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Scientific Stakeholder Meeting on Nanomaterials in the Environment

For our Environment

Umwelt Bundesamt

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Scientific Stakeholder Meeting on Nanomaterials in the Environment

by

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On behalf of the German Environment Agency

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Abstract

This report summarizes the contents and outcomes of the Scientific Stakeholder Meeting on Nanomaterials in the Environment which took place on the 10th and 11th October 2017 at the headquarters of the German Environment Agency (UBA) in Dessau-Rosslau, Germany. The meeting was hosted by UBA and financed by the German Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety. The meeting focused on regulatory relevant results of German and European research projects on nanomaterials in the environment which are carried out or finalised in the current years. By this, it gave a forum to present the state of the knowledge on environmental nanosafety in a regulatory context as well as to discuss the scientific results and their regulatory relevance between affected stakeholders. Therefore, the meeting particularly addressed representatives of science, industry, risk assessors, regulatory experts, and NGOs. It included key note talks, invited platform presentations as well as poster presentations. A Knowledge Café provided the opportunity to discuss selected topics with regard to environmental safety of nanomaterials in smaller groups. The meeting was closed with a discussion on the lessons learned highlighting the outcomes of the meeting by the views of different stakeholders.

Kurzbeschreibung

Dieser Bericht fast die Inhalte und Ergebnisse des „Scientific Stakeholder Meeting on Nanomaterials in the Environment“ zusammen, welches am 10. und 11. Oktober 2017 am Umweltbundesamt in Dessau-Roßlau stattfand. Dieses Treffen wurde durch das Umweltbundesamt (UBA) organisiert und durch das Ministerium für Umwelt, Naturschutz, Bau und Reaktorsicherheit finanziert. Es fokussierte auf regulatorisch relevante Ergebnisse aus laufenden oder kürzlich fertig gestellten, nationalen und Europäischen Forschungsprojekten zur Sicherheitsforschung von Nanomaterialien. Es bot es ein Forum um das aktuelle Wissen zu Nanomaterialien in der Umwelt im regulatorischen Kontext vorzustellen und mit die wissenschaftlichen Ergebnisse und deren regulativen Relevanz mit den betroffenen Interessensvertretern zu diskutieren. Dazu waren Vertreter aus Wissenschaft, Industrie, Risikobewertung, Regulation und Nichtregierungsorganisationen zusammengetroffen. Das Treffen beinhaltete „key note“ Präsentationen, eingeladene Vorträge und Posterpräsentationen. In einem „Knowledge Café” konnten ausgewählte Themen mit Relevanz für Fragen zur Sicherheit von Nanomaterialien in der Umwelt in kleineren Gruppen diskutiert werden. Das Treffen endete mit einer Diskussion zum Thema „lessons learned“, in dem die Ergebnisse des Treffens aus dem Blickwinkel unterschiedlicher Interessensvertreter dargelegt wurden.

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List of Abbreviations

BAuA	Bundesanstalt für Arbeitsschutz und Arbeitsmedizin - Federal Agency for Occupational Safety and Health
BUND	Bund für Umwelt und Naturschutz Deutschland – Friends of the Earth Germany
ECHA	European Chemicals Agency
ERA	Environmental Risk Assessment
GD	Guidance Document
OECD	Organisation for Economic and Technical Cooperation and Development
TG	Technical Guideline
UBA	Umweltbundesamt – German Environment Agency
UK	United Kingdom

1 Introduction

In the past years various national and European research programs funded projects and activities to promote advances in methods, techniques and for a general improved understanding on behaviour, fate and effects of nanomaterials in the environment. Thanks to these initiative, a considerably improvement on knowledge on environmental nanosafety was gained which *inter alia* showed the need for amendments of methods and tools used in current environmental risk assessment of chemicals as well as the need for adapted information requirements within chemical legislations.

This report summarizes the contents and outcomes of the Scientific Stakeholder Meeting on Nanomaterials in the Environment which took place on the 10th and 11th October 2017 at the headquarters of the German Environment Agency (UBA) in Dessau-Rosslau, Germany. The meeting was hosted by UBA and financed by the German Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety. It focused on research results of German and European research projects on nanomaterials in the environment which are carried out or finalised in the current years. By this, it gave a forum to present the state of the knowledge on environmental nanosafety in a regulatory context as well as to discuss the scientific results and their regulatory relevance between affected stakeholders. Therefore, the meeting particularly addressed representatives of science, industry, risk assessors, regulatory experts, and NGOs. It included key note talks, invited plattform presentations as well as poster presentations. A Knowledge Café provided the opportunity to discuss selected topics with regard to environment safety of nanomaterials in smaller groups. The meeting was closed with a discussion on the lessons learned highlighting the outcomes of the meeting by the views of different stakeholders.

2 Welcome Remarks

2.1 Welcome remark by the German Environment Agency

Jutta Klasen, Division on Chemical Safety, UBA

Dear Ladies and Gentlemen,

It is a pleasure for me to welcome you all here at the German Environment Agency, the venue of our International Stakeholder Meeting on Nanomaterials in the Environment, also on behalf of our President Maria Krautzberger. An impressive number of participants from Germany and other European countries, registered for this 2-days meeting to discuss up-to-date research results.

