Opportunities and Risks of Nanomaterials
National Research Programme NRP 64

Engineered Nanomaterials: Impact & Safety Aspects

White Paper

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Contents

1. Introduction

Progress in science shows that nanostructured materials can be found practically everywhere in nature, and that natural nanoscale processes at the cellular level are indispensable for life. Nanotechnology is a key enabling technology used for analysing, for designing and developing new, bio-inspired and synthetic nanomaterials, each with its own specific production methods, characteristics and lifecycle. This has been investigated by the 23 research projects within the framework of National Research Programme «Opportunities and Risks of Nanomaterials» (NRP 64).

Nanomaterials are rapidly being adopted in healthcare and in a broad spectrum of applications in the consumer and industry sectors, for example in the field of energy storage. Notable recent developments include intelligent/functional nanomaterials developed in NRP 62, «Smart Materials»[1]. Nanomedicine is an important application of nanoscience with the goal of exploiting the materials and methods of nanotechnology to the benefit of human health. There is already a broad application in medical in vitro diagnostics, as well as rapidly growing clinical experience with nanostructured therapeutics, and nanotechnology is an enabling technology for personalised medicine. Pharmaceutical and MedTech companies are therefore adopting nanotechnologies and nanomaterials as strategic issues for business development and in order to remain globally competitive.

Nanomaterials and Nanoparticles

The International Organisation for Standardisation (ISO) has defined «nanomaterial» as a «material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale» (ISO, 2010). According to the definition of the EU 50 % or more of the particles need to be in the nanoscale range. «Nanoparticle» are defined as a «nano-object with all three external dimensions in the nanoscale» where nanoscale is defined as the size range from approximately 1–100 nm (ISO, 2008).

An important aspect of every innovation is the assessment of risk and the determination of conditions for safe use. Understanding the potential benefits and risks is important for the general public, as well as for guiding further research, supporting decision-making in government funding and regulation, for the innovative capacity and competitiveness of industry and for the protection of the environment. In 2009, the Federal Council commissioned the Swiss National Science Foundation to implement a national five-year programme focusing on the opportunities and risks of nanomaterials: NRP 64. The total funding amounted to twelve million Swiss francs.

The goal of NRP 64 was to deliver the scientific basis for a deepened understanding of the potential benefits and undesirable effects of nanomaterials. Risk assessment included elucidation of potentially harmful mechanisms, the probability of exposure to such materials due to emissions, their potential persistence in the environment and their biological effects.

1 www.nrp62.ch

In the scope of NRP 64, the promise of innovative solutions based on nanomaterials was analysed, including consideration of their potential risks. For example, the use of nanoparticles in materials for medical implants led to an in-depth study on their biodegradation carried out by Martin Frenz, University of Bern. Other applications that were analysed in-depth in terms of their benefits and risks included the use of nanomaterials as immune modulators (Barbara Rothen-Rutishauser, Adolphe Merkle Institute), for the purification of blood (Beatrice Beck Schimmer, Institute of Anaesthesiology, University Hospital Zurich), and for targeted drug delivery (Francesco Stellacci, Federal Institute of Technology, Lausanne). Several projects focused on the development of methods for assessing the risks associated with nanomaterials.

Risk Assessment:

The risk of a chemical is defined as the product of hazard and exposure: risk = hazard (i.e. toxicity) x exposure (to the hazardous chemical)

NRP 64, «Opportunities and Risks of Nanomaterials», was thus a timely effort to deepen the understanding of these important issues. It yielded an array of complementary information in the areas of healthcare, protection of the environment, energy, food and construction materials. It was also embedded in various past and ongoing national and international initiatives focusing either on nanoscience innovation, for example, SNSF NCCR Nanoscale Science[2] in Switzerland, and the US Nanoscience initiative, as well as recent major European nano-safety programmes, including NanoSafety Cluster,[3] NanoReg[4], NanoImpactNet[5] and the global Working Party on Manufactured Nanomaterials (WPMN, established in 2006).[6] WPMN was an initiative of the Organisation for Economic Cooperation and Development (OECD), where the Mutual Acceptance of Data in the Assessment of Chemicals[7] is an essential agreement.

Switzerland holds a leading position in the field of nanoscience, and NRP 64 supported the maintenance and further development of this position through risk assessment and the examination of issues relating to regulatory control. Switzerland aims to maintain its leading role in responsible and rational risk assessment policies and regulations. In 2008, the Federal Council adopted its Action Plan for «Synthetic Nanomaterials», which requires federal authorities to create the legal bases for the safe handling of nanomaterials as an integral part of technological development. In the scope of this action plan, the Federal Office of Public Health and the Federal Office for the Environment developed a precautionary matrix intended to help companies to comply with the requirement of due diligence and their duty to perform self-regulation versus employees, consumers and the environment. This matrix facilitates the identification of potential risks posed by nanomaterials and the communication of important safety information along the entire production and supply chain.[8] A number of experts from NRP 64 were involved in the development of this action plan.

2 http://www.snf.ch/en/researchinFocus/nccr/nccr-nanoscale-science/Pages/default.aspx

3 horizon2020; http://horizon2020projects.com/industrial-leadership/nanosafety-cluster-releases-2016-compendium/

4 www.nanoreg.eu

5 https://empa.ch/web/s506/nanoimpact

6 Rasmussen K, Gonzalez M, Kearns P, Sintes JR, Rossi F, Sayre P. *Reg. Toxicol. Pharmacol.* 2016; 74, 147-160.

7 OECD. *Decision of the Council concerning the Mutual Acceptance of Data in the Assessment of Chemicals*. 12 May 1981-C(81)30/FINAL.

8 Precautionary matrix for synthetic nanomaterials, Version 3.0, Federal Office of Public Health, Division Chemical Products, 2013

Characteristics of Nanomaterials

Every kind of nanoparticle is characterised by its unique physical and chemical properties which differ from their macroscale counterparts. Nanomaterials can be classified into five different groups:

- **Carbon-based materials:** these can take the form of hollow spheres and ellipsoids (fullerenes), tubes or flakes (e.g. graphene). Carbon-based nanomaterials are being used in many different applications, e.g. in electronics, in films and coatings, or they are being incorporated into various materials to improve different properties.
- **Metal-based materials:** these include metals such as gold, silver and platinum, as well as metal oxides such as titanium dioxide and copper oxide. Quantum dots also belong to this group. They are characterised by their unique electrical, magnetic and optical properties. All materials in this group can be coated with different materials such as organic molecules.
- **Dendrimers:** these nanosized polymers consist of branched units. Their interior cavities can be used, for example, for drug delivery.
- **Self-assembled soft nanoparticles:** nanoparticles formed by the process of self-assembly of individual molecules (unimers) due to specific physicochemical properties.
- **Composites:** materials that combine nanoparticles with other nanoparticles or with other materials. The incorporation of nanomaterials into composites enhances, for example, mechanical, thermal and barrier properties of a material.

Source: http://www.azonano.com/article.aspx?ArticleID=1872

Abbreviations

AgNP:	Silver nanoparticle
CNT:	Carbon nanotube
CBN:	Carbon-based nanomaterial
ENM:	Engineered nanomaterial
ENP:	Engineered nanoparticle
OECD:	Organisation for Economic Cooperation and Development
WPMN:	Working Party on Manufactured Nanomaterials

This White Paper is one of the synthesis products of NRP 64. Its aim is to summarise the opportunities associated with nanomaterials and their safe use in the areas of human health and protection of the environment based on the activities within the individual research projects. It also presents conclusions from an integrative point of view, and takes into account recent progress in international activities in this area. Its purpose is to place the scientific findings of the research into the broader context of societal progress, healthcare policy, and environmental relevance in a language that is more easily accessible than the many individual scientific papers that originated in this research programme. It may serve as an information source for the formulation of future policies in the areas of research funding and environmental legislation, and as background information for developing industrial strategies.

