DRAFT

Nanotechnology:
Health and Environmental Risks of Nanoparticles

– Research strategy –

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1. Summary

As an important future technology, nanotechnology[1] provides the opportunity to promote long-term economic development by means of intensive research and the effective transfer of research findings into innovative products. At present, the toxicological and ecotoxicological risks linked to this expanding technology ("emerging technology") cannot be assessed yet. Nanotechnology is increasingly moving into the centre of public attention. However, currently it is not yet linked to any great degree to concerns about health and the environment. Over the next few years this could change, if the media increasingly will point at components linked with nanotechnology which are harmful to health or the environment (cf. also public debate on genetically modified organisms (GMOs)). It is expected that the importance of nanotechnology will continue to grow and that workers and consumers will be increasingly exposed to it. Hence, there is a need to monitor the development of this new technology, to weigh up the opportunities and risks in a transparent process and compare them with established technologies. Particularly important is co-ordinated and effective research in order to enable the public agencies BAuA, UBA, BfR (downstream of the ministries BMAS, BMU and BMELV) to describe and assess the toxicological and ecotoxicological risks and also to place the resulting recommendations (e.g. classifications, limit values, handling recommendations) on a sound and comprehensive basis.

According to present knowledge, the insoluble and poorly soluble nanoparticles[2] are particularly toxicologically relevant. For that reason and to sensibly define the scope of the subject, this research strategy refers to these nanoparticles and to chemical safety at the workplace and for consumers and the environment. Chemical legislation does not specify any obligation to test (e.g. toxicological studies) or assess widespread nanoparticles like for instance titanium dioxide, zinc oxide, iron oxide, silicon dioxide or "carbon black" that involve a nanoscale modification to a HPV[3] existing substance with the same CAS No. Up to now there has been no specific regulation for nanoparticles in the areas food, consumer goods or cosmetics. For instance, no particle sizes are stipulated in the purity criteria for the approved food additives silicon dioxide (E 551) and titanium dioxide (E 171).

As the exposure of humans and the environment as well as the toxicological and ecotoxicological properties and risks have not yet been characterised, there is a general need to conduct further studies and to close the gaps in knowledge through research and assessment activities. Similar to technologically based research, in safety research we also need a shift away from pure fundamental research and a new direction which facilitates the implementation of results in risk-oriented and comprehensive assessments (or recommended actions) and the covering of the relevant toxicological and ecotoxicological endpoints. Furthermore, the goal is to achieve a balance between *in vitro* and *in vivo* methods which is influenced to a large extent by the validity of the *in vitro* methods.

In order to promote the acceptance of nanotechnology by the public, accompanying social scientific research should be conducted and there should be transparent discussion of the risks with all interested stakeholders in society (cf. for instance http://www.dialog-nanopartikel.de/downloads.html). The establishment of a cross-disciplinary nano discussion

[1] Nanotechnology describes the manufacture, examination and use of structures, molecular materials, inner interfaces with at least one critical dimension below 100 nm.

[2] Nanoparticles are understood as being engineered granular particulates, tubes and fibres with a diameter <100 nm (including their agglomerates and aggregates) for at least one dimension which have been shown to have low solubility in biological systems. Based on knowledge acquired so far these particles are particularly toxicologically relevant.

[3] HPV chemicals: chemicals produced in large volumes. Chemicals which are placed on the market in the EU in volumes over 1000 tonnes per year per manufacturer or importer.

platform for Germany is seen as a suitable way of initiating and co-ordinating research and discussion, and using the results for regulatory practice.

It should, however, also be taken into account that nanoscale particles are not completely new. For a long time naturally formed and unintentionally produced particles of this size have passed into the environment leading to exposure for humans and the environment.

The goal of this research strategy is to structure the research area, to develop the measurement of particles (metrology), to record information on exposure and toxicological/ecotoxicological effects, to promote the development of a sophisticated risk related test and assessment strategy, to identify the existing elements of a test strategy, to move substances of particular importance centre stage of assessment and, in particular, to ensure the suitability of the data recorded from publicly financed research activities for regulatory questions (e.g. limit values, classifications, handling recommendations). Furthermore, this also encompasses risk communication projects.

The following projects and work areas requiring funding have been identified:

Exposure and metrology:

1. **Voluntary reporting scheme for the production, further processing and open use of nanoparticles**

   The goal of the project is to give an up-to-date overview of the state and the development of this innovative economic sector by means of regularly updated information. In addition, the dialogue with the companies concerned will be promoted.

2. **Company survey of workplace exposure and risk management**

   The goal is to record exposure to nanoparticles in companies, to create exposure categories and to allow priority setting when determining the needs for further research on the health impact. Based on this information a code of practice can also be drawn up.

3. **Development, testing and standardisation of measurement methods for nanoparticles at the workplace**

   The goal of this project is to develop, test and standardise measurement methods and to facilitate their routine use. Portable devices have to be improved to measure exposure at the workplace.

4. **Company survey on the use of nanotechnology in the production of foods, packaging materials, cosmetics, clothing and other consumer products**

   The goal of the project is to record the exposure of consumers and the environment to nanoparticles in the areas foods, consumer goods and cosmetics in the companies, to set up exposure categories and, based on the distribution of the particles, to establish priorities when identifying requirements for further research on health effects and environmental impact.

5. **Development of methods to determine and characterise nanoparticles in foods, cosmetics and other consumer products**

   Quantitative and qualitative data on exposure are needed to estimate the health risks. The goal is to develop measurement methods for the quantification and characterisation (chemical composition, particle size and distribution, solubility, agglomeration state, shape, surface, surface charge) of nanoparticles in these products.

6. Development of exposure scenarios and life cycle analyses of nanoparticles

Findings from the projects 2 (company survey workplace) and 4 (company survey consumers) provide information on where which particles are produced, and in which products they are contained in. In a preliminary study on this and on project 8 additional applications are to be identified in which nanoparticles are used directly in the environment. This could identify which nanoparticles should be given priority in assessments. The goal is to examine the methodology of exposure scenarios and life cycle analyses from the angle of the special features of nanoparticles and to adapt them if necessary.

7. Development or adjustment of measurement methods for the areas air, water and sewage sludge and for ecotoxicological test methods

The existing measurement methods and findings from projects 3 (measurement methods at the workplace) and 5 (measurement methods in foods, cosmetics and products) should be examined for their suitability for recording nanoparticles in the environment and adapted if necessary.

8. Study on behaviour and fate (accumulation, persistence) in the environment

Nanoparticles may have the potential to accumulate in organisms and in the food chain. The goal of the project is to clarify bioaccumulation and biomagnification as well as the influence of agglomeration on bioavailability.

Toxicological and ecotoxicological assessment of nanoparticles

9. Development of methods to determine and characterise nanoparticles in biological material

The goal of the project is to develop imaging processes and other detection methods for the determination of nanoparticles in biological material. Reliable quantitative methods are needed that allow the detection in the various body compartments.

10. Development of minimum requirements for information in publications

Results of studies for the assessment of nanomaterials are often difficult to evaluate because basic information is missing or certain aspects have not been considered in the study design. The goal is to formulate harmonised minimum requirements for the information content of studies and publications in order to be able to assess their validity and to improve the comparability of nanostudies.

Toxicological assessment

11. Development of a test and assessment strategy

In principle there is a need to develop a risk-related test and assessment strategy. Here valid physico-chemical (PM) methods, *in vitro* and *in vivo* methods are to be sensibly integrated in order to facilitate a comprehensive assessment of nanoparticles making use of resources as efficient as possible also with regard to animal welfare.

12. Determining the sensitivity and specificity of *in vitro* methods or methods to determine the PC properties for the assessment of chronic toxicity and carcinogenicity of dust

The goal of the project is to use *in vitro* studies in the assessment in an optimum and statistically validated manner. In addition methods are promoted that avoid animal

testing. Furthermore a contribution is made to concretise a test strategy. The validity of the various *in vitro* studies should be proven before further *in vitro* studies are carried out.

13. Assignment of nanoparticles to categories of different toxicity through *in vitro* studies and studies on PC properties

The goal is to establish substance classes for nanoparticles so that not all nanoparticles would have to be examined extensively. Based on similarity a joint assessment will be possible requiring fewer animal experiments.

14. *In vivo* studies with widespread nanoparticles on chronic toxicity and CMR effects

The goal of the *in vivo* studies is to create reference studies by means of which the suitability of *in vitro* studies can be measured. In addition, a sound assessment basis for possible protective measures (e.g. classification, limit values) would be created for representative, widespread nanoparticles. Nanoparticles with a lower level of exposure may be evaluated analogous to the substances tested *in vivo.*

15. Studies on the mechanism of toxicity and the influence of particle size

The goal is to elucidate the mechanism of toxicity and to provide a better description of the quantitative difference in toxicity between nanoscale and microscale particles with the same composition. Information of this kind would help to develop a test and assessment strategy but would also contribute to the respective assessment of the individual substance.

16. Studies on the toxicity of nanoparticles at the workplace

Exposure at the workplace is mainly via skin and inhalation. Since findings so far indicate that systemic availability after inhalation is far higher and damage to the lungs has already been shown, the inhalation route has priority from the angle of occupational safety. Taking into account the unclear validity of the *in vitro* studies, the *in vivo* studies with repeated exposure are of special importance.

