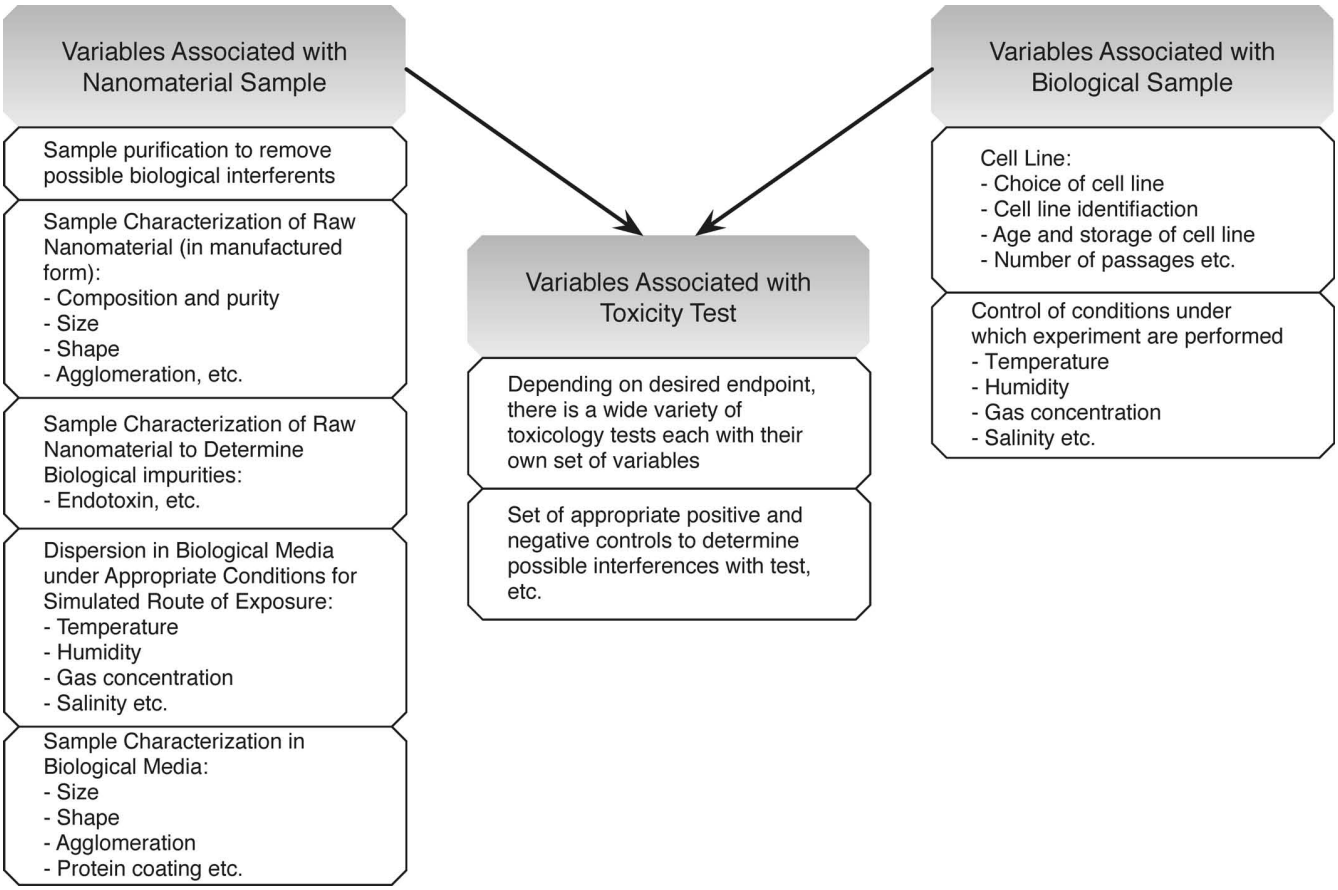

Fig. 2: Most relevant variables associated with *in vitro* toxicity testing.