While a detailed description of each project in this programme is beyond the scope of this paper, a number of exemplary studies are discussed and put into context.

2. Nanomaterials and the Environment

The potential of nanomaterials lies in their new and unique chemical and physical properties, which differ from their macro-scale counterparts and which, at least in part, originate from their large surface area per volume. Interesting new properties are also caused by effects of quantum mechanics. Improved hardness, strength, increased electrical conductivity, different catalytic efficiency and new thermal properties are typical features that make engineered nanomaterials (ENMs) beneficial when incorporated into batteries, computers, cosmetics, sports equipment, building materials, coatings or medical devices. Many such applications may be of benefit to the environment, such as new environmentally friendly construction materials and improved energy storage. Yet the potential uses of ENMs, their diversity and their unique properties, must be evaluated in light of potential risks associated with the manufacture and use of nanomaterials. The more products containing ENMs that are developed, the greater the probability of their release into the environment, which can occur during production, use and (unintentional) disposal of nano-based products. Once released, the flow of nanomaterials, their fate, their bioavailability and their biodegradation have to be studied in order to properly evaluate their risk. All these issues were addressed in NRP 64 in the projects relating to the environment, either from the point of environmental protection or improved knowledge with regard to the lifecycle of the nanomaterials, from production to environmental release.

Nanomaterial Developments to Benefit the Environment

Various projects explored the opportunities of nanomaterials with potential to enhance environmental protection. For example, the group led by Christoph Weder at the Adolphe Merkle Institute in Fribourg investigated porous cellulose nanocomposites as an environmentally friendly alternative to conventional insulation materials. By applying new protocols for the preparation process and by testing different binders they were able to create aerogels with improved and very promising properties,[9, 10] which could lead to new or improved applications. Another field of innovation in which nanomaterials could offer promising benefits is energy storage. Lithium-ion batteries provide excellent opportunities for grid storage of renewable energy, for electric mobility and for energy storage in portable devices. Within NRP 64, the group led by Katharina Fromm at the University of Fribourg investigated various nanocomposites as active materials in order to improve currently used materials and create new materials with improved battery capacity.[11] Further, the group led by Vanessa Wood, Federal Institute of Technology, Zurich, developed an attractive approach to increasing energy density of nano-based lithium-ion batteries by assembling nanoparticles into microspherical particles.[12]

Scenarios Relating to the Emission of Nanomaterials into the Environment

Production

During the manufacture and processing of nanomaterials there are several potential sources leading to environmental and workplace exposure. The most likely route is direct inhalation of materials released into the air. At the University of Lausanne, the group led by Michael Riediker conducted inhalation studies with unintentionally produced NPs occurring in welding fumes and revealed an increase in oxidative stress biomarkers in the blood and urine after such exposure.[13] With the nano aerosol chamber for in vitro toxicity (NACIVT), a method that was improved within the scope of NRP 64 in the project led by Marianne Geiser at the University of Bern, cell cultures replicating the inner lung surface can be exposed to a wide range of engineered nanoparticles (ENPs) out of a continuous airstream in order to assess the toxic effects of these ENPs on lung cells.[14]

Emissions during or after Use

Numerous consumer products comprising nanomaterials are already on the market and their number is growing constantly, increasing the possibility of nanomaterials being unintentionally released into the environment.

9 Mueller S, Sapkota J, Weder C, Foster, EJ *J. Appl. Polym. Sci.* 2015, Volume 132 (13)

10 Mueller S, Weder C, Foster EJ, *Green Materials* 2014, 2, 169-182.

11 Kwon NH1, Brog JP2, Maharajan S2, Crochet A2, Fromm KM3. Chimia (Aarau). 2015;69(12):734-6.

12 Nowack LV, Bunjaku T, Wood V, Adv Sci (Weinh), 2015, 2(6)

13 Graczyk H, Lewinski N, Riediker M, *Ann Occup Hyg.* 2016 Mar;60(2):205-19.

14 Jeannet, N, Fierz, M, Geiser, M, *Nanotoxicology*, 2015 Feb;9(1):34-42.

Silver nanoparticles (AgNPs) are used in commercial products for their antimicrobial properties[15] and during the life cycle of these products the discharge of AgNPs into the environment seems inevitable. As dissolved silver is highly toxic to certain organisms, the discharge of AgNPs is an important field of research and several NRP 64 projects concentrated on the fate and possible toxicity of released AgNPs (see chapter 4).

Carbon nanotubes (CNTs) are often used in composites in view of their great strength and stability. There are ongoing debates on the health risks associated with CNTs and whether their fibrous forms could be as harmful as asbestos if inhaled. The pathogenicity of fibres greatly depends on their length, thickness and biopersistence. As CNTs come in many combinations of sizes, types, purity levels and sources, a comparative study of existing evidence on the toxicity of CNTs remains difficult. More research is needed in order to determine whether CNTs can cause fibrosis and cancer in the long term and whether it is possible to make them safe by design. At the Federal Institute of Technology, Zurich, the project headed by Jing Wang aimed to develop a technology platform on which the toxicity of CNTs can be assessed as a function of their release when the composite materials are subjected to abrasion, weathering processes and elevated temperatures. Amounts of CNTs released after abrasion, weathering or exposure to elevated temperatures were low, but when carbon fibre reinforced polymer cables were stretched to breaking point, respirable carbon fibres were set free.[16, 17, 18, 19, 20] The toxicity of the particles was assessed in different cell culture systems, resulting in no acute negative effects at realistic doses of CNTs (see also chapter 4). The project demonstrated for the first time the cytotoxicity of CNTs incorporated in nanocomposites as a direct function of their release.[21, 22] Although long-term effects still need to be studied, this project suggests that it is possible to develop safe consumer products with CNTs and provides important know-how for their design.[23]

The platform created in the above-mentioned project was also used in the project led by Peter Wick at Empa, St. Gallen, where the release of copper was assessed after abrasion of Scots pine wood that was previously treated with nanocopper-based wood preservatives. Based on the rationale that nanocopper may penetrate wood more efficiently and provide a longer-lasting protection against wood-destroying fungi thanks to a reservoir effect, nanocopper-based formulations were introduced in 2006 in the USA. With this project it was demonstrated that the expected beneficial effect of nanobased formulations was not observed in common European wood species, i.e. that nanobased formulations did not penetrate wood more efficiently than conventional copper-based preservatives. Copper-loaded wood dust was released regardless of the treatment with nano-based or conventional pre-

15 Hansen SF, Heggelund LR, Baun A, 2016, *Environmental Science: Nano*, vol 3, no. 1, pp. 169-180
16 Schlagenhauf L, Kuo YY, Wang J. *J Occup Environ Hyg.* 2015;12(8):D178-83.
17 Schlagenhauf L, Nüesch F, Wang J, 2014, *Fibers* 2, 108-127
18 Schlagenhauf L, Chu BTT, Wang J, 2012 *Environ. Sci. Technol*, 46, 7366 – 7372
19 Schlagenhauf, L, Kuo YY, Wang J, 2015, *Journal of Nanoparticle Research*, 17:440
20 Wang J, Schlagenhauf L, Setyan A, *J Nanobiotechnology*, 2017 Feb 20;15(1)
21 Schlagenhauf L, Kianfar B, Wang J, *NanoScale*, 2015, 7 18524-36
22 Schlagenhauf L, Buerki-Thurnherr T, Wang J, *EST*, 2015, 49,10616-23
23 Schlagenhauf L, Kianfar B, Wang J, *Nanoscale*. 2015 Nov 28;7(44)

servatives.[24] This could pose a health risk as previously recognised.[25, 26] Further, the project demonstrated that, over time, substantial amounts of copper are released into the environment via dissemination of spores. Mechanisms of copper release via wood dust or spores are not specifically related to nanocopper formulations, but rather generally apply to copper-based systems. The findings obtained from this project indicate that while nanocopper may be applied successfully in North America, where timber is readily treatable, in Europe the preservative is not superior to conventional wood preservatives.[27] This finding is of importance for European regulatory bodies when approving or rejecting nanocopper as a wood preservative.