In particular I like to welcome Anke Jesse, who is responsible for *Nanotechnology and Advanced Materials and OECD Chemicals Policy* at the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety and Eva Gerhard-Abozari (Projektträger Jülich, PTJ) who will speak on behalf of Mrs Cottone from the division on *New Materials* of the Federal Ministry of Education and Research.

In addition, I`d like to particular welcome the colleagues from EU, from federal, and member states authorities which are very important partners of UBA's engagement in the safety matters of nanomaterials since years. And of course I welcome our guests from research, industry and non-governmental organisations. We highly appreciate your participation and your willingness to present and discuss the state of knowledge on effects and fate of nanomaterials in the environment in a regulatory context.

Let me start my speech with giving you some information on the venue of this meeting, the German Environment Agency – or just called "UBA" - which is Germany's main federal authority for environmental protection. Since more than 40 years, we act as interface between science, politics and society. As scientific authority we give advice to the federal government. Our daily work includes environmental research, the enforcement of environmental laws and providing information to the general public about environmental protection issues.

Identifying tomorrow's problems today is one of our main goals. We understand UBA as an early warning system which detects potential future adverse impacts or risks for humans and the environment, but also tries to offer proposals for practicable solutions in risk reduction. To that end, experts at the agency carry out research in in-house laboratories in addition to commissioning research projects to scientific institutions in Germany and abroad. UBA is committed to contribute to international processes related to environmental policy. Various members of our staff are actively involved in working groups in the EU, OECD and on a global basis.

Next month there will be the COP 23 in Bonn, and our agency will be involved in the performance of different side-events, amongst others one on the topic of sustainable chemistry, presenting our new International Sustainable Chemistry Collaboration Centre ISC3 at the GIZ in Bonn established in May this year.

In 2005, the headquarters of UBA moved from Berlin to Dessau into this wonderful building. I recommend to those, who will have the time, to get to know Dessau and its surroundings. Dessau is a city of ~80T inhabitants and was one of the centres of the German Enlightenment with focus on social, economic and educational projects. Thanks to this, we still benefit from extensive parks, gardens and castles in the vast meadow landscape of the rivers Elbe and Mulde - the so called Garden Kingdom of Dessau-Wörlitz. Only a few hundred meters from here, the Bauhaus is located which gave worldwide and long term impact on architecture, art and design. And not to forget: This year Germany – and particularly this region – celebrates 500 years of reformation. If you are interested to walk in the tracks of Luther, I recommend to travel to Lutherstadt Wittenberg which is only 35 km from here.

Coming back to Nano: Why are nanomaterials an important topic for us? We believe that nanotechnology can offer chances for environmental protection which include energy and resource efficiency, water purification but also for decontamination of polluted areas. However, the rapid evolution of nanotechnology goes hand in hand with increased amounts produced which may result in increased burdens for both, humans and the environment. Nanomaterials may be released from products and applications and thus, enter the environment. Therefore, confidence in the safety of this technology has to be ensured by a robust and reliable risk assessment and an appropriate chemical legislation.

UBA is in charge with the environmental risk assessment within different chemicals legislations including REACH, the regulation on classification and labelling, on biocidical products, plant protection products and on human and veterinary pharmaceuticals. In consequence, an appropriate environmental risk assessment of nanomaterials is one of our central goals!

Until now, with few exceptions, no specific provisions exist for nanomaterials within the European chemicals legislations. As nanomaterials are considered as special forms of chemicals, they are covered by European chemicals legislations and by the methods to assess and manage risks implemented there. However, as nanomaterials feature specific properties that differ from those of conventional substances of the same chemical identity, amendments of the current information requirements on nanomaterials are needed.

Thanks to you, the scientists in the field of nanosafety, and your intensive research new and important insights about effects and fate of nanomaterials in the environment has been provided. These insights help us to identify the steps which need to be taken to make environmental risk assessment "fit" for nanomaterials and with that to advise policy decision makers.

Considering these scientific insights, UBA has been part of expert groups of ECHA and the European Commission – especially related to REACH – since 2009. However, despite of the support of member state experts, the EU Commission did not succeed yet in presenting concrete and binding measures on nanomaterials most importantly in REACH and also for a harmonized definition.

Cooperation is one mayor key for success! This is especially true when decision making needs scientific expertise, like the excellent expertise of the scientists present today and tomorrow. We believe that strong cooperation between regulatory risk assessment and science is needed to ensure that risk assessment and regulation keep up with the advancements in technology.

So, already 10 years ago we came together with other involved federal authorities [the Federal Institute for Risk Assessment (BfR), the Federal Institute for Occupational Safety and Health (BAuA), the Federal Institute for Materials Research and Testing (BAM) and the National Metrology Institute (PTB)] to identify and address the research needed to answer regulatory questions in an effective manner. And we ended up with a long-term research strategy for nanosafety which is regularly updated. Since 2007, this strategy serves as a roadmap for governmental research in view of occupational safety and health, consumer protection and environmental protection. Backed by that strategy, UBA is contracting authority as well as cooperation partner in several national and European third-party funded projects.

With the great support of the funding program of the Federal Ministry for the Environment - called UFOPLAN or REFOPLAN - we have been in the lucky situation to fund 10 research projects during the last 8 years, focusing solely on environmental safety of nanomaterials. We highly appreciate that many of the needs identified in our governmental strategy found also entry into the research calls of the German Ministry of Education and Research as well as in the research framework programmes of the European Commission.