Throughout the last two years, a handful of publications have started looking systematically into the matter. Locascio *et al.* [54] (2011) from the National Institute of Standard and Technology (NIST, USA) has published the most promising approach to resolve the controversy so far. In chapter 8 of the book "*Nanotechnology Standards*", they summarized numerous variables that are associated with the test system (see Fig. 2) and fall under the following categories:

- Nanomaterial samples
- Toxicity testing
- Biological samples

The nanomaterial samples encompass the following main categories: "Sample purification, sample characterization of the raw nanomaterial, sample characterization to determine biological impurities, dispersion in biological media and sample characterization in biological media". A similar list [28] for nanomaterial characterization was published independently as a minimum set of information on the properties of nanomaterials for each study. This list consists of: "Chemical composition, purity, impurities; particles size and size distribution; specific surface; morphology; surface chemistry, coating, functionalization; degree of agglomeration/aggregation and particle size distribution under experimental conditions; water solubility and surface reactivity and/or surface load". Locascio *et al.* [54] continue with a very general description of variables associated with toxicity testing: "Depending on the desired endpoint a wide variety of toxicology tests exist each with their own set of variables and set of appropriate positive and negative controls to determine possible interferences." Various publications have already stressed the importance of these positive and negative controls and of interference tests. Owing to the particle nature of nanomaterials, interference especially with optical measurements systems is much more likely to occur than with dissolved chemical compounds. Locascio *et al.* [54] finalize their large list of variables with biological-sample-specific issues: "Cell line, which includes choice of cell line, cell line identification, age and storage of cell line, number of passages etc. and the control conditions under which experiments are performed". This comprehensive list of variables affecting results of *in vitro* assays test systems is quite revealing. The following example illustrates this.

Back in 2006 [6] and 2007 [55] overwhelming evidence was published that a specific assay to analyse cell survival – the MTT assay – is strongly affected by interferences when carbon nanotubes are investigated. Therefore the authors of the first publication [6] proposed that at least two, but better more than two test systems should be used to verify any cytotoxicity data. Nevertheless the authors of a recent publication [56] (2012) claim a toxic finding based on their study investigating the cytotoxicity of multi-walled carbon nanotubes (MWCNT), graphene oxide and nanodiamond. But their study does not mention any test for interference between the examined nanomaterial and the MTT assay [57]. In addition the release of LDH [58] was determined as a second cytotoxicity test system. But this test system lacked any internal reference for the effect because positive and negative controls were omitted. Even though two independent test systems were used, important variables that Locascio *et al.* [54] list were not specified and hence they remain undefined. The two cytotoxicity assays *per se* indicate comparable results, but they contradict the results of previous studies that had all those variables (interference, negative and positive control) defined. This failure to define important variables of a test system in accordance with the recommendations of the Joint Committee for Guides in Metrology [59, 60] is the key to understanding the contradiction between the findings of the recent publication with the results of all the previous studies.

On the other hand, over the last decades the use of nanomaterials without side-effects has often been neglected and most reasons for concern have so far come from studies with overdose concentrations. There is an increasing number of publications indicating no difference between micro- and nanoparticles in respect of their biological effects (Auffan, M., Rose, J., Bottero, J.Y., Lowry, G.V., Jolivet, J.P., and Wiesner, M.R. (2009): Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. *Nature Nanotech.*, **4**, 634 – 641) and severe toxic effects are more or less dependent on the aspect ratio and the length of fibre-like materials (Hamilton, R.F., Wu, N., Porter, D., Buford, M., Wolfarth, M., and Holian, A. (2009): Particle length-dependent titanium dioxide nanomaterials toxicity and bioactivity. *Part. Fibre Toxicol.*, **6**, 35).

V. Conclusions

The development of new materials on the nanoscale, especially ceramic materials, for various applications in consumer products raises the question of the safety of these materials for health and environment. Many discussions lead to the general opinion that the small size of these specific materials may lead to a higher transport rate into the body, a higher exchange rate between the organs, the larger surface-to-volume ratio may lead to higher reactivity of the same amount of material and thus, nanomaterials pose a higher risk to human health and the environment. Whereas the reactivity of nanomaterials compared to their bulky counterparts is indeed enhanced and can lead to a greater biological effect, the behaviour of nanomaterials in the body or in cells can be compared to other particulate matter as well. Thus, actually no "nano-regulation" is needed and the existing rules and laws seem to be adequate for the production and use of these materials.

Nevertheless, testing methods specifically for nanomaterials have to be established or existing assay have to be evaluated for their suitability for testing nanoparticles. Projects such as NanoCare [61], Nanommune [62] or VIGO that systematically investigated each test system separately and all their relevant variables have been defined accordingly. Such efforts provide tremendous support in establishing the standardization of test methods worldwide. Afterwards these standardized test methods have to undergo final validation based on interlaboratory comparison.

Such elaborated and validated test methods are the basis for the comparability of measurement results in nanotoxicology on a worldwide scale. Hence they enhance the trust in the findings and promote the acceptance of nanotechnology as well as their application in industry.

References

0 Nanoscience and nanotechnologies: opportunities and uncertainties, The Royal Society and The Royal Academy of Engineering, (2004).

