Conclusions and recommendations from the project „Health assessment, exposure and environmental effects of nanomaterials: literature review and assessment"

Current scientific reviews[1, 2, 3, 4] assess the available literature of recent years[5] on safety research into nanomaterials. These reviews address central scientific publications[6] on the safety of (engineered) nanomaterials (NM)[7] and environmental and health effects incl. specificities of action, toxicological methods, genotoxicity (in vivo/in vitro) and exposure and environmental effects and eco-toxicology of NM and ENM (mechanisms of the fate of particles in the environment, taking into account potential release paths).

In this paper the German Chemical Industry Association (VCI) derives the conclusions and recommendations from these reviews:

[1] Harald Krug: “Nanosafety Research — Are We on the Right Track?; Angewandte Chemie Intern. Ed., Special Issue: Nanotechnology & Nanomaterials, Nanotoxicology & Nanomedicine, Vol. 53, Issue 46, pp 12304–12319, Nov. 10, 2014, http://dx.doi.org/10.1002/anie.201403367

[2] Stephan Wagner, Andreas Gondikas, Elisabeth Neubauer, Thilo Hofmann und Frank von der Kammer: „Spot the Difference: Engineered and Natural Nanoparticles in the Environment – Release, Behavior, and Fate“; Angew. Chemie Intern. Ed., Vol. 53, Issue 46, pp 12398–12419, November 10, 2014, http://dx.doi.org/10.1002/anie.201405050

[3] Lars Michael Skjolding, Sara Nørgaard Sørensen, Nanna Bloch Hartmann, Rune Hjorth, Steffen Foss Hansen, Anders Baun, A Critical Review of Aquatic Ecotoxicity Testing of Nanoparticles – The Quest for Disclosing Nanoparticle Effects, Angewandte Chemie Intern. Ed., accepted for publication June 2016, http://dx.doi.org/10.1002/ange.201604964

[4] Ken Donaldson and Craig A Poland: Nanotoxicity: challenging the myth of nano-specific toxicity, Current Opinion in Biotechnology, Volume 24, Issue 4, Pages 724-734, August 2013

[5] Regarding toxicology as from the year 2000; regarding exposure and environmental effects as from the year 2010 or author-specific.

[6] Nanomaterials (NM) are understood to mean substances according to the EU’s definition recommendation (Commission Recommendation 2011/696/EU, October 2011).

[7] ENM (Engineered Nanomaterial): internationally, this term is used inter alia by OECD for industrially manufactured nanomaterials with specific properties.

Results from the literature reviews

Regarding both human toxicity and potential environmental effects of ENM, it is noted that no statements can be made on ENM per se but that for nanoscale substances a risk assessment on a case-by-case basis is needed for – like for all other substances. The famous theorem by Paracelsus applies today as in the past: *"All things are poison and nothing is without poison; only the dose makes that a thing is no poison."* Consequently, all safety research will also stretch into fields where effects have been proven.

- **Uptake into the body (in vivo):**
  In direct contact with epithelial cells, ENM can enter the organism in principle. But the volumes that reach the organs, e.g. the blood stream or secondary organs, are very small.
  - ENM can pass the lung and the gastrointestinal tract. However, only a very small fraction of the applied dose reaches the bloodstream and is distributed in the body to secondary organs. The vast majority of the applied ENMs is cleared from the lung by macrophages and/or excreted through the feces.
  - ENM are not absorbed via skin.
- **Uptake into cells (in vitro):**
  ENM can basically be taken up by all cells. Out of 6.600 analyzed studies 1.300 studies examined cellular uptake in vitro, describing only a handful of exceptions.
- **„Nanotoxicity":**
  No „nano paradigm" for human toxicological effects can be derived based on the current state of scientific studies.
  - For primary organs, like the lung, there are indications of an effect, e.g. inflammatory processes which, however, depends on the chemical composition of the material or represent unspecific particle effects largely independent from particle size. So far, no systemic effects of the ENM taken up by the organism have been observed in secondary organs in the body and in their cells.
  - The majority of recent toxicological studies of ENM and NM cannot be used to derive risk assessment statements, for a variety of reasons:
    - In many cases, the design of the toxicological studies does not meet the required scientific quality standards which, consequently, leads to misinterpretations: In very many cases, the examined material is not or only poorly characterized; quite often, the work is performed in unrealistic concentration ranges.
    - Many of the test results lack an adequate interpretation: they were not interpretated in accordance with the applied toxicological methods and the underlying toxicological models.[8]
    - In many scientific publications, the test batteries used for gathering toxicological data and the applied measurement and evaluation methods did