Since 2006, the Federal Ministry of Education and Research supports research on safety of nanomaterials, both human health and environmental safety and by this supports the gain of knowledge on fate and effects. Most of the projects materialised from these different initiatives and programmes will be presented at this meeting.

The outcomes of the different scientific projects on nanomaterials feature also great benefit for the OECD: Since 2006, the OECD Working Party on Manufactured Nanomaterials has addressed the different aspects of nanosafety. As part of the German delegation to this working party, UBA supports the activities on environmental impacts and behaviour of nanomaterials. One of the current main emphasis of the working party is the development of new OECD test guidelines or specific guidance for nanomaterials. We highly appreciate the long lasting, close and successful cooperation with this working party.

This meeting brings together the different views of experts on nanomaterial environmental safety.

For you as participants, this meeting offers the possibility to share your knowledge, exchange your views, and discuss the relevance of science on nanosafety in a regulatory context. Coming from science, regulation, industry and civil society organisations, your active contribution is required for the success of this meeting as tomorrow we will ask for your expert opinion on 5 important topics on nanosafety in a knowledge café.

As this conference constitutes a meeting of different stakeholders with different priorities, objectives, and sometimes also speaking different tongues, it may support your knowledge on the broad range of activities of all the players, it may help you in establishing new networks and widens your perspective on the needs for environmental risk assessment and proper regulation of nanomaterials.

I wish you a very pleasant stay, and every success for this meeting. My special thanks go to our colleagues Kathrin Schwirn and Doris Völker, who did the main work to organise the meeting.

Thank you very much.

2.2 Welcome remark by the Federal Ministry of the Environment, Nature Conservation, Building and Nuclear Safety

Anke Jesse, IG II 6, BMUB

Dear Colleagues from Federal States and EU and Member States Authorities, dear Guests from Research, Industry and Non-Governmental Organisations,

It is a pleasure for me to welcome you here at the German Environment Agency in Dessau, the host of the present Scientific Stakeholder Meeting on Nanomaterials in the Environment. Thank you all for taking part in this event which aims to bring together representatives from research, regulators, industry, and NGO to learn about the current knowledge and discussion points related to nanomaterials environmental safety.

Nanotechnology is one of the key technologies of the Hightech Strategy 2020 of the German Federal Government. Nanotechnology involves research and development, production and processing of structures and materials on a nanometre scale. As you know, such nanomaterials can have different or completely new properties and functions in comparison with conventional chemicals and materials.

By using these features, we can improve efficiencies or achieve new functionalities for a wide range of products and applications. Thus, the use of nanotechnology opens up potentials for resource- and energy-efficient products and processes. This includes environmental technologies for the removal and avoidance of harmful substances, product-integrated optimisation of energy- and material flows as well as efficient methods of conversion, storage, distribution and use of energy.

With a high number of applications, nanotechnology has found its way into almost all branches of industry. Nanomaterials find also use in everyday products and consumer goods. This can result in increased burdens for humans and the environment when nanomaterials are released from products and applications. Because of that, beside fundamental research for development and innovation accompanying research to understand the risk of nanomaterials to human and environment is crucial.

Since 2006, eight federal ministries have been in charge with the task of publishing the German government's action plan on nanotechnology at five year intervals. Led by the Federal Ministry of Education and Research, the ministries develop a common approach that pools a strategy for nanotechnology. The third action plan on nanotechnology - Nanotechnology Action Plan 2020 - was adopted by the Cabinet in autumn 2016.

Within the German government's Nano Action Plan the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety leads the German NanoDialogue. The NanoDialogue has started as a national platform were the emerging technology – nanotechnology – is accompanied by a public dialogue. In its dimension the NanoDialogue is probably unique in Germany as well as in Europe. It aims to exchange information across institutional boundaries, to define areas of consensus but also to convey and to understand the positions of other parties. Thus, it helps to promote the responsible and sustainable use of nanomaterials.

More than 200 experts were engaged on a voluntary basis in the discussion on the responsible use of nanomaterials so far. In 2011 participants of the NanoDialogue agreed that it would be appropriate and preferable to approach the questions and tasks at hand through issue-related events. The format was therefore changed into two-day expert dialogues. During these events topics like "Sustainability of nanotechnologies – green nano", "Traceability of nanomaterials" or "Nanomedicine" have been discussed. Thereby, the NanoDialogue considered the chances and risks equally and with its recommendation it has increased the acceptance of nanotechnology in Germany. In fair and open discussions also controversially topics like the adaptation of chemical legislations, reporting obligations or labelling have been debated. In summer last year the NanoDialog celebrated its 10^{th} anniversary.

Also the current two-day event, kindly organised by the German Environment Agency, is a perfect opportunity for a dialogue between the different stakeholders. This meeting will place the research on environmental safety and its recent findings into the centre of our attention.

Research starts with scientific curiosity and follows manifold ideas, hypothesis and aims. This is part of the human being and the base of our progress. From a regulatory point of view, research is always accompanied with the questions how its outcomes can be exploited to protect human and environment! Hereby, one major aim hereby is to facilitate the identification, assessment and mitigation of risk for environment and human health.