17. Studies on the skin penetration of nanoparticles from cosmetics and consumer products

In order to assess the health impact of dermal exposure of consumers to nanoscale particles used in cosmetics or other consumer products, it is necessary to determine the degree of systemic availability of nanoparticles after dermal exposure.

18. Studies on absorption, systemic availability, accumulation and excretion of nanoparticles after oral exposure (foods and food packaging materials)

For the health assessment of oral exposure of consumers to nanoscale particles used in the production of foods and food packaging materials, it is necessary to determine the degree of absorption, systemic availability, accumulation and excretion of nanoparticles after oral exposure. In the assessment the focus is on *in vivo* studies with repeated exposure.

19. Assessment of the toxicity of nanoparticulate zinc oxide

The goal of the project is to examine the indications of a mutagenic potential of a widespread nanoscale substance and to allow a transparent assessment on the basis of studies.

20. Assessment of the toxicity of nanoparticulate silicon dioxide

The goal is to assess indications of hazard potential arising from published studies and to clarify this in further examinations.

Ecotoxicological assessment

21. Grouping of nanoparticles by ecotoxicological effects

In project 13, nanoparticles with similar toxicity are to be compiled in groups in order to examine one representative substance in this group. The goal of this project is to check the grouping based on human toxicological aspects for its transferability to environmental organisms and, where appropriate, to undertake an additional grouping from an ecotoxicological point of view.

22. Analysis of conducted studies with regard to relevant endpoints, elaboration of action hypotheses, identification of suitable test systems

Based on a literature review the studies on environmental impact and human health effects conducted so far are to be analysed and assessed regarding their relevance for ecotoxicology.

23. Examination and adjustment of ecotoxicological test methods and test strategies, standardisation of nano-specific test systems

The results from project 22 are then to be used to examine already validated ecotoxicological test methods and test strategies for their suitability for the assessment of the acute and chronic effects of nanoparticles on the environment.

24. Elaboration of an assessment strategy to determine the risk of nanoparticles in the environment

The information gathered in the above-mentioned projects is initially to be used to elaborate an intelligent test strategy (ITS) in order to determine the concentrations in the environmental compartments as well as the relevant action threshold values. The scale of the studies should be determined by the scale of exposure.

Nanotechnology debate

25. Creation of a joint nano discourse platform

Three areas of action in particular are considered to be relevant for examining the social dimension to nanotechnological research and development:

- Identification and assessment of the positive and negative effects of nanotechnology development on the environment, health and safety
- Development of dialogue offerings as well as initial and continuing training initiatives
- Identification and quantification of the impact of nanotechnology on society, industry, workplaces, education, ethics and the legal system.

The diversity of the projects and research subjects demonstrates the complexity of examining and assessing the risks of a new technology. For occupational safety, consumers and the environment partly similar shortcomings are arising which have led to similar demands (e.g. information on type and scale of exposure). In the fields of occupational safety and consumer protection, ideas of sensible testing are more precise which means that some elements of a test strategy are already available. One major challenge in the near future will be the identification and integration of suitable *in vitro* methods and suitable methods to determine the PC properties in order to predict *in vivo* toxicity. This would justify a renunciation of *in vivo* studies. Up to now, no limit value has been derived from *in vitro* methods as the central database. When it comes to developing a strategically sensible link-up between the projects, it is clear that some projects have priority and are essential for other

projects. For instance, the projects (1, 2, 4, 6), which determine the type and scale of exposure to nanoparticles in a qualitative (or semi-quantitative) manner, are required for identifying substances for priority testing (cf. Chapter 6).

2. Introduction

As an important future technology, nanotechnology provides the opportunity to promote long-term economic development by means of intensive research and the effective transfer of research findings into innovative products. It describes the production, examination and application of structures, molecular materials, inner interfaces with at least one critical dimension below 100 nm. The lower nanometre range adjoins to the size of molecules that has been created for a long time by means of targeted chemical reactions. The upper nanometre range encompasses microtechnology which is also enjoying a dynamic development for instance through computer technology (integrated circuits). Nanotechnology closes a gap and is increasingly attracting the attention of the public. However, currently it is not yet linked to any great degree to concerns about health and the environment. Over the next few years this could change, if the media increasingly will point at harmful ingredients in workplace chemicals and consumer products linked with nanotechnology and their open environmental application (cf. also public debate on genetically modified organisms (GMOs)). It is expected that the importance of nanotechnology will continue to grow and that workers, consumers and the environment will face growing exposure. Therefore, there is the need to observe the development of this new technology and to weigh up the chances and the risks compared to established technologies in a transparent process. Particularly important is co-ordinated and effective research in order to enable the public agencies BAuA, UBA, BfR downstream of BMAS, BMU and BMELV to describe and assess the toxicological and ecotoxicological risks and also to place the resulting recommendations (e.g. classifications, limit values, handling recommendations) on a sound and comprehensive basis.

In 2005, "nanoparticles and ultrafine particles" were already appraised to be the most important "emerging risk" for occupational safety in an EU expert survey. Because of their size nanoparticles have special technological properties that permit their application in the most diverse areas. Here, nanoparticles are understood as engineered granular particles, tubes and fibres with a diameter <100 nm in at least one dimension (including their agglomerates and aggregates) which show low solubility in biological systems. Based on present knowledge, these particles are of particular toxicological relevance. The soluble nanoparticles also show innovative properties. However, they lose their particle character as soon as they are dissolved. Hence, they have different properties to insoluble nanoparticles. In some cases the use of nanoscale organic compounds, like specific liposomes, micelles and vesicles, which amongst other things are used as carriers for other substances in foods, are linked to nanotechnology. The authors of this research concept do not include the use of nanoscale organic carriers in nanotechnology in the narrower sense. In this context solely the question would be relevant whether the bioavailability of the substances transported in these carrier materials is so increased through the use of these carrier materials that acceptable daily intakes are exceeded. Since in particular the poorly soluble or insoluble particles are linked to critical effects and in order to sensibly define the scope of this strategy, we will refer to poorly soluble nanoparticles that are systematically produced (engineered/manufactured nanoparticles) and may be released during production, further processing, use and after consumption (from products). Medical applications (like cytostatics) are also not discussed. Here, the discourse focuses on the area of chemical safety at the workplace, for consumers and the environment.

The current legislative situation on the health protection of workers is limited to the general regulatory framework on national level (ArbeitsschutzG – Occupational Safety Act and GefahrstoffV – Hazardous Substances Ordinance) and on European level (Framework Directive Safety and Health at Work 89/391/EC, Health and safety of workers from the risks related to chemical agents at work 98/24/EC) that assigns fundamental responsibility to the

employers. Particularly in small and medium-sized enterprises shortcomings have been observed when it comes to their implementation regarding the handling of chemicals. There are no special provisions for nanoparticulate substances. As nanoparticles normally have the same CAS number as the according coarser particle fractions, the nanoparticulate fraction has not been specifically assessed in any recent substance-specific regulation (ChemG, AltstoffV). So far, there was no legal substance-specific obligation to carry out studies specifically for nanoparticles. Fullerenes are an exception as they are not an EINECS substance and have their own CAS number and are subject to a more comprehensive testing obligation once they reach a certain production volume threshold. It is not quite clear to what extent nanoparticles will undergo their own assessment under the new chemicals legislation (REACH). It was not until a public agency initiative pointed out that the REACH drafts on granulometry had not yet covered nanoparticles. Also, there has not been any specific regulation for nanoparticles in the areas foods, consumer products and cosmetics. For instance, no particle sizes are stipulated in the purity criteria for the approved food additives silicon dioxide (E 551) and titanium dioxide (E 171). Nanoparticles may be added to products and commodities in order to achieve a biocidal effect (e.g. silver). Biocides are governed by a notification obligation (ChemBiozidMeldeV) which does not, however, record particle size.

As the exposure of humans and environment as well as the toxicological and ecotoxicological properties and risks cannot be assessed yet, there is a general need to conduct further investigations and to close the gaps in knowledge by research and assessment activities. The European Commission, for instance, has published an action plan which envisages amongst other things contributions to research on the health risks (EC 2004, EC 2005). In an overview of European research the European Commission argues that adequate risk assessments must be conducted and risk management must be available before large scale production and application (EC 2006a). Public authorities provide funding for various international and national projects in order to explore the technological opportunities. Until now, the share of funding that was to be used to investigate the risks was less than 5%. Therefore, it is particularly important to spend these funds on co-ordinated and effective research in order to place the resulting recommendations (e.g. classifications, limit values, handling recommendations) on a sound and comprehensive basis. That is why this research strategy identifies and discusses risk-related projects and subjects worthy of funding.

The reports published in the United Kingdom (United Kingdom 2005a, United Kingdom 2005b, IOM 2005, Royal Society and the Royal Academy of Engineering 2004) exemplify a procedure in the fields of risk research, the involvement of the competent public authorities and the general public in a transparent debate. Fair and early communication about the opportunities and risks of nanotechnology will be the decisive factor for how a society deals with this technology. The debate in Germany has been launched with various official public events (BMU-BAuA-UBA-iku 2005, BfR 2006b). The basis for risk communication and social discourse is again sound knowledge about exposure to and the toxicity of nanoparticles including the analytics necessary for the production of knowledge.