1 Donaldson, K., *et al.*: Nanotoxicology, *Occup. Environ. Med.*, **61**, [9], 727 – 728, (2004).

2 Oberdorster, G., Oberdorster, E., Oberdorster, J.: Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles, *Environ. Health Perspect.*, **113**, [7], 823 – 839, (2005).

3 Hristozov, D.R., *et al.*: Risk assessment of engineered nanomaterials: A review of available data and approaches from a regulatory perspective, *Nanotoxicology*, (2012).

4 Davoren, M., *et al.*: In vitro toxicity evaluation of single-walled carbon nanotubes on human A549 lung cells. *Toxicol. in Vitro*, **21**, [3], 438 – 448, (2007).

5 Monteiro-Riviere, N.A., Inman, A.O.: Challenges for assessing carbon nanomaterial toxicity to the skin, *Carbon*, **44**, [6], 1070 – 1078, (2006).

6 Worle-Knirsch, J.M., Pulskamp, K., Krug, H.F.: Oops they did it again! Carbon nanotubes hoax scientists in viability assays, *Nano Lett.*, **6**, [6], 1261 – 1268, (2006).

7 Spohn, P., *et al.*: C60 fullerene: A powerful antioxidant or a damaging agent? The importance of an in-depth material characterization prior to toxicity assays, *Environ. Pollut.*, **157**, [4], 1134 – 1139, (2009).

8 ISO, ISO 14644 – 6:2007 Cleanrooms and associated controlled environments – Part 6: Vocabulary. 2007, ISO: Geneva, 34.

9 Lövestam, G., *et al.*: Considerations on a definition of nanomaterial for regulatory purposes, in European Commission Joint Research Centre, JRC Reference Reports. 2010, European Commission, Joint Research Centre, 36.

10 Maynard, A.D.: Don't define nanomaterials, *Nature*, **475**, [7354], 31, (2011).

11 Sayes, C.M., Reed, K.L., Warheit, D.B.: Assessing toxicity of fine and nanoparticles: Comparing *in vitro* measurements to *in vivo* pulmonary toxicity profiles, *Toxicol. Sci.*, **97**, [1], 163 – 180, (2007).

12 Johnston, H.J., *et al.*: A critical review of the biological mechanisms underlying the *in vivo* and *in vitro* toxicity of carbon nanotubes: The contribution of physico-chemical characteristics, *Nanotoxicology*, **4**, 207 – 246, (2010).

13 Geiser, M., *et al.*: Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells, *Environ. Health Perspect.*, **113**, [11], 1555 – 1560, (2005).

14 NanoDerm. 2008 [cited 2012 25.9.2012]; Quality of skin as a barrier to ultra fine particles. Available from: http://www.uni-leipzig.de/~nanoderm/.

15 Baroli, B., *et al.*: Penetration of metallic nanoparticles in human full-thickness skin, *J. Invest. Dermatol.*, **127**, [7], 1701 – 1712, (2007).

16 Larese, F.F., *et al.*: Human skin penetration of silver nanoparticles through intact and damaged skin, *Toxicology*, **255**, [1 – 2], 33 – 37, (2009).

17 Rouse, J.G., *et al.*: Effects of mechanical flexion on the penetration of fullerene amino acid-derivatized peptide nanoparticles through skin, *Nano Lett.*, **7**, [1], 155 – 160, (2007).

18 Ryman-Rasmussen, J.P., Riviere, J.E., Monteiro-Riviere, N.A.: Penetration of intact skin by quantum dots with diverse physicochemical properties, *Toxicol. Sci.*, **91**, [1], 159 – 165, (2006).

19 Ryman-Rasmussen, J.P., Riviere, J.E., Monteiro-Riviere, N.A.: Variables influencing interactions of untargeted quantum dot nanoparticles with skin cells and identification of biochemical modulators, *Nano Lett.*, **7**, [5], 1344 – 1348, (2007).

20 Riehemann, K., *et al.*: Nanomedicine-challenge and perspectives, *Angew. Chem. Int. Edit.*, **48**, [5], 872 – 897, (2009).

21 Thiesen, B., Jordan, A.: Clinical applications of magnetic nanoparticles for hyperthermia, *Int. J. Hyperther.*, **24**, [6], 467 – 474, (2008).

22 Reddy, S.T., *et al.*: Exploiting lymphatic transport and complement activation in nanoparticle vaccines, *Nat. Biotechnol.*, **25**, [10], 1159 – 1164, (2007).

23 Alyaudtin, R.N., *et al.*: Interaction of poly(butylcyanoacrylate) nanoparticles with the blood-brain barrier *in vivo* and *in vitro*, *J. Drug Target.*, **9**, [3], 209 – 221, (2001).