In that context one has to differentiate between "nice to know" and "need to know". Both are justified and important! The "nice to know" is important to identify emerging issues. But also to gain new knowledge to re-consider old truths. "Need to know" is that part of research that provides reliable and reproducible tools and information to help us to understand and manage risks.

I would like to give you two examples for the exploitation of research outcomes with respect to nanomaterials environmental safety:

In 2006, the Chemicals Committee of the Organisation for Economic Co-operation and Development – OECD – agreed to initiate a specific programme to address the specific safety aspects of manufactured nanomaterials: the Working Party on Manufactured Nanomaterials (WPMN). Already at that time, it was commonly known that nanomaterials are increasing in volumes on the market worldwide as well as in a wide range of products and applications.

From a regulatory perspective, nanomaterials came with a number of unknowns about how they may be interacting with humans or the environment. One of the significant issues that was on the top of the agenda was the question whether the current risk assessment methods could be used for nanomaterials.

Since the 1970ties, the OECD has facilitated the development of harmonised test guidelines and tools to assess chemical safety and enabled good laboratory practice. This work has been the foundation for generating high quality and reliable data on hazards of chemicals in commerce on the market. Thereby, it gives a better understanding of how to safely manufacture and use chemicals at a global level. It also offers benefits for both OECD countries and industry to be able to use commonly accepted test guidelines and tools; in particular when the test guidelines are coupled with another important concept: The agreement on the OECD concept of Mutual Acceptance of Data. This concept reduces trade barriers, prevents duplication of tests, and actively limits the need of animal testing. This in turn has not only positive financial aspects but also contributes to several sustainable development goals.

Back to recent years: To support the work of the WPMN, EU and Member States has invested significant funding in research to answer questions related to nanosafety.

Thanks to the scientific community who supported the work of OECD by providing their research on nanosafety, the WPMN has gained clarity on many issues of relevance for nanomaterial risk assessment. Thus, the OECD Council Recommendation from 2013, states that in general the existing methods and tools may indeed be utilized also for nanomaterials. However, this recommendation also pointed out that there is a need to amend these tools and test guidelines for the specific nature of nanomaterials.

Also in 2013, an OECD Expert Meeting on Environmental Fate and Effects took place where expert on nanomaterial environmental safety agreed on many recommendations regarding the adaptation needs of environmental relevant OECD test guidelines and guidance documents. As follow up to this meeting, several projects were initiated worldwide to address these recommendations. One milestone of these initiatives was achieved this year when the OECD finally adopted the very first test guideline specific for nanomaterials. This test guideline was developed by the University of Vienna on behalf of the German Environment Agency and funded by the German Ministry of Environment.

For an appropriate environmental risk assessment of nanomaterials besides adequate testing and adapted assessment tools we also need to obtain appropriate risk information for regulatory decision making. - And this is my second example. - Here, the European chemicals legislation comes into play. Until now, with few exceptions, there are no specific provisions for nanomaterials within the chemicals legislation. As a result, specific environmental risks cannot be described and assessed adequately and appropriate measures to minimize the risks cannot be taken.

The Federal Ministry for the Environment leads the responsibility for the European Chemicals Regulation REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) in Germany. The REACH Regulation aims to secure a high level of protection for humans and the environment. According to the European Commission, it is the appropriate instrument for regulating nanomaterials. But an adaptation is needed to address the characteristics of nanomaterials. Thanks to the research efforts in the present but also in the past, we meanwhile know what is needed for an appropriate environmental risk assessment of nanomaterials. Based on that, Germany together with a number of other Member States proposed concrete measures for a way forward for nanomaterials under REACH.

Only the European Commission has the right to initiate such a revision. Even though the Member states spent much time and many efforts in the discussion with the European Commission since 2009, an official proposal is still pending.

In 2011 the Commission submitted a recommendation on the definition of a nanomaterial. This recommendation was taken up in the biocide regulation while in other regulations different definitions or so far no definition were implemented. A review of the recommended definition was performed by the Commission's in-house research service, the Joint Research Centre (JRC) in 2014. Based on the review the JRC made several proposals for minor revisions. Still, the Commission do not succeed to conclude on a final definition on nanomaterials, which is also considered to harmonize the definition across the various legislations.

These delays lead to legal uncertainties for all involved parties – producers, users, and authorities – and in consequent obstacles for innovation!

Based on these examples you can see, the implementation of research outcomes for regulatory purposes needs a long breathing!

Looking into the future and learned from the past, we all have to increase our efforts that research output can be easier utilised for regulatory purposes. For the question on how to better harness research output it will start with a stronger network between researcher, risk assessors and regulators to increase the understanding amongst each other. This meeting offers an ideal opportunity to address that need!

It includes structures that enables long-term availability of whole research output. Not only that part, which can successfully be published by peer review, because research is more than just headlines!

And it also needs instruments that motivates the research community to take part in regulation oriented research, for instance by alternative funding models to promote standardisation projects, and new incentives for scientific reputation next to the own name in "Nature Nanotechnology".

With these words, I would like to finish my speech. For you as participants, I wish you two very interesting days with possibility to share your knowledge, fruitful and fair discussions to come to conclusions or even just gain understanding of other parties' needs, and the possibility to establish new contacts.