A reliable assessment of environmental emissions and concentrations of various nanomaterials is possible on the basis of a modelling tool developed by the research group led by Bernd Nowack at Empa, St. Gallen.[28] Based on the production, use and disposal of important nanomaterials such as nanosilver, nano-titanium dioxide, nano-zinc oxide, carbon nanotubes, fullerenes and nano-gold and their behaviour in technical systems, a dynamic probabilistic mass-flow model was developed which estimates the former, current and future flows of these ENMs into the environment for Switzerland and the EU.[29] Estimated concentrations of these nanomaterials in sewage effluent, surface water, sediments and sludge treated soils are several orders of magnitude lower than those of conventional materials.[30] The combination of the mass-flow model with an environmental fate model (see section on environmental fate) and with hazard data taken from the ecotoxicological literature facilitates a probabilistic environmental risk assessment of the above-mentioned nanomaterials.[31]

Detection and Characterisation of Nanomaterials in the Environment

Due to the diversity of types of materials available and being developed, together with the fact that each nanomaterial has its unique properties, their detection, evaluation of their fate in the environment and assessment of their potential risk are very challenging tasks. Before NRP 64 was initiated, it was not yet possible to detect and trace concentrations of nanomaterials in natural systems such as bodies of water, sediments or soils. Substantial progress has been made in the past few years and various projects within the scope of NRP 64 have made a valuable contribution. For example, in order to quantify and detect carbon-based nanomaterials (CBNs) and to identify possible biotransformation products, various new methods and techniques were developed and applied by the group led by Hans-Peter Kohler at Eawag, Dubendorf. The same research group contributed to a crit-

24 Civardi C, Van den Bulcke J, Schwarze FWMR, *PLOS One*, 2016:11 (9)

25 Civardi C, Schlagenhauf L, Schwarze FWMR, *Journal of Nanobiotechnology*, 2016 14:77

26 Civardi C, Schwarze FWMR, Wick P. *Env Poll.* 2015. May; 200:126–132.

27 Habicht J, 26. Holzschut-Tagung. Neue Normen, neue Erkenntnisse; 2010 Apr 22-23; Göttingen, DE. Münster: Self published; 2010. p. 161-188 German

28 Sun TY, Gottschalk F, Nowack B. *Environ Pollut*. 2014, Feb;185:69-76.

29 Bornhöft NA, Sun TY, Nowack B, *Environmental Modeling and Software,* 2016; 76: 69-80.

30 Sun TY, Bornhöft NA, Nowack B, *Environ Sci Technol*, 2016, 3;50(9):4701-1

31 Coll C, Notter D, Nowack B, *Nanotoxicology*, 2016;10(4):436-44.

ical review of methods and techniques used in carbon nanotube (CNT) research.[32, 33] Jing Wang's group at the Federal Institute of Technology, Zurich, implemented new methods for quantifying CNTs after abrasion processes. Further, the project led by Thomas Bucheli (Agroscope, Zurich), contributed tools and methods for monitoring nanomaterials in water and in soil[34, 35, 36], while the group headed by Ralf Kaegi (Eawag, Dubendorf) contributed to an increased understanding of sulphidation kinetics and reaction mechanisms of silver nanoparticles in wastewater systems.

Environmental Fate of Nanomaterials

Predictions based on Computational Modelling

Once released into the environment, the flow of ENMs within the environment, as well as their potential for transformation and their possible interactions with other substances, determine their potential environmental risks and govern their accumulation within the ecosystem. In order to model such processes, the group led by Bernd Nowack at Empa, St. Gallen, developed an environmental fate model in collaboration with the Federal Institute of Technology, Zurich. Concepts of corresponding models for organic pollutants were re-designed to take account of ENM-specific properties and fate behaviour such as aggregation, sedimentation, dissolution and other surface transformations.[37] The results show that ENMs aggregate with naturally occurring suspended particles, which determines their transport in surface waters.[38] Also, the studies confirm that sediments act as sinks for ENMs. This highlights the need to consider regional and site-specific conditions of soils and sediments when assessing the spatial and temporal variability of ENM concentrations and their potential risks for the environment and human health. The mass-flow model described in the previous section was combined with the environmental fate model in order to predict the fate of ENMs across a network of all Switzerland's major rivers.

Processes in Wastewater Systems

The fate of silver nanoparticles (AgNPs) in (waste) water systems was studied by the research group led by Ralf Kaegi at Eawag, Dubendorf. The use of silver nanoparticles in commercial products such as textiles and cosmetics is increasing, and their discharge into wastewater is inevitable. Due to the well-known antimicrobial effects of silver, AgNPs may impair biological processes that are vital to sewage treatment (e.g. nitrification during the activated sludge process). Furthermore, AgNPs may be released into the environment via the effluents of these treatment plants or the use of digested sludge as a fertiliser. AgNPs

32 Petersen EJ, Flores-Cervantes DX, Winchester MR, *Environ Sci Technol.* 2016; 3;50(9):4587-605.
33 Flores-Cervantes DX, Maes HM, Kohler HPE, *Environ Sci Technol*, 2014; 48: 4826-4834.
34 Mortimer M, Gogos A, Slaveykova VI, *Environ. Sci. Technol.*, 2014, 48, 8760-8767.
35 Gogos A, Kaegi R, Bucheli TD, *Environ. Sci. Nano*, 2014. 1, 584-594.
36 Gogos A, Moll J, Bucheli TD, *J. Nanobiotechnol.*, 2016, 14:40.
37 Praetorius A, Scheringer M, Hungerbühler K, *Environ Sci Technol.* 2012;19;46(12):6705-1
38 Praetorius A, Labille J, Bottero JY. *Environ Sci Technol.* 2014; 16;48(18)

were spiked into a sewage system and their changes inside a 5-kilometre-long sewer pipe were studied. Based on total silver concentrations measured in samples collected along the sewer pipeline at increasing time intervals, complemented by results from transmission electron microscopy analyses, an efficient transport of the AgNPs along the sewer channel was shown without substantial losses to the sewer biofilm.[39] Synchrotron based X-ray absorption spectroscopy revealed that AgNPs become efficiently sulphidised during sewage treatment, which reduces their toxicity by several orders of magnitude.[40] Experiments where AgNP reacted with bisulfides in the presence of various amounts of humic acid suggested that the sulfidation of AgNP in wastewater proceeds very fast with half-life times of a few minutes to hours.[41] AgNPs accumulate in the sewage sludge, which results in their very efficient removal from the wastewater (up to 95 percent). Depending on applicable local policies, sewage sludge is used as a fertiliser in agriculture, which represents a possible route for the release of AgNPs into the environment. However, for Switzerland this release route is not relevant, as sewage sludge is combusted. Together, the findings indicate that only a small amount (< 5 percent) of AgNPs are released from wastewater treatment plants and reach surface waters. Experiments where AgNP reacted with other metal sulfides present in urban surface waters (e.g. CuS and ZnS) resulted in the sulfidation of the AgNP and the dissolution of the metal sulfides. Half live times for the sulfidation reaction ranged from hours to days and depended on the AgNP size and the type and concentration of the metal sulfides.[42] Treated sewage can be ozonated in order to degrade remaining micropollutants, whereby sulphidised AgNPs are oxidised, which results in the release of dissolved silver into the effluent (see chapter 4).[43] These effects may become relevant at elevated AgNP concentrations and should be taken into consideration when evaluating ozone treatments of wastewater plants, explicitly in Switzerland where several such plants are planned to be upgraded by ozone treatment. Experiments conducted with different kinds of particles (Ag, Au) of different sizes and with different coatings indicate that sewage treatment plants act as a very efficient barrier for different kinds of (metal) nanomaterials.