[8] On this point, cp. with the guidelines of OECD and for GLP (Good Laboratory Practice).

not undergo quality testing – e.g. in round robin tests with certified laboratories. Toxicological data gathered in this way do not provide a basis for comparisons of data and an adequate risk discussion.

- Many results of these examinations, which are wrongly classified as "toxicological studies", are based on biological-mechanistic experiments (modes of action/models, uptake kinetics, effects on biological endpoints) which do not enable any statements on the real toxicity of a given substance in the meaning of a risk characterization.

- Shape and solubility are two of those material parameters of NM and ENM which have been identified as important for possibly triggering toxicologically relevant effects.
  - As certain NM and ENM can dissolve in the organism or in the cells, after their uptake as particles a purely substance-related toxicity is observed; its effect is based on the toxicity of e.g. ions or molecules. This does not represent toxicity of particles and, consequently, in such cases effects are substantially independent of particle size.
  - The overwhelming majority of studies on pulmonary toxicity can be summed up in the statement that most inert NM and ENM have non-specific effects; i.e. potential toxicity can be attributed to generic particle effects (fine dust).
  - Like in any other risk assessment of chemical substances, High Aspect Ratio Nanomaterials (HARN) and other fibrous substances[9] need to be studied with increased attention, depending on their physicochemical properties.
- So far „no-effect studies", i.e. results from studies where no toxicological effects were observed, have been published only to a small extent and mostly without being titled as such. For this reason, the data published in scientific literature do not reflect the whole picture as the impression may arise that every nanomaterial shows an effect.

- **Genotoxicity:**
  At present, robust indication of genotoxicity does only exist for a small number of ENM – e.g. for carbon nanotubes (CNT) or quantum dots[10]. These effects can be attributed to the shape of the material (CNT) or the chemical toxicity (quantum dots) but not to their size. Results/observations that have been interpretated as genotoxicity effects for other materials can often be explained by:
  - Quite frequently, genotoxic effects are studied in acute toxic concentration ranges; but no statements on genotoxic effects can be derived from in vivo and in vitro studies that work in such extremely high concentration ranges.
  - In vitro studies only enable a derivation of statements on genotoxicity if they do not analyze genotoxicity in the cytotoxic concentration range. This can be

[9] Fibrous substances with a length-to-width ratio larger than 3:1 and an overall length larger than 5 µm.

[10] A quantum dot (QD) is a material structure, mostly from semi-conductor material (e.g. InGaAs, CdSe or also GaInP/InP) at nanoscale.

excluded to the largest extent possible only if at least two independent methods are used for determining a dose-effect relationship so that potential interferences of the ENM with the method can be excluded and the genotoxic effects are studied in the correct dose range.

- Primary genotoxicity is based on direct damage to DNA. However, especially secondary DNA damage is caused also in consequence of inflammatory processes or oxidative stress which, in the organism, do not necessarily lead to permanent damage to the genetic material. DNA damage can be shown in particular by in vivo studies where the repair and protection mechanisms of the organism can become effective or, as an alternative, by recognized in vitro test combinations of good informative value.
- In pulmonary exposure studies no attention is given to working below the "overload dose". Frequently, this is the case especially in instillation experiments[11], to a lesser extent in inhalation experiments. However, an overload of processes to "clear" the lungs ("clearance mechanisms") can lead to tumours, totally irrespective of the material used.

- **Modes of action (MoA):**
  Mode of action studies serve to clarify the process of damage to the organism or to cells. No new nanospecific mechanisms were detected. Studies that may indicate nanospecific mechanisms can often be explained by the following:
  - In mode of action studies for ENM, frequently no difference is made between indirect and direct effects – like e.g. for genotoxicity. Relatively often, publications speak of direct (primary) genotoxic effects, even though indirect (secondary) DNA damage – caused by oxygen radicals or inflammatory processes – was found (over 95 % of studies).
  - Out of the total of all MoA publications reviewed (over 700), studies on inflammatory processes and oxidative stress are by far the most frequent. The reason is that these endpoints correspond to generic particle effects so that neither any "nano-specificity" nor any size dependence can be deduced from this. Moreover, the effects frequently occur in the upper dose or concentration ranges.