I wish you every success for the meeting. My special thanks go to the colleagues of UBA who organized the meeting.

3 Abstracts of Key Note Presentations

3.1 Session 1: Regulatory Key Note Presentations

3.1.1 OECD contribution to the safety of nanomaterials

[This presentation was cancelled due to unavailability of the speaker]

***Mar Gonzalez**, OECD*

The OECD's Chemicals Programme aims at protecting the environment and human health through a collaborative approach by governments to the safe management of chemicals. One of the main features of the programme has been the development of tools to assess the safety of chemicals, while at the same time keeping a flexible approach in order to tackle the emergence of new substances, such as nanomaterials. A specific programme to deal with nanomaterials was established in 2006. The nanosafety programme is a forum involving regulators and experts in the field nominated by delegations. It has boosted the discussion regarding the regulatory needs to address nanomaterials. Its position vis-a-vis the OECD's Chemicals Programme created a model that not only is contributing to the safety of manufactured nanomaterials, but it is also a global source of knowledge that is available beyond the OECD countries.

3.1.2 Regulatory activities in the area of environmental risk assessment of nanomaterials

***Anu Kapanen**, Laurence Deydier, Virginia Rodriguez Unamuno and Jenny Holmqvist, ECHA*

Nanomaterials (NM) are regulated under a broad range of regulatory frameworks in EU such as REACH Regulation, Biocidal Products Regulation (BPR), CLP, Plant protection Products and Cosmetics Regulations. NM are implicitly covered by the substance definition of REACH Regulation 1907/2006 although there are no explicit requirement laying down NM specific obligations. The BPR (528/2012) has partly implemented the Commission recommendation of 18 October 2011 on the definition of nanomaterials article 3(1)(z)). It states that the approval of an active substance does not cover nanomaterials except where explicitly mentioned (Article 4).

To implement REACH, CLP and BPR in the EU, ECHA currently uses multiple tools to implement regulations and to support these processes aiming at ensuring safe use of NM:

- SUPPORT: helpdesk, meetings with stakeholders and with Registrants, Nanomaterials Expert Group (NMEG) [1] and;
- REGULATE: formal processes under regulatory frameworks, whereby ECHA uses the legal instruments available under REACH (substance/dossier evaluation, authorisation and restriction), CLP and BPR, and
- COMMUNICATE: ECHA Nanomaterials [2], conferences, workshops, communication throughout the supply chain and in a broader context e.g. EUON [3] and press.

There are still challenges remaining on how to both generate and assess hazard data from substances at the nanoscale for regulatory purposes. Over the past years, ECHA has engaged in discussion on these issues with member states and stakeholder in the Nanomaterials Expert Group (NMEG). NMEG aims at seeking common ground on relevant scientific and technical issues relating to the implementation of REACH, CLP and the BPR for NM.

As a concrete output, NMEG has actively contributed to the discussion on the needs for new or updated standard methods. These discussions have led to prioritisation of the methods needed to ensure a reliable hazard assessment under REACH and CLP. The need for additional guidance to aid registrants was also the main driver behind the recent publications of a Practical Guide “Registration dossiers for nanoforms: best practices” [4] and following Appendices for nanomaterials applicable to a following ECHA *Guidance on Information Requirements and Chemical Safety Assessment*:

- Appendix R.6-1 to the Chapter R6 on QSARs and grouping of chemicals [5]
- Appendices R7-1 to Chapters R7a and R7b Endpoint specific guidance [6][7]
- Appendix R7-2 to Chapter R7c Endpoint specific guidance [8].

These endpoint specific appendices provide advice on sample preparation, recommendations on how to perform and interpret aquatic, sediment, terrestrial toxicity tests and the bioaccumulation and degradation/transformation testing with NM.

As a response to the perceived lack of information on nanomaterials among stakeholders and member states, a new initiative was launched by ECHA in June 2017 - the European Union Observatory for Nanomaterials (EUON) [7]. EUON is funded by the EU Commission and hosted by ECHA. It offers a platform to find objective and reliable information on NM on the market. Its first version contains general information on uses, safety, regulation, international activities and research & innovation but over the years to come, EUON is expected to grow in content.

In summary, multiple actions have been taken by ECHA to address NM under REACH, CLP and BPR: Dossier and substance evaluation, NMEG, EUON and ECHA’s involvement at OECD level. However, to work efficiently towards safe use of NM and to decrease the uncertainties in the regulatory processes, there is an urgent need to update the REACH Annexes. Commission is currently considering modifying some of the technical provisions in the REACH Annexes. In addition, ECHA highlights the need for good coverage of standard methods applicable to NM to produce adequate information for regulatory risk assessment.