Agriculture

Titanium dioxide and multi-walled CNTs are considered as active ingredients or additives of pesticides and fertilisers. The project led by Thomas Bucheli at Agroscope, Zurich, analysed the mobility of such ENMs in soil, together with their uptake by soil microbes and the effects on the ecological function of these microbes.[44, 45, 46] Both types of nanomaterials show limited mobility due to strong interactions with soil particles.[47] To assess the potential benefits and risks associated with nanomaterials used in plant protection and fertilisation, a detailed review of the current status, foreseeable applications and research priorities was

39 Kaegi R, Voegelin A, Mueller E, *Water Research*, 2013, 47(12), 3866–3877.
40 Kaegi R, Voegelin A, Siegrist H, *Environmental Science & Technology*, 2011, 45, 3902–3908.
41 Thalmann B, Voegelin A, Kaegi R, *Environ. Sci.:*, 2016, Nano 3, 203–212.
42 Thalmann B, Voegelin A, Kaegi, R, Environmental Science & Technology, 2014, 48, 4885–4892.
43 Thalmann B, Voegelin A, Kaegi R, Environmental Science & Technology, 2015 ; 49(18), 10911–10919
44 Moll J, Gogos A, Bucheli TD, van der Heijden MGA, *J. Nanobiotechnol.*, 2016, 14:36
45 Moll J, Okupnik A, Bucheli, TD, Widmer F, *PLoS ONE.* 2016, 11(5)
46 Moll J, Gogos A, Bucheli TD, van der Heijden MGA, *J. Nanobiotechnol.*, 2016, 14:36.
47 Gogos A, Moll J, Bucheli TD, *J. Nanobiotechnol.*, 2016, 14:40.

carried out within the scope of this project. This review was highly recognised by international experts in the field and was discussed at international meetings, workshops and conferences.[48]

Biodegradation

Biodegradation of nanoparticles may occur via routes typically observed in the biodegradation of organic molecules. However, it may also result in changes in the physical structure or surface characteristics of the material, depending strongly on the chemical and physical nature of the particle. When NRP 64 was initiated, there was a great need to find evidence of possible biotransformation mechanisms, including potential structural defects of CBNs acting as reaction sites. Familiarity with such processes is of great importance as the production of CBNs steadily increases, with an estimated worldwide production of more than 300 tonnes per annum of CNTs and fullerenes alone. CBNs are known for their very high thermal, chemical and mechanical durability. Based on these characteristics it is expected that CBNs are very persistent in the environment and undergo biotransformation at very slow rates. The project led by Hans-Peter Kohler (Eawag, Dubendorf) systematically analysed various CNT materials and developed methods to quantify and assess the extent of enzymatic transformations of CNTs. In this *in-vitro* setup, peroxidase mediated degradation was very slow.[49] In contrast, in biological systems such as macrophages, neutrophilic and eosinophil leucocytes, much faster degradation is reported in the literature.[50, 51, 52] However, CNT characteristics that make materials more or less resistant to biological transformation could be identified – a finding with implications for CNT design. Also, the findings provided information about enzymes that could play a key role in degradation processes and therefore might be used for the long-term treatment of CNTs in water or soils. The key outcome of this project emphasised the importance of the systematic characterisation of CBNs in various settings in order to draw reliable conclusions with regard to transformation processes. A review was therefore carried out regarding the status of techniques for the qualitative and quantitative analysis of CBNs and their applications.[53]

48 Gogos A, Knauer K, Bucheli TD, *J. Agric. Food Che*m, 2012, 60, 9791-9792
49 Flores-C ervantes DX, Maes HM, Kohler HPE, *Environ Sci Technol*, 2014; 48: 4826-4834
50 Andón FT, Kapralov AA, Kagan VE. Small 9.16 (2013): 2721-2729.
51 Farrera C, Bhattacharya, Fadeel B. *Nanoscale* 2014,6 (12), 6974-6983.
52 Kotchey GP, Zhao Y, Kagan VE, Star A. Advanced drug delivery reviews (2013), 65 (15), 1921-1932.
53 Petersen EJ, Flores-Cervantes DX, Bucheli TD, Winchester MR, *Environ Sci Technol.*, 2016; 3;50(9):4587-605.

3. Nanomaterials for Human Health Applications

The development and study of nanomaterials for medical applications is a key focus of nanomedicine research and development (where «nanomedicine» denotes the use of nanoscience methods, materials and tools to the benefit of human health). The design of ENPs is seen as one of the most promising applications of nanomaterials, because such innovative materials open the door to new ways to protect human health and prevent disease by allowing the monitoring of health and the early detection of diseases. Furthermore, they are suitable for delivering therapies that overcome problems with conventional drugs.[54] This was also successfully demonstrated by projects within the scope of NRP 64.

Various projects focused on specific types of nanoparticles, including magnetic, gold and polystyrene nanoparticles. Magnetic nanoparticles are currently used together with a magnetic force for cell sorting,[55] e.g. to gather circulating stem cells that might then be used after leukaemia therapy to replenish blood cells. Such nanomagnets are also used in medical imaging and permit the new therapeutic modality of targeted hyperthermia in cancer.[56] By combining diagnostic and therapeutic capabilities, they are an enabling technology for theranostics, e.g. in simultaneous detection and treatment of a disease such as cancer.[57] Gold nanoparticles are already widely used in rapid diagnostic tests in clinical practice worldwide due to their characteristic optical properties. Multifunctional gold-based nanocomposites have the potential to improve theranostics of cancer and other diseases.[58] Other types of nanoparticles include polystyrene nanoparticles used as biosensors in rapid tests, nanoparticle vaccines[59, 60] and nanoparticles for targeted drug delivery.[61]

Research results can contribute to our understanding of the world around us, but they can also contribute towards societal goals such as public health, medical applications and industrial progress. In order for a scientific finding to have an impact, the process of industrial «translation» is of the utmost importance. This transition from theory to practice in medicine often requires 4 to 6 years in diagnostic applications and devices, and 6 to 12 years (or even longer) for applications for new therapies. Current concepts for the development of new therapies are highly regulated in order to assure safety and ethics when human participants are involved. While NRP 64 was not designed as a product development programme, but rather for elucidating the scientific bases for the safe use of ENMs in human

54 Kagan CR, Fernandez LE, Weiss PS, *ACS-Nano*, 2016, 10 (10), 9093–9103.
55 Plouffe BD, Murthy SK, Lewis LH, *Rep Prog Phys.*, 2015, 78(1)
56 Hayashi K, Nakamura M, Ishimura K. *Theranostics*. 2013;3:366-76
57 Gobbo OL, Sjaastad K, Prina-Mello A. *Theranostics*; 2015; 5(11): 1249–1263.
58 Dykman LA, Khlebstov NG. *Biomaterials*; 2016, 108, 13-34
59 Zhao L, Seth A, Middelberg AP. *Vaccine*; 2014, 32 (3), 327-337
60 Powles L, Xiang SD, Plebanski M, *Vaccines*, 2015, 3(4): 894–929
61 Masood F. *Materials Science and Engineering*, C, 2016; 60, 569 - 578

health and the environment, it nevertheless resulted in project outcomes that approximate medical applications.