However, it should also be borne in mind that nanoscale particles are not completely new. For a long time naturally formed and unintentionally produced particles of this size have reached the environment and led to exposure of humans and the environment. When it comes to the particle problem it is not enough to break them down into inhalable and respirable dust; further distinctions are needed in respect of respirable dust. Within the nanoscale which extends from 1 nm to 100 nm, there are major differences in size which may also have a toxicological and ecotoxicological impact. The engineered nanoparticles should not be regarded as an isolated research area. Given their size, similarities with

unintentionally produced (welding smoke, diesel engine emissions) and naturally formed particles of this size must be taken into account. Furthermore, the data on the larger and better examined fine dust particles are of importance as there are similarities regarding lung toxicity between the two particle fractions. However, the toxicity potency of the nanoscale particles is currently estimated to be higher.

A series of reviews on the harmful effects, exposure and the risks of nanotechnology (in particular insoluble nanoparticles) have been published that summarise the currently available information and define future tasks (Allianz (2005), BIA (2003), Borm *et al.* (2004), Borm and Kreyling (2004), Colvin (2003), IOM (2004), HSE (2004), Hurt *et al.* (2006), Luther (2004), Maynard and Kuempel (2005), Maynard (2006a), Meili (2006), Nanoforum (2005), Nel *et al.* (2006), Oberdörster *et al.* (2005a), Oberdörster *et al.* (2005b), The Royal Society and the Royal Academy of Engineering (2004), Swiss Re (2005), United Kingdom (2005a), United Kingdom (2005b), U. S. EPA (2005)). Various authors have discussed the subject of a research strategy for nanomaterials (Balshaw *et al.* 2005, Borm *et al.* 2006, Holsapple *et al.* 2005, NIOSH 2005, Oberdörster *et al.* 2005b, Powers *et al.* 2006, Thomas *et al.* 2005, Thomas *et al.* 2006, Tsuji *et al.* 2006). Up to now, no concrete strategy, which would facilitate a comprehensive risk assessment and recommendations for measures, has been developed. NIOSH (2005) has structured the various research activities in a general strategy plan. Besides the health risks linked to nanotechnology, the focus of research is on preventing workplace-related diseases through the application of nanotechnology, support for practice and international co-operation. In a research strategy, Maynard (2006b) holds the view amongst other things that public agencies engaged in risk assessment have to get more involved in the steering of research than in the past and that international co-ordination is necessary.

In a review, Oberdörster *et al.* (2005b) examines for instance a screening strategy for nanoparticles. According to this, a detailed and concrete test strategy for assessing the health effects cannot yet be developed. However, major elements can be named which can be assigned to three areas:

- physico-chemical characterisation,
- *in vitro* methods (cellular and cell-free),
- *in vivo* methods.

Besides information on chemical composition an exact description of the physico-chemical properties (including metrology) encompasses the determination of particle size and distribution, solubility, agglomeration state, shape, crystal structure, surface, surface chemistry and charge as well as porosity. As these parameters can influence toxicity, their determination is a major precondition for the interpretation of experimental studies. Cell-free *in vitro* studies provide information on biopersistence in biological media, interaction with proteins, activation of the complement system and induction of oxidative stress. The cellular systems provide information, for instance, on the translocation of the particles, genotoxicity and the biological mechanism of action in cells of the portal of entry and systemic target organs. In *in vivo* studies the toxic reactions at the portal of entry or at the inner organs are observed after single and repeated exposure and information collected on inflammatory and fibrogenetic reactions, oxidative stress and cell proliferation. Furthermore, information is obtained on deposition, intake into the blood circulation, toxicokinetics, toxicodynamics and biopersistence. The histopathological study and studies of BAL[4], oxidative stress and cell proliferation may contribute to elucidating the mechanism. The additional determination of acute phase proteins and coagulation factors provide insights into the effects on the cardiovascular system.

[4] BAL: Bronchoalveolar lavage: lung washing with physiological salt solutions to obtain lung fluid

The EU expert body SCENIHR (2005) stresses that the conventional toxicological and ecotoxicological OECD test methods may not suffice for particle characterisation and that modifications to the test design may be needed. The concrete incorporation into updated test guidelines has not yet been undertaken. To describe the exposure of humans, the environment and laboratory animals in experimental studies, data on particle number, surface, mass concentration, solubility and persistence are required. However, the measurement methods will have to be improved further before they can be used as routine methods. There is a particular need for further research on the distribution of nanoparticles in the human organism and on the mechanism of action on the cellular, sub-cellular and molecular level. The monitoring of workplace exposure and epidemiological studies on the effects of nanoparticles are other important areas of research (SCENIHR 2005). However, it is not yet clear what supplements are needed in the OECD methods used so far for sub-acute, sub-chronic and chronic toxicity which encompass in some cases extensive histopathological studies. This also applies to ecotoxicological test methods. In a following public consultation procedure SCENIHR informed about the range of opinions on the SCENIHR position (SCENIHR 2006b). VCI, for instance, does not agree with the SCENIHR position and believes that the current test guidelines and assessment methods are suitable aside from some supplements (VCI 2005). In a review, SCENIHR has undertaken a more in-depth depiction and added further aspects. For instance, it is stipulated that a careful characterisation of the physico-chemical properties of nanotechnology products is necessary, that the assessment of nanoparticles may not be done solely on the basis of studies on larger particles of the same substance and that new test strategies are required. Previous test methods have already proven to be useful but some methods have to be supplemented or new methods have to be developed. The assessment methods must also be supplemented. For instance, besides mass concentration, also particle concentration and surface have to be taken into account.

Against the backdrop of the various concepts and for the purpose of structuring this area, definitions of terms are needed. In a research strategy the generic term is understood as a comprehensive risk-related research programme which creates the preconditions for the establishment of the test strategy, assessment strategy and an effective and consistent examination of individual substances. In addition, it encompasses projects on communication and other subjects in order to express the overall research needs on safety research in a holistic approach. A test strategy is understood to be a test programme defined in the individual phases which is modified in line with interim results and information on individual substances about their PC properties, *in vitro* and *in vivo* test results. The test strategy is applied to individual substances. In this way an information base is created which normally permits comprehensive risk characterisation and assessment. The assessment strategy (here a synonym for the assessment concept) is understood in this context to be a defined procedure for the interpretation and assessment of the generated substance data leading to a consistent characterisation and assessment of the risks taking into account specific characteristics of substances. The results of assessments of individual substances are for instance limit values for air at the workplace, limit values for substances in foods and cosmetics, classifications, warnings or handling recommendations.

The goal of this research strategy is to structure research activities, to develop particle measurements (metrology), to collect information on toxicological and ecotoxicological effects, to promote the development of a sophisticated risk-related test strategy and assessment strategy, to identify the existing elements of a test strategy, to place substances of special importance into the focus of assessment and, particularly, to guarantee the suitability of data collected with publicly funded research for regulatory questions (e.g. limit values, classifications, handling recommendations). Hence, in safety research there also is a

demand for moving away from pure fundamental research towards a new orientation which facilitates the integration of the results into risk-oriented and comprehensive assessments by taking into account the exposure conditions of humans and the environment as well as the covering of the relevant toxicological and ecotoxicological endpoints. Furthermore, a balance is sought between *in vitro* and *in vivo* methods which will be considerably influenced by the validity of *in vitro* methods.

In Chapters 3 to 6 the research projects and areas are described and discussed for which we see a special need for financing. Current research projects of public agencies are described in Chapter 7.

3. Exposure and Metrology

3.1. General

Voluntary reporting scheme for the production, further processing and open use of nanoparticles

- Project 1 -

The innovation potential and the special technical properties linked to nanotechnology products are stressed by various sides. However, there is no overview of the various products and their composition. Normally it is not possible to tell whether a "nano" product actually contains nanoparticles. A dynamic development is expected over the next years. Against this backdrop and based on the activities in the United Kingdom a voluntary reporting scheme should be agreed with the companies who produce nanoparticles or who produce, process and market nanoparticles in products for open use (see also DEFRA 2006). The goal of the project is to provide, by means of regularly up-dated information, a topical overview of the position and development of this innovative business sector and to be able to undertake an exposure-based setting of priorities. In addition, the dialogue will be promoted with the companies involved.

3.2. Workplace exposure

Modifications of titanium dioxide, zinc oxide, iron oxides, silicon dioxide or carbon black are indicated as substances which may be widely distributed at the workplace also in a nanoparticulate form. Concrete information on the type and scale of exposure or toxicologically relevant modifications (e.g. coating) is not available. Risk management and protective measures on site are not clear either. More exact understanding is necessary in order to be able to determine a graduated need for information concerning health risks for the different nanoparticles depending on the scale and level of current or envisaged exposure. The higher and more far-reaching human exposure is, the more intensively the particles should be examined for their health impact. It is interesting to note that the measurement devices which are used and developed to detect and measure nanoparticles, could in some cases be seen themselves as products of nanotechnology.