    Beside the endpoints of inflammation, oxidative stress and genotoxicity, several further mechanisms were analyzed with relatively high frequency; these include, inter alia, apoptosis, gene expression and various signal transduction paths in the cell. Histopathological examinations of impacted tissue (lung, liver, spleen, kidney …) were carried out very frequently too.

    Inflammatory processes are detected for almost all ENM, depending on the concentration/dose. For the endpoint "oxidative stress" there are ENM (amorphous SiO_2 and CeO_2) where no such effect was found in the majority of the evaluated studies.

    Overall, the studies can be divided into three categories:

[11] In instillation, the substances under examination are introduced directly into the lung.

1. Studies without effect independent of the particle size;
2. studies **with** an effect independent of the particle size;
3. studies **with** effect dependent on the particle size.

In terms of numbers, category 2 is the largest group.

- If a change in toxicological effects is observed in studies of category 2 this may be explained with better transport/uptake and higher surface activity (specific surface) of the ENM due to reduced particle size.
- The shape plays a role for HARN and other fibres: For both CNT and other (nano) fibres, those fibres that correspond to the WHO definition are generally critical in regard to health effects.

- **Exposure of the environment:**
  Environmental concentrations of NM and ENM in air, soil and water are very difficult to determine; this applies to both soils and aquatic systems, with a transport of ENM being most likely in surface waters, where soluble NM will be transported, but for low soluble NM that agglomerate fast there will be limited transportation and rather sedimentation.
  - For almost all inorganic ENM, analogous substances exist in nature in the form of colloidal particles.[12] There are very few inorganic ENM without natural counterpart – like e.g. CNT or quantum dots. Naturally occurring nanomaterials – for example, some metals and many metal compounds[13] – are ubiquitous in the environment. Differentiating between ENM and natural particles (colloids, natural NM) is not easy and often can be done only at the level of individual particle analytics, e.g. by the use of electron microscopy.
  - For example, matrix bound ENM are not released from end products in the event of mechanical stress. Instead, nanoparticles are broken off together with the binder matrix[14]. No passage through a matrix can be observed, either[15].
  - As far as can be estimated, the environmental concentrations of ENM are by orders of magnitude below the concentrations of naturally occurring NM. Therefore, up until now the environmental concentrations of ENM can be

[12] Colloids are particles or droplets which are finely dispersed in the dispersion medium. Sizes of individual particles are typically in the nanometre or micrometre range. In colloidal chemistry, particles in the size range of 1-1.000 nm are assigned to the colloidal size range; most recently, frequently also to the nanoscale size range.

[13] For example, metal oxides, sulphides, carbonates and phosphates.

[14] Göhler, Stintz: Nanoparticle release studies under laboratory conditions Particle and Fibre Toxicology 2011, 8:22; Vorbau M, Hillemann L, Stintz M, TU Dresden, Method for the characterization of the abrasion induced nanoparticle release into air from surface coatings, J. Aerosol Science, 2009, Vol. 40, No 3, 209-217

[15] Johannes Bott, Angela Störmer, Gerd Wolz, Roland Franz, Fraunhofer Institute for Process Engineering and Packaging (IVV),Studies on the migration of titanium nitride nanoparticles in polymers, and Migration potential of nanoscale silver particles in food contact polyolefins; Poster presentations at the 5th international Symposium on Food Packaging, 14-16 November 2012, Berlin

determined only for particles which have no analogues in the environment. Analytical methods for a fundamental differentiation between ENM and NM are still being developed. But the concentration of a specific ENM can locally exceed the concentration of its natural analogue.