The following projects focusing on health applications demonstrate the benefits of ANMs and raise awareness of the associated risks, and illustrate the multidisciplinary character and translational aspects of this field. The majority of projects used nanoparticles, except the last one listed here, which used cellulose nanofibres and was the result of an unexpected discovery.

The first project presented here focused on the development of a new extracorporeal blood purification system by exploiting biocompatible carbon-coated magnetic nanoparticles developed by Beatrice Beck Schimmer at the Institute of Anaesthesiology, University Hospital Zurich. It offers the possibility of target molecule isolation and removal from the body, e.g. in a detoxification procedure used after a drug overdose or in case of an imbalance in inflammatory mediators. This was demonstrated by the removal of the drug, digoxin, and the toxic heavy metal, lead, from the blood of living rats[62] and by the rapid elimination of endotoxins from human blood in vitro.[63] Potential risks of this technique were examined by studying the possible interaction of the nanoparticles with blood cells and plasma, including inflammation reactions and toxicity. Coagulation was not affected to a clinically relevant degree and no inflammatory reaction could be shown during the experiments. Long-term exposure that could, for example, result from a device failure of the magnetic trap while eliminating toxins in extracorporeal circulation, did not reveal any sign of inflammation, necrosis or induction of malignancy in liver and lung tissues. This blood purification system based on carbon-coated magnetic nanoparticles developed in this NRP64 project has the potential to be translated to clinical medicine. The nanoparticles were extensively tested in the extracorporeal blood compartment, thus permitting the next step towards a clinical phase I study.

The immune-modulatory potential of gold nanoparticles with different surface coatings was explored in the project led by Barbara Rothen-Rutishauser at the Adolphe Merkle Institute by using a sophisticated 3D lung model that emulates the human airway tissue barrier (in vitro) and a mouse model (in vivo). The findings indicated that specific surface properties, including charge and composition of surface modified gold nanoparticles, can modulate the uptake of the nanoparticles by human monocyte-derived dendritic cells, but this does not affect the phenotype, cytotoxicity or cytokine secretion.[64] The influence of the surface charge on the interaction between the nanoparticles and antigen presenting cells in different respiratory tract compartments was then further investigated in vitro as well as in vivo.[65, 66] Positively charged particles coated with a poly-vinyl-alcohol polymer were taken up to a larger degree in comparison with particles with negative surface charge in both models. In addition it was demonstrated that the positively charged gold nanoparti-

62 Hermann IK, Schlegel A, Beck-Schimmer B, *Nanoscale* 2013; 5: 8718-23
63 Hermann IK, Urner M,, Beck-Schimmer B, *Adv Healthc Mater*, 2013, 6: 829-35
64 Fytianos K, Rodriguez-Lorenzo L, Rothen-Rutishauser B. *NBM*, 2015, 11(3):633-44.
65 Fytianos K, Chortarea S, Rothen-Rutishauser B, *ACS Nano*. 2017 Jan 24;11(1):375-383
66 Seydoux E, Rodriguez-Lorenzo L,, von Garnier C. *NBM*; 2016, 12 (7), 1815 - 1826

cles induced T-cell activation in draining lymph nodes of the respiratory tract in vivo. Thus, surface charge determines the uptake by antigen presenting cell population in different respiratory tracts and plays a role in the modulation of downstream immune responses such as the proliferation of CD4+ T cells in lung draining lymph nodes. These findings are examples of particle-specific properties, which are important for the development of, for example, immune-modulatory nanoparticles.

A Federal Institute of Technology, Lausanne, project led by Francesco Stellacci explored new composite nanoparticles that combine a metallic core with a coating of organic molecules. Remarkably, these are able to cross the cell wall.[67] This involves an energy independent interaction of the ligand of the metallic nanoparticle with the plasma membranes in diverse biological systems. Proteins are able to adsorb in a corona-like structure on the particles' surface. Modification of this surface results in changes in the corona and clever design could therefore optimise the interaction of the particles with, for example, cell membranes, proteins or viruses[68] when such an interaction is required. While the design of such nanoparticles contributes towards progress in science, the effective and safe use of such materials for targeted drug delivery will require more research. Nevertheless, such achievements in early phase innovative projects require a wise research funding policy, which ensures that academic innovations can ultimately lead to competitive applications to the benefit of patients, as well as provide impulses for Swiss industry.

In the project led by Peter Wick at Empa, St. Gallen, the translocation mechanism of nanoparticles across the human placenta barrier was studied using the *ex-vivo* placenta perfusion model. It was demonstrated that unmodified polystyrene nanoparticles are able to cross this barrier in a size dependent manner[69] and that their surface modification plays a significant role in the translocation rate.[70] Understanding such quantitative structure-effect relations opens the door to «Safety-by-Design» approaches for future products. Safety studies demonstrated that specific particles were accumulated in placental tissue without affecting the viability and functionality of the placenta within the period of observation.[71, 72] Such nanoparticle-based transport systems will require further study in order to identify detailed mechanisms and could also open up another possible route for future nanomedical applications.

A project led by Martin Frenz at the University of Bern studied the use of nanoparticles for suture-less blood-vessel repair in surgery by laser. Dye-enhanced nanoparticles were incorporated into a biodegradable implant for the purpose of transforming laser light into localised heat for the suture-less laser soldering of blood vessels, in particular in the brain. Two types of nanoparticles (gold and silica) with different surface properties were developed and investigated. Thorough safety studies were carried out and included the study of

67 Carney RP, Carney TM, Stellacci F. *Biointerphases;*, 2012, 7, 17
68 Huang R, Carney RP, Stellacci F, Lau BL. *Nanoscale*; 2013, 5 (15), 6928-35
69 Grafmüller S, Manser P, von Mandach U, *J. Vis. Exp.* 2013, 76, e50401
70 Grafmueller S, Manser P, Wick P, *Environ Health Persp*, 2015 123:12 1280-1286
71 Grafmueller S, Manser P, WickP, *Sci Technol adv Mat*, 2015(16) 044602
72 Grafmueller S, Manser P, Wick P, *Environ Health Pers*p, 2015 123:12 1280-1286

biodegradation of the implants containing nanoparticles. The effects of the nanomaterials on enzymes, cell lines and primary cells *ex-vivo* were examined and an organotypic model was used to emulate an *in-vivo* situation.[73] The nanoparticles were mostly absorbed in a concentration- and time-dependent manner by the immunoreactive cells of the brain. Confirming the findings by others in NRP 64, less negatively charged nanoparticles were taken up more rapidly. Among several involved endocytosis pathways, macropinocytosis was the most prominent. Studies to determine the safety of the material indicated that cell viability was not affected, but at the highest tested nanoparticle concentration a brief increase in oxidative stress was detected. Among several tested particle types, gold nanoparticles proved to be the best candidates for laser assisted vascular soldering in the brain. In addition, several particle types, including polycapsulated nanoparticles for laser tissue soldering, were also developed.[74]