At workplaces inhalation and dermal exposure are to the fore. The level of exposure is influenced considerably by the operations and protective measures on site. The production of particles in closed systems, further processing and use in a non-dust form or in a liquid suspension (solid in liquid) which are not sprayed, the incorporation of particles into a solid matrix (solid in solid) can considerably reduce inhalation exposure. Dermal exposure results, amongst other things, from manual activities involving dust-like or suspended particles. If the nanoparticles are bound in a solid substance matrix, skin exposure is low. The example of sunscreens which have a nanoparticulate zinc oxide or titanium dioxide base, shows that contact with skin can indeed correspond to the proper use of nanoparticulate-containing products.

3.2.1. Company survey on workplace exposure and risk management

- Project 2 -

A company survey on workplace exposure and risk management and protective measures is to determine which particles in which modifications lead to exposure at the workplace. The number of exposed persons, the level and duration of exposure are for instance important parameters in order to be able to assign nanoparticles to exposure categories. This

information is essential for the selection of the particles to be examined and for justifying a graduated need for information about health effects. The information on workplace exposure is available to manufacturers, formulators and users of products or can be generated by them. Given the high number of companies a standardised questionnaire campaign in co-operation with VCI, is to be used to collect information on exposure, protective measures and risk management. A part of the project is already running. Since there are plans to continue this, the overall project is mentioned here.

The goal of the project is to identify exposure to nanoparticles in companies, establish exposure categories and, based on the distribution of the particles, to facilitate priority setting when it comes to determining the needs for further research on health effects. On the basis of this information a code of practice can also be developed.

3.2.2. Development, testing and standardisation of measurement methods for nanoparticles at the workplace

- Project 3 -

The quantitative measurement of particles in the nano range is still in the development phase and far from being routine. However, it is very important in order to be able to assess environmental and health risks. No national or international standardisation of measurement techniques are currently available and the measurement strategies for workplace measurements or epidemiological studies have yet to be elaborated. Therefore almost no measurement data with engineered nanoparticles have been published. Besides mass concentration, other parameters like for instance particle number concentration, agglomeration, size and surface are relevant.

The starting points for further research plans should be a review of the measurement technology currently available for determining exposure (NanoCare). Furthermore emphasis should be placed more particularly on the (further) development of portable measurement devices. This is the decisive precondition for carrying out epidemiological studies at a later stage. At the same time, in-company measurements should be stepped up using the available measurement technology in order to obtain initial findings on exposure to nanoparticles and secondly to create the knowledge base for the elaboration of measurement strategies for "routine workplace measurements" and for epidemiological studies. Taking measurements in plants must offer advantages to the companies who produce, process or use nanoparticles as their co-operation is the precondition for the carrying out of the measurements.

The goal of the projects is to further develop and standardise measurement methodology and to make possible routine application with portable devices.

3.3. Consumer exposure

Like at the workplace nanotechnology is also increasingly being used in the production of foods, packaging materials for foods and food supplements and in other consumer products. Consumers may in principle be exposed to nanoparticles via various routes (dermal, oral, inhalation).

Dermal uptake is possible from cosmetics for instance but also from correspondingly treated textiles. Nanomaterials in the formulations of cosmetics may also influence the skin penetration of other ingredients. The use of nanoscale titanium dioxide and nanoscale zinc oxide as UV filters in sunscreens has been common knowledge for a long time. Although it could be recently demonstrated in an *in vitro* study with sunscreens that these two

substances do not penetrate through the skin (Gamer *et al.* 2006), it cannot currently be estimated whether intact skin is a barrier to all nanomaterials. There are already indications that besides size, form and surface coating may also play a role when it comes to the penetration of nanoparticles through the skin. Furthermore, the condition of the skin must also be taken into account as damaged skin is more permeable. For that reason it is felt that there is a need for information and research on the possible applications of other nanoparticles in cosmetics and other consumer goods which come into contact with the skin or mucosa.

Oral exposure may occur through the consumption of nanoparticle-containing foods. However, it may also occur when nanoparticles migrate from packaging material to food. In this context, in addition to the need for information about foods and packaging materials, there is also a need for research into the question of absorption in the gastrointestinal tract, related systemic availability and possible accumulation in specific compartments or organs as well as into the migration behaviour of various nanoparticles from food packaging materials.

The problem of a possible inhalation exposure of consumers draws attention to the occurrence of severe lung disease following use of a "nano" sealing spray. However, nanoparticles were not contained in the sealing spray (BfR 2006a). From the consumer health protection angle knowledge about the occurrence of nanoparticles in household products (products as well as preparations) is of considerable interest. It would therefore be extremely helpful if the use of nanoparticles in consumer products were sufficiently documented.

3.3.1. Company survey of the use of nanotechnology in the production of foods, packaging materials, cosmetics, clothing and other consumer products

- Project 4 -

There are no reliable data about the actual distribution of "nanoproducts". Products are also sold with the claim "nano" without nanotechnology having been used in their production and without them containing nanoscale ingredients. Corresponding information is necessary in order to estimate the scale of existing and expected exposure to nanoparticles and in order to be able to undertake an exposure-based fixing of priorities for the examination of the health effects of particles. A database for consumer products and foods that contain nanoparticles should be set up. The BfR product database can be used to document products of this kind.

When it comes to exposure assessment in the fields of foods, consumer goods and cosmetics, the oral and dermal exposure routes are of particular importance. However inhalation exposure may also take on importance when nanoparticle-containing products (for instance in cosmetics) are used as sprays. When assessing exposure, a distinction must be made between free and bound nanoparticles. In the case of bound nanoparticles as for nanoparticle agglomerates, the question is raised about a possible release of nanoparticles. Special relevance must be attached to free, insoluble, inorganic nanoparticles.

3.3.2. Development of methods to determine and characterise nanoparticles in foods, cosmetics and other consumer products

- Project 5 -

Quantitative and qualitative exposure data are needed to assess the health risks resulting from the use of nanoparticles in foods, cosmetics and other consumer products. Up to now

the necessary measurement methods for the array and characterisation (chemical composition, particle size and distribution, solubility, agglomeration state, design, surface, surface charge) of nanoparticles in these products have not been available. In order to be able to determine the possible exposure of consumers to nanoparticles via this uptake path, studies are needed on products already on the market which were manufactured using nanotechnology.

Methods of this kind are also needed in conjunction with the use of nanotechnology in the production of food packaging in order to examine the portions which migrate to the foods.

3.4. Exposure in the environment

In future, the growing use of synthetic nanoparticles is likely to lead to increased input into the environmental media soil, water and air. Research findings on the behaviour and impact of natural ultrafine dust or ultrafine dust formed during incineration can only be partially applied to the risks of engineered nanoparticles. Further studies are needed for the adequate assessment of potential risks. In the case of "natural" formation the nanoparticles vary considerably in terms of their form, composition and size whereas artificially - "intentionally" - engineered nanoparticles are normally manufactured and designed in a uniform way based on desired properties. The wide application possibilities for nanotechnology and the highly diverse nanoparticles require a differentiated procedure when it comes to assessing a possible threat to the environment.

When assessing the exposure risk for nanoparticles, the decisive factor is the form in which these materials come into contact with man and the environment. The main information is how nanoparticles are released from the materials, how they behave in the environment, how stable and long-lived these forms are, whether they for instance disintegrate or agglomerate, are soluble in water or body fluids, interact with other nanoparticles, chemicals, surfaces or are degraded and how their properties change in these processes.

Given their small size nanoparticles can be widely distributed by air. In soil because of their large, active surfaces nanoparticles can bind and mobilise impurities like heavy metals or organic substances and therefore pose a threat to ground water.

Stable nanoparticles can reach living cells and possibly accumulate there.

So far no findings have been reported about how organic nanomaterials are degraded in the environment. Fullerenes and nanotubes are not naturally occurring carbon modifications and they are stable. There are no indications about whether and how these carbon nanomaterials are degraded, disintegrated, aggregated.

3.4.1. Development of exposure scenarios and life cycle analyses of nanoparticles

- Project 6 -

Findings from projects 2 (Company survey at the workplace) and 4 (Company survey consumers) provide information on which branches produce nanoparticles and which products contain them. In a preliminary study on this and on project 8, additional applications are to be identified in which nanoparticles are directly released into the environment (e.g. treatment of waste water, soils, insect control). This is how to identify the nanoparticles that should be examined first.

The methodology of exposure scenarios and life cycle analyses should be examined with regard to the special requirements of nanoparticles and adapted if necessary. The examination should focus on possible release during production via waste air, waste water

and solid waste as well as during transport and during further processing into an end product. Exposure during use for instance through abrasion, wear and tear or washing should be examined. An important aspect is the behaviour of nanoparticles after their use during disposal, landfilling, incineration or recycling. The goal of the project is to develop exposure scenarios and life cycle analyses. The aim is to identify relevant parameters which could lead to exposure to nanoparticles over the entire life cycle and exposure probabilities in order to be able to take mitigation opportunities into account already at an early stage within the production processes.

3.4.2. Development or adjustment of measurement methods for the areas air, water, and sewage sludge and for ecotoxicological test methods

- Project 7 -

The existing measurement methods and findings from projects 3 (measurement technology at the workplace) and 5 (measurement technology in foods, cosmetics and products) should be re-examined for their suitability for the recording of nanoparticles in the environment. These methods are needed for the risk assessment of exposure. When carrying out ecotoxicological test procedures the actual level of exposure can be determined. As in project 5 (measurement technology in foods, cosmetics and products), besides the quantification, the characterisation (e.g. chemical composition, particle size and distribution, solubility, agglomeration state, design, surface, surface charge) of nanoparticles should also be determined.