24 Koziara, J.M., *et al.*: In situ blood – brain barrier transport of nanoparticles. *Pharm. Res.*, **20**, [11], 1772 – 1778, (2003).

25 Kreuter, J.: Nanoparticulate systems for brain delivery of drugs. *Advanced Drug Deliver. Rev.*, **47**, [1], 65 – 81, (2001).

26 Lockman, P.R., *et al.*: *In vivo* and *in vitro* assessment of baseline blood-brain barrier parameters in the presence of novel nanoparticles, *Pharm. Res.*, **20**, [5], 705 – 713, (2003).

27 Wick, P., *et al.*: Barrier capacity of human placenta for nanosized materials, *Environ. Health Persp.*, **118**, [3], 432 – 436, (2010).

28 Krug, H.F., Wick, P.: Nanotoxicology: An interdisciplinary challenge, *Angew Chem. Int. Edit.*, **50**, [6], 1260 – 1278, (2011).

29 Beyersmann, D., Hartwig, A.: Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms, *Arch. Toxicol.*, **82**, [8], 493 – 512, (2008).

30 Conner, S.D., Schmid, S.L.: Regulated portals of entry into the cell, *Nature*, **422**, [6927], 37 – 44, (2003).

31 Kanno, S., Furuyama, A., Hirano, S.: A murine scavenger receptor MARCO recognizes polystyrene nanoparticles, *Toxicol. Sci.*, **97**, [2], 398 – 406, (2007).

32 Nagayama, S., *et al.*: Time-dependent changes in opsonin amount associated on nanoparticles alter their hepatic uptake characteristics, *Int. J. Pharm.*, **342**, [1 – 2], 215 – 221, (2007).

33 von Zur Muhlen, C., *et al.*: Superparamagnetic iron oxide binding and uptake as imaged by magnetic resonance is mediated by the integrin receptor Mac-1 (CD11b/CD18): implications on imaging of atherosclerotic plaques, *Atherosclerosis*, **193**, [1], 102 – 111, (2007).

34 Rothen-Rutishauser, B.M., *et al.*: Interaction of fine particles and nanoparticles with red blood cells visualized with advanced microscopic techniques, *Environ. Sci. Technol.*, **40**, [14], 4353 – 4359, (2006).

35 Simon-Deckers, A., *et al.*: Size-, composition- and shape-dependent toxicological impact of metal oxide nanoparticles and carbon nanotubes toward bacteria, *Environ. Sci. Technol.*, **43**, [21], 8423 – 8429, (2009).

36 Kreyling, W.G., Hirn, S., Schleh, C.: Nanoparticles in the lung, *Nat. Biotechnol.*, **28**, [12], 1275 – 1276, (2010).

37 Kreyling, W.G., *et al.*: Differences in the biokinetics of inhaled Nano- versus micrometer-sized particles, *Acc. Chem. Res.*, (2012).

38 Semmler-Behnke, M., *et al.*: Biodistribution of 1.4- and 18-nm gold particles in rats, *Small*, **4**, [12], 2108 – 2111, (2008).

39 Nel, A.E., *et al.*: Understanding biophysicochemical interactions at the nano-bio interface, *Nat. Mater.*, **8**, [7], 543 – 557, (2009).

40 Oberdorster, G., *et al.*: Acute pulmonary effects of ultrafine particles in rats and mice, *Res. Rep. Health Eff. Inst.*, **96**, 5 – 74; disc 75 – 86, (2000).

41 Stoeger, T., *et al.*: Instillation of six different ultrafine carbon particles indicates a surface area threshold dose for acute lung inflammation in mice, *Environ. Health Perspect.*, **114**, [3], 328 – 333, (2006).

[42] Zhang, Q., *et al.*: Comparative toxicity of standard nickel and ultrafine nickel in lung after intratracheal instillation, *J. Occup. Health*, **45**, [1], 23 – 30, (2003).

[43] Schrand, A.M., et al.: Are diamond nanoparticles cytotoxic?, *J. Phys. Chem. B*, **111**, [1], 2 – 7, (2007).

[44] Vial, S., *et al.*: Peptide-grafted nanodiamonds: preparation, cytotoxicity and uptake in cells, *Chembiochem.*, **9**, [13], 2113 – 2119, (2008).

[45] Monteiller, C., et al.: The pro-inflammatory effects of low-toxicity low-solubility particles, nanoparticles and fine particles, on epithelial cells *in vitro*: The role of surface area, *Occup. Environ. Med.*, **64**, [9], 609 – 615, (2007).