The group led by Christoph Weder at the Adolphe Merkle Institute focused on the study of new high-performance polymer nanomaterials based on natural cellulose nanofibres isolated from renewable sources such as plants or wood. Here, one of the goals was to assess the potential risks of these materials following inhalation,[75] while another was to gain control over the synthesis of morphologies by understanding and exploiting nanofibre-to-nanofibre or nanofibre-to-polymer interactions. Such architectures could be key materials for an abundance of applications including tissue engineering. Multi-zonal polymer / nanofibre composite scaffolds were developed, emulating the structure, chemical cues and mechanical characteristics of mature articular cartilage.[76, 77] Thes e scaffolds bear phosphate moieties at the surface that nucleate and promote the formation of hydroxyapatite, which is required to integrate with the subchondral bone. Further studies led to the production of poly (D,L-lactide) / nanofibre composites. In vitro studies showed that such multi-zonal scaffolds promote the growth of neo-cartilage. Most importantly, the characteristics are similar to mature native tissue. Even though the findings were extremely promising, significant additional research and reliable in vivo data will be required in order for this method to be translated from research into practical application.

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74 Schönbächler A, Andereggen L, Reinert M, *J Neurol Surg A Cent Eur Neurosurg*, 2012, 73 - P048
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76 Camarero Espinosa S, Rothen-Rutishauser B, Foster EJ. *Biomaterials*, 2016, 42 - 52.
77 Camarero Espinosa S, Kuhnt T, Weder C, *Biomacromolecules*, 2013, 14, 1223-30

4. Safety Aspects and Risk Assessment

Gaining greater insight into nano safety in the context of applications was a declared goal of NRP 64. It is well known that nanomaterials may lose their specific properties after their release into the environment due to the simultaneous occurrence of numerous potential transformation processes.[78] Life cycle considerations are an important aspect for the safe use of new materials in general, including nanomaterials. The rapidly increasing number of newly discovered and synthesised nanomaterials in various fields of application, and the growing number of scientific methods that are suitable for studying the safety and risks associated with a given material, are producing a large body of knowledge in this field.[79, 80] However, not every available analytical method can, should or will be applied to every material. As this is a rapidly evolving field, standardisation and development of testing guidelines for nanomaterials is an ongoing activity in international organisations such as the ISO and OECD. In this context the main aim of the NRP 64 studies is to provide fundamental, mechanistically based scientific insight that in the long term will be of great value for the development of assessment methods for existing or newly developed materials.

Impacts of Nanomaterials on Freshwater and Soil Organisms

Due to their small size and high surface reactivity, engineered nanomaterials (ENMs) have the potential to interact with organisms in different ways compared with the corresponding bulk substances or dissolved forms of the same material. This interaction depends on a combination of factors such as the chemical composition of the material, the size and surface properties that can influence how the material behaves in the environment, with possible implications for biological impacts.[81] Biological effects caused by ENMs include oxidative stress due to formation of reactive oxygen species (ROS) and inflammatory cytokine generation, alteration of membrane integrity, composition and permeability, and effects due to the toxic metal ions as a result of dissolution of metal and metalbased ENMs.

In the scope of NRP 64, a new, highly sensitive bio-sensing platform was developed by Olivier Martin at the Federal Institute of Technology, Lausanne, that enables the real-time and non-invasive measurement of several oxidative stress markers when aquatic microorganisms are exposed to ENMs.[82] This project focused on the fact that the characterisation of ENPs as well as realistic exposure conditions is crucial in order to derive meaningful information from ecotoxicological studies. Also, it became clear that the environmental impacts of ENMs strongly depend on the environment in which they are measured, including environmental conditions that are usually neglected, such as illumination. The developed

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82 Koman VB, Santschi C, Martin OJ, *Biosens Bioelectron*, 2015 Jun 15;68:245-52.

platform facilitates dynamic toxicity assessments in model organisms for different types of ENPs under various realistic environmental conditions.

It is the antimicrobial properties of nanosilver (AgNPs) that pose the greatest environmental risk for systems dominated by microorganisms. Renata Behra (Eawag, Dubendorf) focused on the effects of AgNPs on food webs and key ecosystem functions. Two systems were studied that depend on healthy microorganism communities. Firstly, microbial biofilms dominated by algae and various other microorganisms, and secondly, a system dominated by leaf-decomposing fungi that interact with bacteria and aquatic fauna such as shrimps. In short-term exposure, AgNPs and ionic silver showed both the potential to disrupt important functions for both systems [83], while long-term exposures also resulted in changes of community composition, with a disruption of key stream functions driven by either periphyton or by microbial litter-decomposers[84]. The efficient AgNP affected the behavior of organisms feeding on these biofilms corroborating their role of algal biofilms as an important entry of nanomaterials and metals in the trophic chain. The results indicate that AgNPs can have direct and indirect effects on aquatic communities and thus challenge the use of simple toxicity assays as a sole basis for risk assessment.[84, 85] The fact that some effects were found to be caused specifically by AgNPs and cannot be explained by the presence of ionic silver implies the need to reconsider the adequacy of current regulations for water-quality with regard to nanoparticles. Finally, within this project it was demonstrated that ozone treatment of waste water, a process used in sewage treatment plants to degrade residual micropollutants, increases the toxicity of wastewater plant effluents through the release of toxic Ag ions.[86]

Whereas the project described above concentrated on the effects of AgNPs on microorganisms, the group led by Kristin Schirmer at Eawag, Dubendorf, set out to understand how AgNPs specifically interact with cells of aquatic organisms. This project studied algal cells, which are protected by a cell wall, and fish cells, which only have a cell membrane. It was found that algal cells do not internalise the nanoparticles, and the toxicity of AgNPs was explained by the ionic silver that was dissolved from the particles.[87] In contrast, fish cells take up AgNPs quickly via endocytosis mechanisms..[88, 89, 90] In a unique approach, the group characterised the proteins in the protein corona of the particle and demonstrated that the corona comprises a footprint of the destination of a particle within a living cell and reveals mechanisms underlying the particle toxicity.[91] This method will be of great value for the identification of protein-particle interactions, not only in the field of environmental protection, but also in the areas of human health and biomedical sciences. Together, the findings demonstrated the dynamic nature of nanoparticle-cell interaction, underlined the

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84 Tlili A, Jabiol J, Gessner MO, *Environmental Science & Technology*, 2017: 51 (4), pp 2447–2455.
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88 Yue Y, Behra R, Schirmer K, *Nanotoxicology*, 2015, 9(1):54-63.
89 Yue Y, Behra R, Schirmer K, *Environmental Science*, 2016, Nano 3 (5), 1174-1185
90 Yue Yang Y, Li X, Schirmer K, *Journal of Nanobiotechnology*, 2017, accepted.
91 Yue Y, Behra R, Schirmer K, *Environmental Science*, 2016, Nano 3 (5), 1174-1185

importance of studying particle characteristics as closely as possible in the environment they truly encounter, and highlighted the importance of considering potential long-term effects. Using a newly developed fish cell model that is suitable for long-term experiments the group found that AgNPs inhibit cell proliferation.[92] Additional research will be required in order to better understand the long-term effects. However, a suitable *in-vitro* model is now available.[93, 94]

Possible adverse effects of ENMs on soil bacteria and crops were investigated by the group led by Thomas Bucheli at Agroscope, Zurich. In this project, titanium dioxide and multi-walled CNTs both indicated limited mobility in soil due to strong interactions with soil particles.[95] In liquid culture experiments, the growth rate of Rhizobium trifolii, a soil bacterium which is able to fix nitrogen in symbiosis with clover, was decreased significantly when exposed to high concentrations of titanium dioxide.[96] In soil systems however, high concentrations of titanium dioxide did not affect symbiosis of R. trifolii with clover or wheat.[97] Although the experiments conducted here concentrated on specific ENPs and did not legitimate an extrapolation of results to other agricultural systems, the results indicated that the low mobility and availability of NPs in soil reduces their risk for soil organisms. Overall, the project provided important exposure and effect data as well as methods for a risk assessment of ENP applications in the agro-environmental context. However, there are still important knowledge gaps. Taking into consideration the fact that research and development activities in the field of ENP-containing pesticides and fertilisers are constantly progressing, it is highly important to further investigate fate, exposure and mechanisms of toxicity of various ENPs in the soil.