3.4.3. Examination of behaviour and fate (accumulation, persistence) in the environment

- Project 8 -

The decisive factor when assessing the exposure risk from nanoparticles is the form in which these materials come into contact with humans and the environment. Therefore, it should be examined how stable and long-lived these forms are, whether and if so under what conditions they for instance disintegrate or agglomerate, are soluble in water or body fluids, interact with other nanoparticles, chemicals and surfaces or are degraded and how their properties change thereby. This should primarily be examined for nanotubes and fullerenes as they do not occur in this form in nature.

A further research area is the use of nanoparticles to remove environmentally harmful impurities from soil and water (e.g. removal of arsenic-containing substances with the help of nanoparticulate iron oxide from drinking water). Hence, those nanoparticles should also be considered which are intentionally released into the environment, for instance to control pests (e.g. silver) or to decontaminate soil or water or to remove inorganic or organic impurities.

Nanoparticles may reach living cells. Therefore, they have the potential to accumulate in organisms and along the food chain. It should, consequently, be examined whether bioaccumulation and biomagnification may occur.

4. Toxicological and Ecotoxicological Assessment of Nanoparticles

4.1. General

4.1.1. Development of methods to determine and characterise nanoparticles in biological material

- Project 9 -

The detection of nanoparticles in biological material is a special challenge. Because of their small size nanoparticles cannot be rendered directly visible with light microscope methods and special methods (e.g. electron microscopes, fluorescence microscopes) are needed in order to identify the particles outside and inside the cell or the cell compartments (e.g. nucleus). There is a need for research in order to standardise imaging methods in a reproducible manner. Furthermore, there is a need to determine distribution in the organism in kinetic studies. The goal of the project is to develop imaging methods and other detection methods for the determination of nanoparticles in biological material. Reliable quantitative methods are needed in order to facilitate detection in the various body compartments.

4.1.2. Development of minimum requirements to be met by information in publications

- Project 10 -

Published studies on the toxicological and ecotoxicological effects of nanomaterials are also frequently and controversially discussed by experts. It is often difficult to assess the results because information is missing or specific aspects were not taken into account in the study design. This impedes the interpretation and comparability of the findings. Therefore work should be done to establish what information is needed to assess the validity and comparability of nanostudies (e.g. stipulation of characterisation of nanomaterials, impurities). These minimum requirements should be co-ordinated around the world. A similar concept has already been adopted in toxicogenomic methods (MIAME = Minimum Information about a Microarray Experiment). The goal of the project is to ensure that a publication of toxicological and ecotoxicological studies contains this information as a quality criterion. This would facilitate the discussion and recognition of study findings.

4.2. Toxicological assessment

The health effects of nanoparticles which are poorly soluble or only soluble to a minor degree in biological systems are often unclear. The central toxicological endpoints are acute and chronic toxicity, irritation, corrosion, sensitisation and the CMR endpoints carcinogenicity, mutagenicity and reproduction toxicity (for a more detailed description please refer, amongst other things, to EC 2006b, EC 2003, EC 2001). For the establishment of limit values chronic toxicity and CMR effects are the most important endpoints. A systemic availability of nanoparticles after inhalation can be assumed to a certain extent at present. Chronic lung toxicity (inflammation, fibrosis) and the formation of tumours through nanoparticles and "microparticles" (fine dust) have already been observed in animal experiments under specific exposure conditions (*inter alia* Mohr *et al.* 2006). Additional toxicological information from *in vivo* and *in vitro* studies is described in the overview articles mentioned in the introduction. Generally speaking further experimental studies are deemed necessary in these reviews in order to be able to sufficiently assess the risks.

The next chapters examine the assessment of the health effects from three angles and identify the corresponding need for research. Firstly the fundamental need is stated to develop a suitable test and assessment strategy or to adapt the existing ones (cf. 4.2.1.).

Then elements of a test strategy for nanoparticles are discussed, which are already available and can be used (cf. 4.2.2.). At the end individual substances are indicated whose previous data situation requires a targeted review and assessment (cf. 4.2.3.).

4.2.1. Development of a test and assessment strategy

- Project 11 -

At the present time there is no consensus on the national or international level concerning appropriate test guidelines and an appropriate test and assessment strategy (definition see introduction) to be used for research into and assessment of the health risks of nanoparticles. Test guidelines are already available for industrial chemicals (EC 2006b), as well as test and assessment strategies (*inter alia* EC 2003, BMA 1998, BUA 2003, IPCS 2005), classification criteria (EC 2001) etc. These are used for the assessment of risks and possible establishment of measures to reduce risks on the national and international level (classification, limit values, handling recommendations, measure concepts etc.). It makes sense to use the existing system for the testing of toxicological properties, assessment and containment of health risks for nanoparticles as well and to modify them where necessary. It would also make sense to supplement the test guidelines with study parameters that reflect specific aspects of nanoparticulate mechanisms of action for instance following inhalation exposure in the lung (BAL studies).

It would be desirable to have cost effective studies that use fewer laboratory animals and supply rapid and reliable results. Ideally *in vitro* methods or PC data for instance on solubility, size or surface coating, should permit reliable conclusions about the effects on the human organism. The extrapolation of the findings to humans is frequently done in the following series with growing uncertainty, the further the measurement system is removed from humans:

PC data[5] → *in vitro* animal/(human) → *in vivo* animal → *in vivo* human

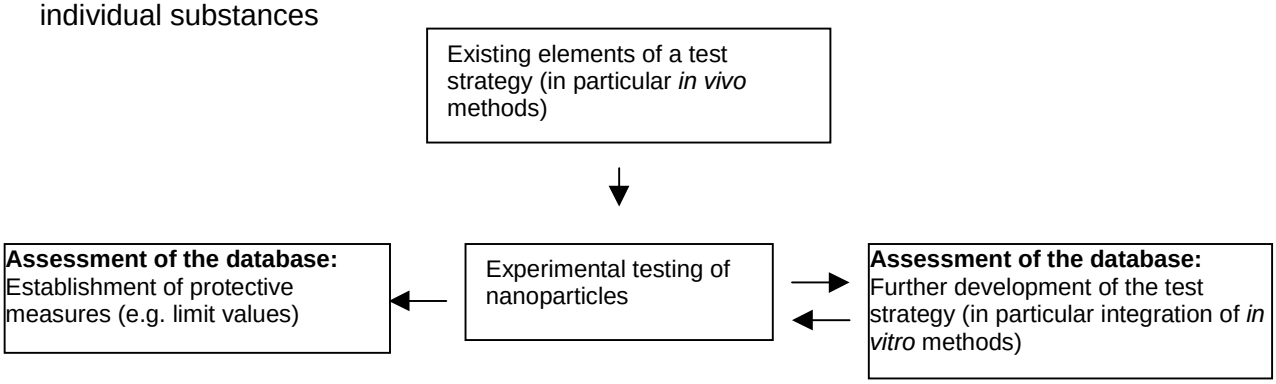
On the other hand simple PC studies and *in vitro* methods are not useful unless they predict effects in humans with sufficient sensitivity and specificity. Furthermore, the predictive value of cell culture studies with (transformed) cells of the target tissue is disputed as a qualitative and quantitative indicator test for chronic effects. If the existing *in vitro* methods are not sufficient, then existing *in vitro* tests must be developed further or new ones designed. However, this only makes sense when it has been demonstrated that the present *in vitro* tests are inadequate. The validation and establishment of *in vitro* test methods is seen as a central task of this research strategy. Up to now no limit value has been established with *in vitro* methods as the central database. Reliable descriptions of the health effects in humans triggered by nanoparticles have not yet been provided. In case of effects with a longer latency period such data only supply clear results on morbidity and mortality when larger groups have already suffered irreversible damage (cf. asbestos). Hence long-term test methods involving laboratory animals take on particular importance in a test strategy. Long-term studies with inhalation exposure and intratracheal instillation, which also encompass special studies on lung toxicity and cardiovascular effects in addition to full histopathology, already constitute a high standard as reference methods against which simpler test methods must be measured. Sub-chronic studies can also provide reliable information (but not on carcinogenicity).

As a test strategy is already available for industrial chemicals and is being further developed under REACH and furthermore elements for a test strategy for nanoparticles are already

[5] Substance identity, solubility, size, coating, agglomeration, lipophily, crystallinity etc.

available (cf. 4.2.2.), the development of a sophisticated test strategy for nanoparticles should not be seen as a new development but as a further development of the existing strategy. Figure 1 shows the dependencies of individual steps which lead up to the assessment of nanoparticles and recommendations on handling them (e.g. limit values). It becomes clear that this is not a linear but an iterative development process. Further experimental data improve the existing database. The assessment of these data also facilitates the further development of the test and assessment strategy, and their application to substances improves the data situation in turn. Furthermore it becomes clear that the reasonable integration of suitable *in vitro* methods is a major challenge when it comes to the further development of the test and assessment strategy.