- **Transport and behaviour in the environment:**
  The environmental behaviour of ENM can be described based on the knowledge about naturally occurring, analogous nanomaterials (e.g. metal oxides).
  - NM and ENM have a very high propensity to form agglomerates. In particular, this holds true for NM and ENM in natural environmental conditions. The ability of an ENM to form discrete particles depends on the surface coating[16], the electrostatic conditions and the concentration conditions of environmental media. For this reason, aggregates consisting of different particles are formed very often. Then, such aggregates consist of both ENM and NM or larger natural particles and aggregates.
  - The environmental behaviour is determined to a high extent by the kinetics of the dissolution process and of the dissolution products. Here, the ENM change and no longer maintain their original form. But another precipitation of the dissolved components can once more lead to the formation of NM; in this setting, it is arguable and the subject of ongoing discussion whether the thus formed NM should still be deemed to be called an “ENM”.
  - In certain cases, a surface coating of ENM can strongly determine the environmental behaviour of the particles. In cases of persistent coatings which neither degrade nor detach from the surface, the particle behaviour is controlled mainly by the coating and not by the core material of the particle. The stability of coatings considerably depends on the coating processes, the coating material and the type of binding of the coating material on the surface. At present, relatively few or insufficient data are available so that the influence of surface coating on the environmental behaviour cannot be estimated fully as yet.[17]
    On the case that naturally occurring, analogous materials exist, the environmental effects of ENM can be derived (also see below “Conclusions and consequences for the safety of and the regulatory framework for nanomaterials”, bullet “Risk assessment of ENM”). Materials with perfectly new surface properties are mostly still under development and would need to be tested as to their environmental behaviour under controlled environmental conditions. At present, standardised test methods for determining the environmental behaviour are not yet available, even though the OECD methods can be used in principle – with a need for further adaptation in some cases.

[16] Coating means a production process where, depending on the intended use, a firmly adhering substance layer is applied onto the surface chemically, mechanically, thermally or thermomechanically, in various thicknesses, compositions and structures.

[17] Here, it needs to be distinguished from the commercially relevant silylation e.g. of metal oxides where the reactive OH-groups at the particle surface are chemically converted with Si-organic groups.

- Therefore, models and model processes can be developed which enable a description of the environmental behaviour of ENM in realistic exposure scenarios.

- **Eco-toxicology**
  The review of current literature of more than 750 papers indicate that physical effects make it at present very difficult to determine whether potential eco-toxicologic effects are specific for nanoparticles or are more general particle effects. It has to be noted that physical (particle)effects are not a new phenomenon and have been discussed for many decades. They are not specific for NM and certainly do not reflect a “nano-effect”. Therefore it is not possible to answer the question whether nanoparticle effects exists and if so, how to distinguish them from other biological responses. The most important parameter that give rise to eco-toxicologic effects are dissolution processes in aquatic test systems which are not specific to nanomaterials.
  - In most cases it has not been possible to distinguish between physical effects, the toxicity resulting from non-predictable dissolution processes in aquatic media and differences in sites of action due to internalisation of particles in the test organisms.
  - In scientific literature many (eco-)toxicological „artefacts“ are described.
  - Like in toxicology „oxidative stress“ (ROS) is supposed to be an important factor of eco-toxicology (especially in the case of metal oxides) – but there is no clear conclusion about the particle size dependency of ROS-effects in eco-toxicology.
  - The establishment of clear dose-response relationships has proven to be challenging due to their dynamic behaviour during ecotoxicological testing. Generally the plethora of different ENPs in terms of chemical core composition, size and Coating/functionalization complicates the comparison between studies.

Conclusions and consequences for the safety of and the regulatory framework for nanomaterials

- **Assessment of the hazard potential of ENM:**

  No clear indications were found of a specific (eco)toxicity of NM and ENM by taking the “confounding factors” (artefacts) into account. As compared with other substance forms or substances, the studies did not reveal a higher hazard potential for ENM per se, either.

  - Taking special precautions is not justified scientifically. Therefore, ENM should not be governed by different regulation than other substance forms or substances.
  - The material parameters of shape and solubility of NM should be given special consideration in risk assessment.
    - Potential (eco)toxicological effects of ENM also depend on the specific

dynamics of solubility in biological media and on a possible intrinsic toxicity of the substance.

- In risk assessment, much attention should be given to a possible particle toxicity of poorly soluble ENM. This also applies for fibrous substances, depending on their physicochemical properties.