Impacts of Nanomaterials on Human Health

Nanomaterials may have direct or indirect (e.g. silica particles via food) impacts on human health and could therefore pose risks. Risk depends on multiple factors, including the produced quantity of a given material, the exposure of individuals (duration of exposure, time after exposure), uptake by the body, interaction with the organism, stability of a material and its biodegradation, excretion from the body and distribution and accumulation in the environment. In order to develop nanomaterials for applications in human health, a proactive approach to mechanistic understanding of nano-bio interaction is advisable because it allows a more reliable assessment of risk and opens the door to a «safe-by-design» approach in which materials are designed so that they cannot lead to undesirable effects outside their location of application and the time window of choice.
The project led by Hanspeter Nägeli (University of Zurich) focused on the development of an *in-vitro* test to assess the biological activity of food-borne nanomaterials and their

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93 Schirmer K, *Nanoscience and the Environment*, 2014, Vol 7, *FNS*, UK: Elsevier, 195-222.
94 Schirmer K, Behra R, Zweck A, *Pan Stanford Publishing Pte. Ltd. Singapore*, 137-158
95 Gogos A, Moll J, Bucheli TD, *J. Nanobiotechnol.*, 2016, 14:40.
96 Moll J, Okupnik A, Widmer F, *PLoS ONE*, 2016, 11(5):e0155111.
97 Moll J, Gogos A, van der Heijden MGA, *J. Nanobiotechnol.*, 2016, 14:36.

capabilities to disrupt gut-associated immunity activity of food-borne nanomaterials. Dendritic cells embedded in the intestinal mucosa play a critical role in provoking an immune reaction against invading pathogens and need to maintain tolerance towards normal innocuous luminal commensals and nutrients. An *in-vitro* system developed in NRP 64 for the risk assessment of nanomaterials comprises the two main components, namely well-characterised nanoparticles and primary steady state dendritic cells. Analytic methods in this system included flow cytometry, electron microscopy, enzyme linked immuno assays and Western blotting. It was demonstrated that synthetic amorphous silica nanoparticles activate dendritic cells that mediate pro-inflammatory reactions in the gut. No comparable pro-inflammatory activities were observed in response to titanium or iron nanoparticles. It was concluded that nanoparticles do not pose a completely new threat, thus supporting similar findings by other groups. The potential risk of such materials can be assessed by following established procedures for conventional chemical hazards.[98] Nevertheless, some fundamental properties of food-borne nanoparticles should be further investigated by using *in-vitro* tests with decision-making cells of the immune system, in order to conduct *in-vivo* studies in the future.

A related project led by Michael Bruce Zimmermann at the Federal Institute of Technology, Zurich, focused on gastrointestinal exposure to nanoscale iron compounds in foods: absorptive pathways and potential toxicity. Similar to the above project, the nanoparticles were well characterised, and in order to investigate the mechanism of uptake from the gastrointestinal tract, three different non-cancerous gastrointestinal cell lines and animal studies were performed. The non-cancerous cells used were epithelial cells derived from biopsy of healthy epithelium. In contrast to many commercial standard cell lines, such cells are not tumorigenic, and therefore are not expected to have any genetic mutations in hot spot genes and they express both epithelial as well as stem cell markers. The administration of diet enriched nanoparticles in two dose ranges did not result in adverse excess accumulation of tissue iron or other significant effects. In addition, direct toxicity or oxidative stress did not occur in the cell lines. In collaboration with the above project, the potential of the nanoparticles to induce an inflammation reaction by dendritic cells was assessed. Even though so far results point to an absence of toxicity in food, the researchers recommend further investigation. The developed methodology in NRP 64 is suitable for assessing the toxicity of any nanoparticle and non-nano materials that might come into contact with the gastrointestinal tract.

The group led by Jing Wang at the Federal Institute of Technology, Zurich, set out to determine the potential health and environmental risks associated with carbon nanotube composites. Nanotubes are long, thin fibres, typically of pure carbon, that are thought to behave very differently than spherical particles, and can convey strong mechanical properties,[99] e.g. for more durable medical implants. A new measurement method was developed in this project in which multi-walled CNTs were labelled with lead ions allowing quantification despite their tiny size. This method involved the production of the nanocomposites, abra-

98 Winkler HC, Suter M, Naegeli H, *J. Nanobiotechnol.* 2016; 14; 44
99 Wang J, Schlagenhauf L, Setyan A, *J Nanobiotechnology*, 2017, 20;15(1):15.

sion and the collection of the inhalable particle fraction. The protruding and freestanding nanotubes were subsequently quantified by measuring the concentration of released lead ions. Assessment of the safety of the nanotubes was performed with two different cell lines: human alveolar epithelial cells and blood monocyte-derived macrophages.[100, 101] The cell viability, genotoxicity and formation of reactive oxygen species were investigated and did not reveal that the abraded particles induce any acute cytotoxic effects.[102, 103]

5. Summary and Recommendations

Engineered nanomaterials are high-performance, high-value materials. They have become a key technology platform in research and industry resulting in a broad spectrum of innovative applications in the healthcare, industry and consumer sectors.

More specifically, the key sectors of application for new, engineered nanomaterial-based products are as follows:

1) In medical technology, engineered nanomaterials are widely used for in vitro diagnostics, in particular for point-of-care diagnostic tests.
2) In the pharmaceuticals industry, nanomaterials have led to targeted therapies for severe diseases.[104] Nanomaterials offer improved efficacy and reduced toxicity, and are enabling clinical molecular imaging for earlier and more accurate diagnosis.
3) In personalised medicine, nanotechnology is the key enabling technology for molecular diagnostics and for personalised, molecularly targeted therapies.
4) In environmental applications, engineered nanomaterial-based technologies offer great potential for innovations that could be beneficial for the environment, such as photocatalytic surfaces that degrade environmental contaminants, efficient water cleaning technologies and nanoparticles for remediation of contaminated soil and groundwater, and may also have an indirect beneficial effect on the environment by optimising product lifecycle or efficiency.
5) In energy applications, engineered nanomaterials play a fundamental role and will become a key technology for enabling grid storage of renewable energy.

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101 Schlagenhauf L, Kianfar B, Wang J, *NanoScale*, 2015, 7 18524-36
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103 Schlagenhauf L, Buerki-Thurnherr T, Wang J, *Environ. Sci. Technol.*, 2015, 49, 10616 - 10623
104 Lehner R, Wang X, Hunziker P. *Nanomedicine* 2013, 9:742–57.