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[7] Appendix R7-1 for nanomaterials applicable to Chapter R7b - Endpoint specific guidance, version 2. May 2017, DOI: 10.2823/72973
[8] Appendix R7-2 for nanomaterials applicable to Chapter R7c - Endpoint specific guidance, version 2. May 2017, DOI: 10.2823/647499

3.2 Session 2: Scientific Key Note Talks

3.2.1 Importance of physical-chemical properties of ENM (intrinsic/extrinsic) for environmental hazard and risk assessment

Iseult Lynch

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Nanomaterials (NMs) are a highly diverse group of chemicals, defined mainly by their small size, which ranges from 1 to 100 nm, but varying enormously regarding their physico-chemical properties, such as composition, shape, surface charge, crystallinity, and reactivity, among others[1]. Significant progress has been made over the last 10 years towards understanding those characteristics of nanoscale particles which correlate with enhanced biological activity and/or toxicity, as the basis for development of predictive tools for risk assessment and safer-by-design strategies. However, primary physicochemical descriptors of NMs may not be the most appropriate to predict their toxicological behaviour, in part as many of these are "context dependent", i.e. are affected by the surrounding matrix (pH, ionic strength, biomolecules or macromolecules etc.), the route of exposure, etc.[2] There is growing awareness that NMs age and are transformed throughout their lifecycle, further adding to the challenge of predicting their toxicity. While some context-dependent physico-chemical property changes are obvious and well documented, e.g. size and surface charge alterations as a result of biomolecule binding, others such as redox or photochemical activity are less obvious and require experimental assessment and verification.

Thus, three main NM-associated concerns have been implicated in making regulation, read-across and hazard prediction of NMs problematic: (1) the fact that many properties are non-scalable, (2) the need to distinguish between intrinsic versus extrinsic (i.e., context dependence) properties, and (3) the fact that many properties are interlinked (e.g., changing one property may induce changes to another) properties which renders the description of property-activity relationships arduous and makes the development of systemic libraries of NMs challenging.[3] Identification of critical properties (physico-chemical descriptors) that confer the ability to induce harm in biological systems *under the relevant exposure conditions* is central to hazard and risk assessment, in order to enable both prediction of impacts from related NMs (via quantitative property-activity or structure-activity relationships (QPARs/QSARs)) and development of strategies to ensure that these features are avoided in NM production in the future ("safety by design"). Table 1 shows a first attempt to link properties with mechanisms of action, as a basis for discussion and further elucidation.

Table 1: Contributions of various physico-chemical properties to four different toxicity mechanisms. Two ticks indicates strong contribution, 1 tick indicated some contribution, ~ indicates not clear as yet, while – indicates likely no significant contribution. Note that these are opinion rather that quantitative values. Measuring the relative contributions quantitatively is challenging and has yet to be achieved. From Lynch et al. (2017).

Measured parameters	**Dissolution**	**NM Interactions**	**NM (surface) specific effects**	**Trojan Horse**
Size /size distribution	✓✓	✓	✓	✓✓
Surface area	✓✓	✓✓	✓✓	✓✓
Purity (particle / dispersant)	~	✓	-	-
Photochemical activity	✓	✓	✓	-
Surface charge / chemistry	✓	✓✓	✓	✓✓
Hydrophobicity	~	✓✓	-	✓✓
Redox activity	✓	-	✓	-
Shape	✓✓	✓	✓✓	✓
Crystal structure	✓✓	✓✓	✓✓	✓✓
Porosity / surface defects	✓	✓	✓✓	✓

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3.2.2 Environmental release modelling in contexts of high uncertainty for engineered nano-CeO_2, -SiO_2 and –Ag in Germany

***Fadri Gottschalk*[1]*, Bernd Giese*[2]**

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CeO_2-, SiO_2- and Ag-engineered nanomaterial (ENM) partially represent nanomaterial with long-term applications.[1] High uncertainty of quantities and ways of use in past, current, and future contexts go together with extreme challenges to assess release into natural and technical systems.

Such a release must be understood in order to evaluate environmental risks that result from environmental exposure and toxicity. Some dynamic models are developed to cover such ENM release and exposure. These also depend on ENM in economic circulation, as well as ENM already present in natural and technical environments.

An insight is given for long-term period predictions (1950 -2050) into uncertainties and expected 'natural' variability, as well as modeling limits for German contexts. The focus of such epistemic analysis is on uncertain and missing data for ENM production and use that has to be linked to a difficult-to-approach variety of different ENM applications with some potential subsequent releases. Temporal dynamics, including shifts in release due to long-term ENM product application are modeled, as well as stock and end-of-life (EOL) treatments. In doing so for the first time, we also distinguish predicted concentrations and risks caused by ENM product-use phase release from those caused by release from EOL treatment of ENM products.

Plausibility of results and validity of predictions are evaluated for CeO_2 environment concentration predictions as a premiere by cautiously comparing modeling outputs to newly available ENM measurements in Bavarian water-courses. The problem with such comparisons are that very diverse, measurements of engineered material are limited, if not impossible, if very high natural background concentrations of the target compound have to be expected. The fact that CeO_2-ENM particles have been detected in river waters does not necessarily mean that those particles had their origin in emitted engineering material. On the modeling side, we are statistically challenged to understand to what extent the use and stochastic combination of unrealistic and poor model input data may lead to acceptable model outputs. Ag possibly occurs in rivers and other waters as nanoscale Ag_2S, regardless of whether it was released as a dissolved species or as nano-sized particles.

Evenly distributed ENM concentrations throughout Germany do in principle not account for local spikes in high exposure levels and subsequent potential risks, particularly those that occur daily or over a short period of time. Local river water concentrations are significantly influenced by highly variable (local) water levels, the geographic location of release sources, and sewage treatment infrastructure (e.g. treatment plant network density).[2] Recently proposed multi-media fate modeling accounts for local extremes by discriminating freely aggregated, as well as dissolved, material.[3] The future focus will e.g. be on mechanistical modeling and a reduction of uncertainties regarding the ENM attachment on other colloids, including advection and sedimentation processes in the air (soils and other), dissolution and hetero-aggregation, erosion, and other processes in waters (sediments and soils).