Figure 1: Further development of a test and assessment strategy and assessment of individual substances



In a risk-oriented test strategy the distribution of a nanoparticle and the type and level of exposure of humans and environment should play a major role. The lower the exposure is, the lower are the requirements. The findings of the projects on exposure (see above) make a central contribution when it comes to selecting the substances for priority examination and the administration route. As the existing test strategy and that envisaged under REACH contains elements of exposure, this paves the way for a comparison of the requirements. A higher need for information on risks of nanotechnology compared with other industrial chemicals is partially substantiated by the fact that nanotechnology is seen as a new technology (emerging technology). New risk scenarios arise for which there are as yet no experience values and where there is special public interest. The new chemicals legislation REACH no longer makes any distinction between existing substances and new substances as was the case in the previous regulations Chemicals Act (ChemG) and Existing Substances Regulation (AltstoffV). Up to now more studies were needed for new substances with the same tonnage than for existing substances. Here it becomes clear that the attitude towards a dynamically changing technological world also constitutes an adjustable variable which influences the need for information about health and environmental risks. In order to be able to compare in a transparent manner the risks of substances from "existing" and "new" technologies, the test and assessment strategies should have common basic structures but reflecting also the specificities of nanoparticles. Then it becomes possible to undertake a comparative assessment also within the context of discussions about substance substitution. Hence the existing test and assessment strategies (EC 2003, BMA 1998, BUA 2003, IPCS 2005) should be considered as the foundation and, where necessary, adapted to the specific situation of nanoparticles.

The following projects are considered to be important components for the development of a sophisticated test and assessment strategy.

4.2.1.1. Determination of the sensitivity and specificity of *in vitro* methods or methods to identify the PC properties for the assessment of chronic toxicity and carcinogenicity of dust

- Project 12 -

Because of the high costs, lengthy duration and for reasons of animal welfare, there is seen to be a need to use *in vitro* methods as a supplement to *in vivo* methods. For that reason reliable *in vitro* methods are important (low-cost high throughput *in vitro* assays (Luther 2004, p. 74)), in order to be able to estimate the effects of nanoparticles on humans with sufficient certainty. Besides *in vitro* studies, studies on PC properties could provide results which permit conclusions about effects in humans. However, there has been no comprehensive description of which *in vitro* method (including PC methods) constitutes a statistically sensitive and specific indicator for which long-term effects. The published literature on nanoparticles, fine dust particles and fibres must be systematically evaluated in order to review the qualitative and quantitative reliability of *in vitro* methods on the basis of statistical parameters (sensitivity and specificity). Commonalities with natural particles of this size and particles that were generated unintentionally (e.g. combustion products) should be taken into account. Furthermore, the data on the larger and better examined fine dust particles are of relevance since there are similarities between the two particle fractions concerning lung toxicity. The extent to which the data on fine dust and fibres in the micrometer range can be transferred to nanoparticles (including nanofibres etc.) results from the analysis of the data and plausibility considerations of the mechanism of toxicity. Commonalities were observed between nanoparticles and fine dust with respect to lung toxicity. The potency of the nanoscale particles is, however, considered to be higher.

The goal of the project is to use *in vitro* methods in an optimum, statistically validated manner for assessment, to promote methods involving no laboratory animals and to make a contribution to concretising the test strategy. The validity of the various *in vitro* studies should be confirmed before further *in vitro* studies are conducted.

4.2.1.2. Classification of nanoparticles in categories of different toxicity by means of *in vitro* studies and studies on PC properties

- Project 13 -

In regulatory toxicology substances are jointly subjected to specific regulations (classification, limit values) if it is plausible that they have a similar toxicity. These groups are established particularly when a representative substance has been sufficiently examined and toxicologically similar ones have only been inadequately examined. For instance for coarser particles (fine dust, granular particles with no specific toxicity) various substances were classed together within the framework of setting limit values as it can be assumed that the various particles of the same size have similar toxicity. The basis for the decision to set up groups is toxicological similarity which should be rendered plausible through valid *in vivo* studies or *in vitro* studies.

At the present time it is not clear whether a classification according to physico-chemical criteria or results from *in vitro* studies makes biological sense. What would be conceivable would be a classification according to PC properties like size, tendency towards agglomeration, hydrophily/lipophily, solubility etc.

Furthermore, a classification in groups through suitable *in vitro* studies would be desirable in order to examine the various aspects of the mode of action. The Comet assay and the measurement of 8-hydroxy-deoxyguanosine are mentioned for the determination of a

genotoxic activity (Oberdörster *et al.* 2005b, Greim *et al.* 2001, Schins 2002a, Schins *et al.* 2002b). The oxidative stress that is described as a prevailing hypothesis concerning the mechanism for damage to and activation of cells (Oberdörster *et al.* 2005b) can be proven in the opinion of various authors by measuring dichlorofluorescein, oxidised gluthathione and determination of nitrosated proteins (Hess *et al.* 2005, Janssen *et al.* 1993, Quinlan *et al.* 1995). The reactivity of alveolar macrophages can be examined using the "vector model“ that supplies information on metabolism, secretion of inflammation mediators and reactive oxygen species (Luther, 2004, p. 74). A larger number of physico-chemically exactly characterised nanoparticles should be examined with validated screening tests which look at various endpoints of toxicity in order to be able to assess the influence of physico-chemical properties on toxicological properties. If the validity of screening methods is poorly confirmed, then only the results of *in vivo* studies remain as the toxicological reference parameter. The selection of the nanoparticles to be examined should depend on exposure and distribution of the nanoparticles (cf. projects 1, 2, 4 and 6). The better examined and more widespread substances like silicon dioxide, zinc oxide, iron oxides, carbon black and titanium dioxide should be used as benchmark particle control. Before further *in vitro* studies are conducted, the statistical validity of the respective *in vitro* study for predicting effects in man should be known. Before new *in vitro* tests are developed, evidence should be provided that the previous *in vitro* tests are not suitable for prediction.

The goal of the project is to establish substance classes for nanoparticles so that not all nanoparticles have to be extensively examined but joint assessments are possible on the basis of their similarity which require fewer animal experiments.

4.2.1.3. *In vivo* studies of the chronic toxicity and CMR effects of widespread nanoparticles

- Project 14 -

The suitability of *in vitro* methods depends on whether the results can sufficiently reliably predict the results of appropriate animal studies and finally possible effects in humans. In order to be able to assess this, suitable *in vivo* studies must also be conducted as reference studies for selected nanoparticles if they are not already available. Studies on larger particles (e.g. microscale particles) may also possibly be taken into account if it is plausible that the results can be transferred to the nanoscale fraction. The choice of particles for priority examination should be oriented towards distribution and level of exposure. Long-term animal experiments, as the reference parameter for the assessment of effects in man, take on particular importance as they are the precondition for the assessment of effects after chronic exposure. As nanoparticles have been detected in internal organs following inhalation administration it must first be clarified whether there is a need to examine systemic target organs. Kinetics studies considering the exposure route may provide insight here. OECD long-term test methods, which also encompass specific studies for instance on lung toxicity and cardiovascular effects in addition to complete histopathology, are a good foundation for examining chronic toxicity. Sub-chronic (and perhaps also sub-acute) studies may also supply reliable information (but not on carcinogenicity). From the angle of occupational safety the particles should preferably be examined by inhalation as dermal absorption is comparatively low. Intoxications after inhalation exposure to a "nano" sealing spray, which didn’t in fact contain any nanoparticles, draws attention at least to the importance of the inhalation route (BfR 2006a). From the angle of consumer and environmental protection dermal and oral studies are also of interest.

Besides clarifying chronic toxicity, the endpoints mutagenicity, carcinogenicity and reproduction toxicity are of particular importance. Information on mutagenic potential can be obtained *in vitro*. If corresponding indications are available, *ex vivo* or *in vivo* studies are necessary for the further clarification of mutagenicity, especially in case of systemic availability. Positive mutagenicity data can provide indications about a possible carcinogenic potential. A chronic study would be necessary to clarify suspicions. Studies of this kind are already available on a few nanoparticles; a carcinogenic potential was detected under specific test conditions following exposure of the respiratory tract in the rat. In the case of systemically available nanoparticles reproduction toxicity also constitutes an important endpoint. The examinations of gonads in studies on repeated exposure can supply initial results. A definitive assessment is only possible on the basis of teratogenicity studies and generation studies. Examinations of chronic toxicity and mutagenicity should be produced prior to those on reproduction toxicity.

The goal of *in vivo* studies is to create reference studies against which the suitability of *in vitro* studies can be measured. In addition, this will also create a solid database for possible protective measures regarding representative, widespread nanoparticles (e.g. classification, limit values). Nanoparticles with lower exposure can possibly also be assessed on the basis of the substances tested *in vivo*, if toxicological similarity is made plausible with sufficient certainty.

4.2.1.4. Studies on the mechanism of toxicity and the influence of particle size

- Project 15 -

So far the mechanism of toxicity of nanoparticles has not been elucidated. For instance the hypothesis was advanced for the lung as the target organ after inhalation exposure that lung tumours caused by particles are the consequence of a chronical inflammatory reaction and secondary genotoxicity. For some dusts it was observed that potency is influenced by the size of the particles. Studies show that nanoparticles have a higher potency related to mass than particles from the same substance in the micrometer range (fine dust). It is under discussion that there is a lower qualitative but relative quantitative difference between the toxicity of nanoscale and microscale particles. Furthermore, a theory does claim that the larger surface of the smaller particles is the main parameter for higher potency. The influence of size on lung cell toxicity should be examined by measuring the parameters of inflammation, genotoxicity and immunotoxicity. Furthermore, the influence of agglomeration on the mobility and toxicity of nanoparticles should be examined.