- **Exposure assessment of ENM:**

  Like the exposure assessment for every other subtance an exposure assessment of ENM needs to take into account the concentration of naturally occurring substances and forms. Here, realistic exposure scenarios (inter alia, regarding concentrations, environmental conditions/media) should be taken as a basis. Concerning an exposure scenario for an ENM which is firmly bound in the solid matrix in the end product, it can be assumed that ENM can be released not at all or only under extreme environmental conditions.

- **Risk assessment of ENM:**

  - Usually, the risk of many surface-coated ENM cannot be derived from the risk of non-coated ENM. Like for other substances, it needs to be examined in individual case decisions whether risk assessments can be derived from comparable materials.

    - In the risk assessment it needs to be differentiated between stable and degradable surface coatings.

  - The OECD test protocols for substance examination can be used for NM and ENM taking particle characteristics like agglomeration and dissolution into account. Partly, they may need further adaptation in the testing of non-soluble/poorly soluble particles; this holds true especially for questions concerning the physicochemical sample preparation and dosage. Currently, this is being addressed at OECD level.[18]

  - Like for other substances, a differentiated examination is necessary also for different ENM.

    - For soluble metals or metal oxides, the toxicity of the metal ions or of dissolved molecules needs to be taken into account.

    With todays methods and tools a Risk Assessment is possible. With further information the Risk Assessment can be refined i.e. by further investigation on
    - Mode of action
    - Dose-effect relations ship and dose metrics

    of (nano-) particles in ecotoxicity studies.

  - It will be possible to refine the description of environmental behaviour of ENM in realistic exposure scenarios based on models and model processes that still need to be developed.

[18] Sponsorship Programmes of the OECD Working Party on Manufactured Nanomaterials (WPMN); Guidelines at http://www.oecd.org/ehs; also see „Six Years of OECD on the Safety of Nanomaterials, OECD, 09/2012.

- If effects of NM can be observed they have to be distinguished from other reactions of biological systems. Therefore possible particle effects of other analogous non-nanomaterials have to be known.
- Background particle concentrations during the tests have to be characterised; in nearly all test systems reported in the literature this prerequisite has not been matched.
- (Nano-)particles, inlusively the dynamics of particle transformation, have to be characterised during tests.
- However, it should be considered that for insoluble/poorly soluble materials the pelagic tests may be not relevant. The sediment would be the compartment of concern.

General recommendations on safety research

The results from the studies show that safety assessment is possible for NM and ENM. Essential scientific findings on health and risk assessment are available.

But the study results also highlight a clear need for improvement and the need to comply with quality criteria when performing toxicological studies.

- **Performing toxicological studies:**
  Scientifically substantiated quality criteria should be the essential prerequisites for the carrying out of toxicological studies. The study results can be taken as a basis for discussion on the risk assessment of ENM only if such quality criteria are complied with.[19] Furthermore, only those projects should receive public funding which fulfil the prerequisite quality criteria.
  - Such quality criteria include, inter alia
    - a clear description of the study design (examination of the toxicological mode of action or examinations of the toxicological potential),
    - an adequate material characterization,
    - endpoint-adequate dosage/metrics, and
    - the inclusion of adequate toxicological know-how for the evaluation and interpretation of toxicological results.
  - For the gathering of toxicological data, the test batteries and measuring methods as well as the evaluation procedures need to undergo international harmonization (e.g. by way of round robin tests between internationally recognized laboratories).

[19] Also see the quality criteria of the DANA project of the German Federal Ministry of Education and Research (BMBF) which can be taken as an excellent example (http://www.nanopartikel.info/en/dana-start-en) and the recommendations in the article in Angewandte Chemie Internat. Ed. (http://dx.doi.org/10.1002/anie.201403367).

- Publicly funded projects should communicate their findings in an adequate manner. Here, it should not be forgotten that toxicological research cannot guarantee “absolute certainty”.
- Study participants should be called upon – also by ministries and public authorities – to publish “no effect studies”. Such activities should be taken to European and international levels.

Like for other chemical substances, the above also applies for ecotoxicology.