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4 Abstracts of Platform Presentations

4.1 Session 3: Effects and Accumulation

4.1.1 Adaptation of OECD test guidelines on ecotoxicity tests – experience gained from the EU project MARINA and further considerations

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Testing of chemicals is labour-intensive and expensive. To avoid duplicate testing for regulatory purposes the OECD Council decided 1981 that test data generated in any member country in accordance with OECD test guidelines and GLP (good laboratory practice) shall be accepted in other member countries for assessment purposes and other uses relating to the protection of health and the environment. For nanomaterials (NMs) the test conditions influence significantly the results on fate and effects. Therefore, specific guidance documents (GDs) and test guidelines (TGs) have to be developed in order to fulfill the requirements for the mutual acceptance of data. An OECD expert meeting on ecotoxicology and environmental fate was held in Berlin in January 2013 to discuss the applicability of OECD TGs for chemicals to NMs. Based on this workshop and further activities of the OECD WPMN the update of existing TGs or supplementation by developing specific GDs (e.g. for TG 312: Leaching in soil columns) as well as the development of new ones (e.g. on dissolution rate of nanomaterials in aquatic environment; for nanomaterial removal from wastewater) was induced. While TGs are legally binding and provide specific and detailed information on how to perform the testing, GDs address critical subjects more generally and provide general recommendations on how to deal with the challenges of the testing. The requirements necessary for the implementation of GDs are lower compared to those for TGs. Regarding the hazard characterization of NMs with respect to the environment, a GD on aquatic and sediment toxicological testing of NMs is currently developed. Aim of this new GD is to provide support for existing OECD TG on aquatic and sediment ecotoxicity when deployed for hazard testing of NMs.

Additionally, significant progress on providing experimental data to support proposals for the adaption of several often applied OECD TGs was achieved by the project MARINA (project period 2012 – 2016) funded by the 7th European Framework Program. Eight OECD TGs were adapted based on the testing of at least one ion-releasing NM (Ag) and two non-releasing TiO_2-NMs. With these NMs applied, two main variants of NMs (ion releasing vs. non-releasing NMs) were addressed. As the modifications of the test guidelines refer to general test topics (e.g. test duration or measuring principle), it can be expected that the described approaches and modifications are suitable for the testing of further NMs with other chemical compositions. Firm proposals for modification of protocols with scientific justification(s) were presented for the following tests: growth inhibition using the green algae *Raphidocelis subcapitata* (formerly: *Pseudokirchneriella subcapitata*; TG 201), acute toxicity with the crustacean *Daphnia magna* (TG 202), development toxicity with the fish *Danio rerio* (TG 210), reproduction of the sediment-living worm *Lumbriculus variegatus* (TG 225), activity of soil microflora (TGs 216, 217), and reproduction of invertebrates (*Enchytraeus crypticus, Eisenia fetida*, TGs 220, 222). For every test system the protocol had been developed individually as the demands of the various test organisms differ. This compilation of recommendations instead of several separate publications addressing every test system individually should support the implementation. It has to be noted, however, that within MARINA no validation of the recommendations (i.e. round robin testing) was performed.

4.1.2 What is the meaning of pristine nanoparticles, their lifecycle and fate? An overview and forward look

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[This talk was presented by Iseult Lynch, University of Birmingham (UK) due to unavailability of the originally intended speaker]

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Over the recent years it has become clear that ranking toxicities of nanomaterials through the testing of “pristine” as made particles in clean media may not provide much relevance in terms of the environmental risk their released forms potentially represents. While it is clear that dealing with detailed phys-chem characterisation of the multiple forms in which the nanomaterials may be released from all stages (particle production; incorporation; use and disposal phases) of a nano enabled products life-cycle is impossible. Then it is equally clear that adequate, realistic and efficient risk assessment cannot be done by simply comparing PNECs from “short lab test with pristine NM forms” with the PECs from mass flow based models that do not take the transformations of nanomaterials both pre and post release to the environment into account. For one the fate processes and behaviour of the released materials depend on the new phys-chem properties developed in such transformations. Again tracking such transformations in detail and doing so in environmentally relevant media and concentrations is technically challenging and resource intensive beyond most available means. Therefore, we propose to move focus away from the physical/chemical properties of pristine ENMs and to aim to understand the functional and behaviour patterns of release relevant ENMs in exposure relevant environments. The need for this will be highlighted through presentation of a series of recent non-standard experiments that aim to get as relevant nanomaterial exposures as possible, each addressing a different element of fate, transformation or aging. Through these experiments we will show how presenting an exposure into a system as nano vs ionic metals lead to diverse and in some cases unexplainable response patterns. Finally the presentation will outline the vision of the NanoFASE project focused on developing a catalogue of options to supplement the current mainly mass-based lifecycle and release flow approaches to enable spatial and temporal variability of ENM release, environmental transport and fate to be included in exposure modelling and assessment. The framework, will incorporate (i) the behaviour of the actual relevant ENM forms released from ENM products; (ii) how reactions in waste management and environmental compartments transform such release-relevant ENMs; and (iii) the consequences of these transformations for transport and fate and among the different environmental compartments including organism uptake.