The goal of the project is to explain the mechanism of toxicity and to provide a better description of the quantitative toxicity difference between nanoscale and microscale particles with the same composition. Information of this kind can contribute to the development of the test and assessment strategy but also to individual substance assessment.

4.2.2. Existing elements of a test strategy

As already mentioned the development of a sophisticated test strategy is seen as an iterative process. Individual elements of a test strategy are already available as a consequence of previous experience with industrial chemicals and can be used. The test results of these studies and further accompanying research (see above) contribute to the development of a sophisticated test strategy. In the previous chapters it already became clear that *in vivo* test methods are available which can be supplemented through reasonable modifications and can provide important information on the toxicity of nanoparticles. In particular the studies on

sub-chronic and chronic toxicity are of importance and may be considered as existing elements of a test strategy. If systematic availability is ruled out in preliminary studies on kinetics, then there is no need to examine all internal organs. Further assessment will then concentrate on the effects at the portal of entry. Similarly, studies on reproduction toxicity are not necessary for substances which show no systemic availability. At the present time *in vitro* methods can only be considered in individual cases (e.g. tests for skin permeation, see below) as validated elements of a test strategy.

When choosing the exposure route for *in vivo* studies and selecting the cell system for *in vitro* methods, as already mentioned, the exposure route in humans should be taken into account (see above). Different study requirements may therefore emerge depending on the exposure cohort. This is discussed below.

4.2.2.1. Studies on the toxicity of nanoparticles at the workplace

- Project 16 -

Exposure at the workplace is mainly via skin and inhalation. The current database indicates that the skin is largely impermeable for nanoparticles if it maintains its barrier function and is free from damage or stronger mechanical stress. In the case of skin lesions, stronger mechanical stress and small nanoparticles (< 5 – 10 nm) the protective function could, however, be impaired. Systemic availability after inhalation revealed to be far better than after dermal contact. In addition, harmful effects have already been proven in the lung (similarly to fine dust in the micrometer range). Thus the inhalation route has priority when selecting the administration route in animal experiments studies from the angle of occupational safety. As repeated daily exposure can be assumed, studies are needed which are also suitable for assessing chronic toxicity. Against the backdrop of the currently uncertain reliability of *in vitro* methods (see 4.2.1.1), sub-chronic and chronic *in vivo* studies take on special importance at present. Should it be proven that there is no systemic availability, a detailed histopathological study could be restricted to the respiratory tract.

4.2.2.2. Studies on the skin penetration of nanoparticles from cosmetics and consumer products

- Project 17 -

For the health assessment of dermal exposure of consumers to nanoscale particles used in cosmetics and in other consumer products, it is necessary to determine the scale of systemic availability of nanoparticles after dermal exposure. Skin penetration could initially be examined using suitable *ex vivo in vitro* skin models and could then be checked *in vivo*. *In vivo* studies are needed to examine systemic availability and toxicity. In this context the influence of modifications (e.g. to the coating) on systemic availability and on the toxicological properties of nanoparticles should also be examined. In this way further animal experiments could also be avoided.

4.2.2.3. Studies on absorption, systemic availability, accumulation and excretion of nanoparticles after oral exposure (foods and food packaging materials)

- Project 18 -

For the health assessment of the oral exposure of consumers to nanoscale particles used in the production of foods and food packaging materials, it is necessary to determine the scale of absorption, systemic availability, accumulation and excretion of nanoparticles after oral

exposure. The necessary *in vivo* studies should in any case be integrated into the necessary toxicological studies in order to avoid unnecessary animal experiments. In this context the influence of modifications (e.g. to the coating) on the kinetic parameters and on the toxicological properties of the nanoparticles should also be examined.

4.2.3. Individual substances requiring testing and assessment

4.2.3.1. Assessment of the toxicity of nanoparticulate zinc oxide

- Project 19 -

It is considered necessary to assess and/or improve the database on the already widespread zinc oxide which is used, amongst other things, in sunscreens. In the report of the Royal Society and the Royal Academy of Engineering (2004, p. 44) attention is drawn to the phototoxic effect of nanoparticulate zinc oxide in mammalian cells *in vitro* (DNA-effects) and to the further need for tests and information (cf. also Nanoforum 2005, section 7.2.1; SCCNFP, 2003). Furthermore a genotoxic effect is under discussion (Dufour *et al.* 2006). In the opinion of the BfR interim Committee for Cosmetics, no risks can be derived from dermal use in sunscreens because of low skin permeation (BfR, 2006c). Therefore, the need for assessment and testing (in particular genotoxicity) focuses on inhalation exposure as can be encountered at the workplace. Inhalation exposure to nanoscale zinc oxide not only results from engineered nanoparticles but also from nanoparticulate welding smoke. However these particles are frequently fused into larger aggregates.

The goal of the project is to examine the suspicions linked to a widespread nanoscale substance and to facilitate transparent assessment on the basis of experimental studies.

4.2.3.2. Assessment of the toxicity of nanoparticulate silicon dioxide

- Project 20 -

There are indications that silicon dioxide nanoparticles can disrupt functions of the nucleus (Chen and von Mikecz 2005). This study showed that fluorescence marked silicon dioxide particles (diameter 40 – 5000 nm) can penetrate human epithelial cells in cell cultures. Only nanoparticles (40 – 70 nm) but no larger particles (> 200 nm) were found in the nucleus. They led to protein accumulation in the nuclei. Cell functions like DNA replication and transcription were impaired. In a conventional cytotoxicity test (Trypan blue exclusion assay) no clear effect was identifiable. The results of this study are indications of a hazard potential. Silicon dioxide is used as a food supplement. It is unclear whether comparable effects occur *in vivo* and whether such effects could then also be expected from other particles. The published data would have to be examined *in vivo* to clarify the situation.

4.3. Ecotoxicology

There are only very few studies and overviews on the effects of nanoparticles on the environment (Krug 2005). The results of these studies trigger a considerable need for discussion by experts of the assessment and validity of studies of this kind.

So far, only a few organisms in aquatic ecosystems have been examined. For instance, depending on the type of application, C60 molecules (buckminster fullerenes) and nanoscale titanium dioxide are lethal to water fleas already at relatively low concentrations in water (Lovern *et al.* 2006). Experiments with young largemouth bass show that C60 nanoparticles

are taken up via the gills and penetrate the blood brain barrier. The brain is already damaged at low concentrations of C60 molecules (Oberdörster E. 2004). In zebra fish embryos carbon nanotubes delay hatching (Cheng & Cheng 2005). The bactericidal effect of some nanomaterials like silver could also trigger negative effects in sewage treatment plants by leading to a change in the microbial composition of water.

For terrestrial ecosystems, studies on the effects of nanoparticles are also scarce. In mammals, the results of laboratory studies for modelling the effects on human health may also be applied to wild animals. However, there are no studies up to now on non-mammals or invertebrates. Experiments with aluminium nanoparticles showed reduced root growth in various crops (maize, cucumber, soy, carrot); this effect did not occur with larger particles (Yang & Watts, 2005). The microbial composition of soil can be impaired through a biocidal effect of nanoparticles.

Similar to the considerations for toxicology, existing test systems have to be examined for their validity and transferability to other organisms.

4.3.1. Grouping of nanoparticles with regard to their ecotoxicological effects

- Project 21 -

In project 13 nanoparticles with similar toxicity are to be compiled into groups in order to examine a representative substance of this group. Nanoparticles are to be compiled into classes with similar effects and suitable reference sizes (for instance mass, particle number, surface) are to be determined. In this way, it will be possible to achieve an efficient evaluation and the comparability of findings.

The goal of this project is to examine the grouping according to human toxicological aspects for their transferability to environmental organisms and, where appropriate, the adaptation of this group to the specific ecotoxicological requirements. This could help to reduce the number of animal experiments needed.

4.3.2. Analysis of studies conducted with regard to relevant endpoints, elaboration of action hypotheses, identification of suitable test systems

- Project 22 -

Based on a literature review, the studies previously conducted on effects in the environment and on human are to be analysed and assessed regarding their relevance for ecotoxicology. The goal is to determine whether the study design is suitable for making statements on acute and chronic effects on environmental organisms. Here, based on the literature review, relevant endpoints are to be identified and action hypotheses are to be proposed.

4.3.3. Examination and adaptation of ecotoxicological test methods and test strategies, standardisation of nano-specific test systems

- Project 23 -

Using the results from project 22, already validated ecotoxicological test methods and test strategies are to be examined for their suitability for assessing the acute and toxic effects of nanoparticles. Here, it should be examined whether the existing standardised test methods suffice in their existing form or with an adaptation to the specific properties of nanoparticles, or whether new endpoints need to be taken into account for assessment or test methods have to be developed and standardised.

4.3.4. Elaboration of an assessment strategy to determine the risk from nanoparticles in the environment

- Project 24 -

The risk of environmental chemicals is generally determined by comparing exposure with effects (PEC/PNEC). With the information gathered in the above-mentioned projects, an intelligent test strategy (ITS) is initially to be elaborated in order to determine the concentrations in the environmental compartments as well as relevant action threshold values. For animal welfare and cost reasons, a suitable test strategy for acute and chronic tests should be developed in order to determine the risk using suitable (Q)SAR estimates, substance group considerations, read-across and *in vitro* tests. The scale of the tests should reflect the scale of exposure.