[46] Sager, T.M., Castranova, V.: Surface area of particle administered versus mass in determining the pulmonary toxicity of ultrafine and fine carbon black: Comparison to ultrafine titanium dioxide, *Part. Fibre Toxicol.*, **6**, [15], (2009).

[47] Sayes, C.M., *et al.*: Comparative pulmonary toxicity assessments of C60 water suspensions in rats: Few differences in fullerene toxicity *in vivo* in contrast to *in vitro* profiles, *Nano Lett.*, **7**, [8], 2399 – 2406, (2007).

[48] Donaldson, K., *et al.*: Carbon nanotubes: A review of their properties in relation to pulmonary toxicology and workplace safety, *Toxicol. Sci.*, 92, [1], 5 – 22, (2006).

[49] Donaldson, K., *et al.*: Asbestos, carbon nanotubes and the pleural mesothelium: A review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma, *Part. Fibre Toxicol.*, **7**, [1], 5, (2010).

[50] Donaldson, K., Poland, C.A., Schins, R.P.F.: Possible genotoxic mechanisms of nanoparticles: Criteria for improved test strategies, *Nanotoxicology*, **4**, [4], 414 – 420, (2010).

[51] Wick, P., *et al.*: The degree and kind of agglomeration affect carbon nanotube cytotoxicity, *Toxicol. Lett.*, **168**, 121 – 131, (2007).

[52] Warheit, D.B.: How meaningful are the results of nanotoxicity studies in the absence of adequate material characterization?, *Toxicol. Sci.*, **101**, [2], 183 – 185, (2008).

[53] Haynes, C.L.: The emerging field of nanotoxicology, *Anal. Bioanal. Chem.*, **398**, [2], 587 – 588, (2010).

[54] Locascio, L.E., et al.: Nanomaterial toxicity: emerging standards and efforts to support standards development, in nanotechnology standards, Murashov, V. and Howard, J., Editors. 2011, Springer: New York. 179 – 208.

[55] Pulskamp, K., Diabate, S., Krug, H.F.: Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. *Toxicol. Lett.*, **168**, [1], 58 – 74, (2007).

[56] Zhang, X., *et al.*: A comparative study of cellular uptake and cytotoxicity of multi-walled carbon nanotubes, graphene oxide, and nanodiamond, *Toxicol. Res.*, **1**, 62 – 68, (2012).

[57] Berridge, M.V., Herst, P.M., Tan, A.S.: Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction, in Biotechnology Annual Review, El-Gewely, M.R., Editor. 2005, Elsevier. 127 – 152.

[58] Mitchell, D.B., Santone, K.S., Acosta, D.: Evaluation of cytotoxicity in cultured cells by enzyme leakage, *Methods in Cell Science*, **6**, [3], 113 – 116, (1980).

[59] JCGM, International vocabulary of metrology – Basic and general concepts and associated terms (VIM). 2012, JCGM: Paris, 108.

[60] JCGM, Evaluation of measurement data – Guide to the expression of uncertainty in measurement. 2008, JCGM: Paris, 134.

[61] NanoCare. 2012; Available from: http://www.nanopartikel.info/cms/Projekte/NanoCare.

[62] Nanommune. 2011 [cited 2012 26.9.2012]; Available from: http://ki.projectcoordinator.net/–NANOMMUNE.

[63] Ekimov, A.I., Onushchenko, A.A.: Quantum size effect in three-dimensional microscopic semiconductor crystals, *JETP Lett.*, **34**, 345 – 349, (1981), see: Masanori Ohya, Igor Volovich, Mathematical Foundations of Quantum Information and Computation and Its Application to nano- and Bio-systems, Springer Dordrecht Heidelberg London New York. (2011)

[64] Wautelet, Michel (Editor): Nanotechnologie, Oldenbourg Wissenschaftsverlag GmbH, Munich, p. 190 (2008) (Original edition: Dunod Éditeur, Paris 2003)

[65] Meissner, T., Kühnel, D., Busch, W., Oswald, S., Richter, V., Michaelis, A., Schirmer, K., Potthoff, A.: Physical-chemical characterization of tungsten carbide nanoparticles as a basis for toxicological investigations, *Nanotoxicology*, **4**,196 – 206, (2010)

[66] Kühnel, D., Busch, W., Meissner, T., Springer, A., Potthoff, A., Richter, V., Gelinsky, M., Scholz, S., Schirmer, K.: Agglomeration of tungsten carbide nanoparticles in exposure medium does not prevent uptake and toxicity toward a rainbow trout gill cell line, *Aquat. Toxicol.*, **93** [2 – 3], 91 – 99, (2009).