- **Performing eco-toxicological studies:**
  Scientific findings on environmental exposure through NM and ENM should be further expanded, based on newly developed analytical methods. For refinement of Risk Assessment further investigation of (nano-)particles in ecotoxicity studies on Mode of Action (MoA) and dose-effect relationship and dose-metrics is advisable.
  - There is no common mode of action following the eco-toxicologic path that has been described to derive nano-specific toxicological effects in water organisms.
  - A dose-effect relationship cannot be derived as transformation processes are – in dependency of the testing media – non-predictable and cannot be characterised. The application of an test effective dose metric has to be studied referring to the parameter mass, particle number or specific surface area (SSP).
  - An adaption of the environmental test guidelines of the OECD should be considered where necessary as described in OECD WPMN: „Guidance Manual for the Testing of Manufactured Nanomaterials“ (2009), „Guidance Document on Sample Preparation and Dosimetry“ (2012). Special attention should be given to sample preparation (dissolution processes), stable conditions of exposition (transformation, aggregation/ agglomeration, interaction with the testing media).

Recommendations for projects for “safety research into nanomaterials” from industry’s viewpoint

In the following, recommendations are given for projects which are necessary from industry’s viewpoint for “safety research into nanomaterials”. Safety research is needed for the further development of ENM and should be shaped as effectively as possible.[20]

- **Priority topics for toxicological studies:**
  - Quality improvement in toxicological studies for NM and ENM
    - Setting up of round robin tests among recognized certified laboratories regarding toxicological methods and evaluation of results, extending activities into international cooperations, development of standard operating procedures (SOPs) as contributions to the development of methods within the OECD Working Party on Manufactured Nanomaterials.
  - Application of toxicological test systems

[20] Safety research into nanomaterials – Priority topics for projects which are necessary from industry’s viewpoint, as proposals for funding by the BMBF, VCI, September 2012

- Examining the applicability of toxicological test systems to ENM substance groups and development of SOPs, possibilities for the categorization of toxicological potential of ENM and ENM substance groups.
- Systematization of studies on toxicological effects of artificially surface coated ENM, in dependence of physicochemical surface parameters.
- Contributions to examining an endpoint-adequate dosage/metrics.

- Studying toxicological effects
  - Studying long-term effects, on the example of selected model substances,
  - studying agglomeration in biological media,
  - studying the modes of action of ENM – as a contribution to the “safety-by-design” approach.

- **Priority topics for examining environmental effects:**
  - Exposure
    - Estimate, i.e. modelling and/or measuring of exposure (emission into and fate in the environment, in particular in aquatic systems) of ENM of industrial relevance.
    - Development of measuring methods for measuring the emission of ENM in the environment, for transport and fate in the environment, and for the identification on NM,
    - studying agglomeration / transformation / dissociation in natural (model) media.
  - Transport and behaviour in the environment
    - Studying the behaviour (transport, transformation, fate) in non-aquatic systems (soils/sediments and sewage sludge),
    - possibilities for categorization (development of models, gathering/measuring of specific data) of the ecotoxicological potential of ENM and of behaviour and fate in the environment, development of substance flow models for ENM on the basis of chemical-physical processes – with the goal of a quantitative description of the behaviour of ENM in the environment.
  - Environmental effects
    - Studying the environmental effects of artificially surface coated ENM with an examination as to “unexpected effects”, in dependence of physicochemical surface parameters and of the particle size,
    - application and comparison of various (eco) toxicological test-systems, (eco)toxicological studies with “aged” particles[21] and loosely bound particles which were washed off again,

[21] This means particles which are already subject to conversion processes, depending on the biological media.

- long-term studies stretching over several species generations.

- Ecotoxicological effects
  - Further investigation on effects on
    - Mode of Action (MoA)
    - Dose-effect relations ship and dose metrics

    of (nano-) particles in ecotoxicity studies.
  - Studies to reveal a possible nano-specific mode of action in environmental test systems.
  - Studies on a possible nano-specific dose-effect relationship and dose metric for different environmental test systems.
  - Distinction of possible nano-specific effects from other reactions in biological systems.
  - Characterisation of possible particle effects of other analogous non-nanomaterials.
  - Characterisation of background particle concentrations in different environmental test systems.
  - Studies on the dynamics of (nano-)particle transformation in different environmental test systems.