5. Discourse: Nanotechnology

The three federal agencies believe there is a need for research and action in the discursive accompaniment to the further development of nanotechnology. Besides innovation research and safety research, accompanying social scientific research including dialogue processes should be the third column of nanotechnology promotion. However, dialogue cannot be restricted to the communication of risks but must also incorporate benefit aspects of nanotechnology into the public debate.

Already today there is a series of discourse activities. Mention should be made more particularly of the dialogue on the assessment of synthetic nanoparticles at the workplace and in the environment which was initiated by the Federal Environmental Ministry, the Federal Environmental Agency and the Federal Institute for Occupational Safety and Health (BMU-BAuA-UBA-iku 2005). This dialogue is currently being continued with other activities. BfR has launched various projects in this area. Within the framework of its risk communication activities an expert Delphi survey and a consumer conference on the risks of nanotechnology in the fields food, cosmetics and consumer goods are to be staged this year.

The social dimension to the development of nanotechnology, however, calls for comprehensive concepts of accompanying social scientific research and diverse dialogue offerings which bring together scientists, politicians, stakeholders from industry, NGOs and consumers. Therefore a proposal has been made to set up a joint nano discourse platform involving all competent bodies. The aim of the nano discourse platform is to organise processes and provide fora in which nano stakeholders from research and production can come together in order to discuss the health and environmental risks of nanotechnology. Furthermore, within this framework participation and dialogue methods can be developed and tested which smooth the way for the early involvement of various stakeholders in the nanotechnology debate. In order to examine the factors that influence the perception of nanotechnology and to dare forecasts about the direction that public opinion will take, it is also necessary to undertake regular representative surveys and media analyses.

5.1. Creation of a joint nano discourse platform

- Project 25 -

Consideration of social issues should be done within the framework of a joint nano discourse platform. Three areas of action in particular are deemed to be relevant for the processing of the social dimension to nanotechnological research and development:

- Recording and assessment of the positive and negative effects of nanotechnology development on the environment, health and safety
- Development of dialogue offerings as well as initial and continuing training initiatives
- Identification and quantification of the effects of nanotechnology on society, industry, the workplace, education, ethics and the legal system

6. Comparison and Linkage of the Various Projects

The various projects and research subjects highlight the complex nature of the examination and assessment of the risks of a new technology. In some cases there are similar deficits regarding occupational safety, consumers and the environment which have led to similar demands. The relevant nanoparticles (and their modifications) and the exposure conditions are relatively unclear. The quality requirements to be met by publications must also be defined. A test and assessment strategy must be developed for the various protection goals. In the field of occupational safety and consumer protection the ideas on appropriate tests are more concrete which means that some elements of a test strategy are already available. Furthermore, problematic substances have been identified for occupational safety and the consumer area. The *in vivo* studies with repeated exposure currently constitute a major component in the assessment of the risks and the establishment of limit values. A key challenge in the near future will be to identify and integrate suitable *in vitro* methods and methods for the determination of PC properties in order to justify a renunciation of *in vivo* studies. In addition, low tonnage or restricted distribution and exposure can lead to a reduced need for information. A tiered need for information depending on exposure and properties of the substance is favoured.

When it comes to developing a reasonable strategic linkage of the projects it becomes clear that certain projects have priority and are the preconditions for other projects. The projects (1, 2, 4, 6) that determine the type and scale of exposure to nanoparticles in a qualitative (or semi-quantitative) are one precondition for identifying the substances which are to be tested first. More precise quantitative information facilitating fine tuning of the procedure for selecting substances can be obtained with a developed measurement technology (3, 5, 7) but will not be available in the short-term in a sophisticated form. Decisions about test requirements can already be taken on the basis of qualitative or semi-quantitative information (see above). The assessment of the existing *in vitro* methods, including methods to determine PC properties, is a central project (12) which decides on the suitability and further development of *in vitro* methods. As the *in vivo* reference studies are needed to assess the *in vitro* methods, they are also required in addition to single substance assessment for the development of the test and assessment strategy and should at least be available in the medium-term (14). As a sophisticated test and assessment strategy can only be achieved in the medium or long-term, the existing elements of a test strategy (16, 17, 18) take on special importance if a rapid clarification of effects is needed. What is also important is elucidating systemic availability in a quantitative manner in order to be able to decide about the need to examine systemic target organs. The same applies to studies on reproduction toxicity which are not necessary where there is no systemic availability. The projects on ecotoxicology, which lead to the development of the test and assessment strategy (22, 23, 24), build on one another and are to be conducted in ascending order. The nano discourse platform (25) is designed as accompanying social scientific research and therefore extends over the entire phase of processing of the various projects.

7. Ongoing Research Projects

BAuA:

1: Exposure to ultrafine particles (UFP) at the workplace (F 2055)

In the project a measurement and analytical method for nanoparticles is to be tested as the basis for the conduct of later occupational medical epidemiological studies at relevant workplaces. The method is based on measurements taken with a portable thermal precipitator, scanning electron microscopic analysis and image processing software. Within the project shift-related and personal measurements of UFP will be taken in various industrial sectors within relevant test person cohorts in order to obtain initial statements about the number concentration of UFPs and their size classification.

www.baua.de/de/Forschung/Forschungsprojekte/f2055.html

2: Characterisation of ultrafine dust for the workplace - Part 2 (F 2075)

A mobility spectrometer is currently used as the routine measurement device to determine ultrafine particles (UFP) in the environment as well as in work areas. This device provides information on number concentrations of agglomerates – without any further characterisation of primary particles. This means that because of their size and methodological shortcomings these devices are only suited to a limited degree for the characterisation of UFPs in respect of occupational medical questions. Far more information is needed in the opinion of experts on the assessment of UFP including:

- statements on the morphology of UFPs;
- information on agglomerate formation;
- relationship between agglomerate and primary particle concentration;
- number and surface concentrations of these primary particles and
- statements on the solubility of UFPs.

For that reason the portable sampling devices for UFPs, which are based on an electron microscopic evaluation and which can therefore provide initial answers to the above questions, have to be modified, adapted and validated. By means of the measurement method developed in Part 1 of the project and validated in Part 2, ultrafine dust can be characterised in terms of its mass concentrations, total primary particle number including diameter distribution as well as the number of its aggregates and its size distribution. It is to be expected that these primary particle concentrations (amongst other things per cubic metre air and also per mg inhalable dust) as well as the information on size distribution of primary particles are characteristic for specific situations of the formation of ultrafine aerosols and can therefore be generalised. Besides routine measurements a supplementary toxicity-oriented characterisation of UFPs is available which is an important component for a possible occupational medical assessment of UFPs.

http://www.baua.de/de/Forschung/Forschungsprojekte/f2075.html

3: Studies on the carcinogenicity of granular dust in rats

- Results and interpretations –

Experiments with intratracheal instillation in rats showed lung toxicity after higher exposure to nanoparticles and particles in the µm range and the formation of lung tumours. In the current project the data obtained are to be extensively interpreted and evaluated taking into account

current literature and furthermore processed for discussion in regulatory agencies (limit values, classifications). More particularly the histological diagnoses of previous projects are to be taken into account and new result tables are to be drawn up with information on the statistical parameters for the individual test groups. An analysis of the dose-risk relationships and the establishment of a dust property is necessary which seems most plausible for carcinogenic potential.

UBA:

1: Statutory framework conditions

The Federal Environmental Agency has commissioned an expert opinion, the goal of which is to analyse the current legal framework and to elaborate proposals for possible regulatory measures. The results are to be presented in the autumn of 2006.

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9. Abbreviations

AltstoffV	Existing Substances Ordinance
ArbeitsschutzG	Occupational Safety Act
BAL	Bronchoalveolar lavage
BAuA	Federal Institute for Occupational Safety and Health
BfR	Federal Institute for Risk Assessment
BMAS	Federal Ministry of Labour and Social Affairs
BMELV	Federal Ministry of Food, Agriculture and Consumer Protection
BMU	Federal Ministry for the Environment, Nature Conservation and Nuclear Safety
ChemBiozidMeldeV	Biocide Notification Ordinance
ChemG	Chemicals Act
CMR	Carcinogenicity, mutagenicity and reproduction toxicity
EC	European Commission
GefahrstoffV	Dangerous Substances Ordinance
GMO	Genetically modified organisms
HSE	Health and safety executive
HPV	High production volume
IOM	Institute of Occupational Medicine (UK)
IPCS	International programme on chemical safety
ITS	Intelligent Test Strategy
MIAME	Minimum information about a microarray experiment
NGO	Non-governmental organisation
NIOSH	National Institute for Occupational Safety and Health
OECD	Organisation for Economic Co-operation and Development
PEC/PNEC	predicted environmental concentration versus predicted no-effect concentration
PC	Physico-chemical
(Q)SAR	(Quantitative) structure activity relationship
REACH	Registration, Evaluation and Authorisation of Chemicals
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
UBA	Federal Environment Agency
UFP	Ultrafine particles
U. S. EPA	Environmental Protection Agency (USA)
VCI	German Association of the Chemical Industry