Highlights: New applications or materials studied within the scope of NRP 64:

1) A blood purification system based on carbon-coated magnetic nanoparticles has the potential to be translated to clinical medicine and pave the way for a clinical phase I study.
2) New composite nanoparticles that combine a metallic core with a coat of organic molecules can be designed to optimise the interaction of particles with, for example, cell membranes, proteins or viruses, providing an enabling technology for targeted drug delivery.
3) New high-performance polymer nanomaterials based on natural cellulose nanofibres offer new opportunities for tissue engineering. Very promising results for neo-cartilage have to be upscaled for future application, though this is still in the planning stage and its continuation has not been secured.
4) In the energy sector, synthetic nanomaterials could open up a significant opportunity for energy storage.

Switzerland's Impact on Nanotechnology

Academic and industrial researchers from Switzerland were instrumental in triggering the era of nanoscience, and there is now an active community of academics in the field of nanomaterials, encompassing the two Federal Institutes of Technology (in Zurich and Lausanne), Empa and the majority of cantonal universities. Switzerland's cooperation between the regulatory authorities, universities and industry has been a pioneering approach aimed at securing the safe industrial translation of engineered nanomaterials.

Switzerland has various internationally relevant organisations (e.g. the International Society of Nanomedicine) and organises special events (e.g. the CLINAM conference for nanomedicine, SwissNanoconvention), and Swiss research is contributing to major international initiatives such as the NanoSafety Cluster, the OECD Working Party on Manufactured Nanomaterials and numerous EU FP7 Horizon 2020 projects, including NanoImpactNet, SUN, NANoREG, ProSafe and Nanocalibrate.

Risks and Potential Environmental Impacts of Nanomaterials

Technological innovations always carry intrinsic risks that need to be identified and assessed, and this also applies to engineered nanomaterials. Quantification of risk is based on the inherent toxicity of the material, the level of exposure, the probability of release and the stability or reactivity of the material after its release. The potential risks of nanomaterials do not only depend on their size, but are mainly determined by specific material characteristics, i.e. biocompatibility, biodegradability, interaction with the complex biological environment, stability and accumulation in biological organisms and the environment, all of which are highly material-dependent. Therefore a widespread consensus has evolved both in Switzerland and abroad that the existing principles that apply to risk assessment and risk management in the field of industrial products in the macro range are also applicable to nanomaterials. However, some of the guidelines require specific adaptation because materials in the nano range do not (always) behave in the same way as the bulk material. In addition to chemical differences, this also applies for interactions of the material with biological systems.

In 2008, the Swiss government, universities and industry jointly developed the Action Plan for «Synthetic Nanomaterials», which represents a pioneering approach to assessing the potential risks associated with nanomaterials.

Key facts that determine the risks associated with nanomaterials

1) Many nanomaterials can be biodegraded, although the kinetics of this process is highly variable between specific materials.
2) Long-term safety depends on the potential for persistence and bioaccumulation of a material in living organisms or in the environment.
3) Some nanomaterials can be designed to be low-risk: biocompatible, biodegradable, soluble (thus increasing their propensity to agglomerate), consisting of non-toxic building blocks, tested for known toxicity pathways, controlled quantity, controlled exposures.

In this respect, the most important findings of NRP 64 are as follows:

1) The creation of new mass-flow and environmental fate models enables a realistic estimation of concentrations of nanomaterials in the environment.
2) Carbon nanotubes vary strongly in their potential for persistence in the environment, depending on their specific characteristics. Data from NRP64 showing slow degradation in certain setups, combined with recent reports in the scientific literature documenting the capability of biological cells to degrade them, have implications for the rational design of future carbon nanotube applications.
3) Sewage treatment plants act as a very efficient barrier to the emission of silver nanoparticles and only a small fraction of sulphidised silver nanoparticles are discharged into surface waters. The ozonation of treated waste water oxidises the sulphidised AgNPs which results in the release of silver ions into sewage treatment plants where effluents are ozonated. These findings should be taken into account when evaluating different options for further improving the quality of treated wastewater.
4) The characterisation of the protein corona of nanoparticles provides information on the destination of the particles within living organisms and reveals mechanisms underlying the particles' toxicity.
5) Adverse effects on microorganisms were only detected at concentrations of AgNP about one magnitude higher than those expected to be found in nature. However, long-term experiments are still needed, as well as further studies on the mechanisms of bioaccumulation in microorganisms and along food webs.

6) Possible risks to human health caused by nanoparticles should not be limited to cytotoxicity, but should include end points such as immunoreactivity and genotoxicity.
7) Surface characteristics such as surface charge play an important role in the modulation of immune responses. These particle-specific properties are important for the development of, for example, immune-modulatory nanoparticles.
8) Multi-walled carbon nanotubes released from nanocomposites due to abrasion show no acute cytotoxic effects on human lung cells or macrophages in vitro.
9) The anticipated beneficial effects of nanobased wood preservatives were less pronounced than hypothesised in common European wood species, because they did not penetrate wood more efficiently than conventional preservatives – a key finding for regulatory purposes.
10) Synthetic silica nanoparticles activate dendritic cells and mediate inflammatory reactions in the gut. The next steps are *in vivo* studies and comparison with human exposure data, which in turn could result in a new definition of food toxicity affecting the digestive system. However, results of this project suggest that concentrations of silica nanoparticles in food additives should be reduced.

General findings of NRP 64:

In addition to the specific results from individual projects listed above, the NRP 64 led to various overarching scientific and sociopolitical findings:
1) Creation of an "early warning" group with a sentinel function for rapidly evolving potential threats in the field of synthetic nanomaterials.
2) Enhancement of our knowledge base concerning properties, techniques, benefits and risks of synthetic nanomaterials.
3) Resulting implementation of a number of devices and methods that allow the quantification and characterisation of nanomaterials in living organisms and the environment.
4) Pioneering of a number of promising nanomaterials for progress in medicine with documented low potential toxicity.
5) Contribution to international efforts aimed at assessing the risks associated with nanomaterials.
6) Contribution by participants in NRP 64 towards the standardisation of methods in the context of international organisations such as the International Organisation for Standardisation (ISO) and the Organisation for Economic Cooperation and Development (OECD).

Research carried out within the scope of NRP 64 also highlighted a number of limitations:

1) The development of synthetic nanomaterials is a rapidly progressing field. The body of knowledge is still increasing and was not finalised at the time of completion of NRP 64.
2) In contrast to other industrialised nations, in Switzerland there is still only limited recognition of the role of synthetic nanomaterials as a strategic platform technology.

3) Switzerland does not have an institution that is dedicated, committed, and funded to maintain and advance excellence in the assessment and characterisation of nanomaterials in a manner that is independent of short-term academic projects and industry.
4) There is a need for strongly quality-oriented institutions that are not subject to publication pressure and commercial considerations, which can deliver reliable, reproducible and certifiable results concerning specific materials, and identify new methods for research and industry to develop material innovations in a timely manner.

Recommendations:

1) In the sense of a service for the economy, an excellence laboratory for the characterisation and evaluation of nanomaterials should be established, for testing and certifying their quality in applications, and to promote their use in a safe manner, particularly in the areas of healthcare and protection of the environment.
2) To establish a contact point making the knowledge gained in NRP 64 accessible for smaller and bigger Swiss companies.
3) To institutionalise the «Issues Monitoring» of the NRP 64 which observes new developments in the sector of nanotechnology to a permanent task force.
4) To maintain the exemplary cooperation between universities, government and industry in the action plan and the «Vorsorgeraster für synthetische Nanomaterialien» through periodical reviews and reports.

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