4.1.3 Ecotoxicity and Fate of nanomaterials in laboratory and outdoor lysimeter experiments – experiences from the BMBF project DENANA

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Nanomaterials (NM) will enter the environment via diverse pathways. Sewage sludge for example is repeatedly applied as fertilizer on farmland due to its high nutrient content. This may lead to a significant increase of NMs in soil over years. However, there are other scenarios like the exposure of the terrestrial environment via runoff. Therefore, our aim was to investigate the ecotoxicity and fate of CeO2-NM and Ag-NM under environmentally relevant conditions in outdoor lysimeters over 2 years (CeO2-NM) and 3 years (Ag-NMs).

Nanomaterials of the OECD Sponsorship Programme, namely NM-212 (CeO2) and NM-300K (Ag), which are well characterised, were used for the experiments. Two concentrations for each CeO2-NM and Ag-NM were applied via sewage sludge into the top 20 cm of lysimeter soil. In addition, CeO2-NM were applied via simulated rainfall over 4 weeks on the surface of the lysimeter soil and afterwards mixed into the top 20 cm simulating a plough. Subsamples of the soil were incubated under laboratory conditions for 180 days to study the comparability of outdoor and laboratory results regarding ecotoxicity.

The results from our long-term lysimeter experiments showed no detectable horizontal displacement in combination with very low remobilization for both tested NM over 2 to 3 years. Thus, indicate that the sludge applied NM and the NM applied via simulated rainfall remained nearly immobile in the pathway between soils and leachate. However, Ag uptake in the roots of wheat, canola and barley indicates that the chemical conditions in the rhizosphere induce Ag-NM remobilization from the incorporated sewage sludge even after three harvesting cycles.

No effects on the bacterial activity of *Arthrobacter globiformis* and the collembola *Folsomia candida* reproduction were detected during the whole test period for any of the tested nanomaterials. The CeO2-NM did not induce any adverse effect on the investigated soil microorganisms and the plant growth. At the higher Ag-NM concentration, a constant inhibition of the soil microflora (ammonium oxidizing bacteria and substrate-induced respiration) was observed over about 3 years in the lysimeter study, while there was no effect at the lower Ag-NM concentration. The ecotoxicological results of the laboratory experiment over 180 days reflect the findings of the lysimeter study. For Ag-NM and CeO2-NM the results indicate that a hazard assessment based on data from laboratory tests is acceptable.

4.1.4 Investigating effects of silver nanoparticles on the soil community – An outdoor TME study

Monika Hammers-Wirtz, *Johanna Oellers, Anette Fürste, Susanne Miller, Steffi Peeters, Nadine Willius and Andreas Toschki*

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The production and use of silver nanoparticles (AgNP) have markedly increased in the recent years resulting in an increasing release of AgNP into the environment. The main entry of AgNP into the environment occurs via the use of sewage sludge on arable land making the soil the compartment receiving the highest load of AgNP compared to the aquatic environment. Nevertheless, so far, most studies investigating the potential effects of AgNP have focused on the aquatic environment, while investigations of effects on soil organisms are scarce. The increasing number of studies comprising soil organisms have been conducted with single species exposed to AgNP for a short-term period. However, there is a lack of knowledge as to how a natural complex soil community is affected by AgNP over a longer period of time.

Ecotoxicological impacts on soil communities can be studied using Terrestrial Model Ecosystems (TME). This higher tier approach has been developed to investigate the impact of pesticides on the soil fauna under outdoor conditions over a time period of about one year. It has proven itself as a valid method to detect both effects on the community level as well as on the population level and to observe a potential recovery during the study period.

In our project "Nanomobil", funded by the German Federal Ministry of Education and Research (BMBF), we have been using the TME approach to investigate potential long-term effects of two differently stabilized AgNPs (AgPURE, PVP coated AgNP) on the soil community in comparison to $AgNO_3$. The TME study was performed with intact soil cores sampled from an untreated grassland (diameter of 0.47 m, height of 0.40 m). The AgNP and $AgNO_3$ were applied once by spraying the test solutions on the surface of the soil followed by irrigation. Afterwards soil cores and pitfall traps were sampled several times (after 14 days, 28 days and after 3, 6 and 12 months) to investigate short-term and long-term effects on collembolans and oribatid mites, two important mesofauna groups. Furthermore, after 1 year effects on the earthworm community were investigated by destructive sampling. The study addressed the effects of environmentally relevant concentrations (1 and 10 mg Ag/kg soil). In this presentation the results of the TME study are shown.

4.1.5 NanoUmwelt - Risk analysis of engineered nanomaterials in the environment: identification, quantification and analysis of the human- and ecotoxicological effects *(Florian Meier, Postnova Analytics GmbH, GER)*

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With rising numbers of nano-enhanced consumer products, an increased release of engineered nanomaterials (ENM) into the environment is obvious with consequences yet difficult to predict. However, questions on the fate and behavior of ENM in the environment as well as their human- and ecotoxicological effects are challenging and still widely unexplored. This is mainly due to the lack of appropriate analytical tools for the investigation of ENM and their interactions at trace level concentrations in complex human and environmental matrices.

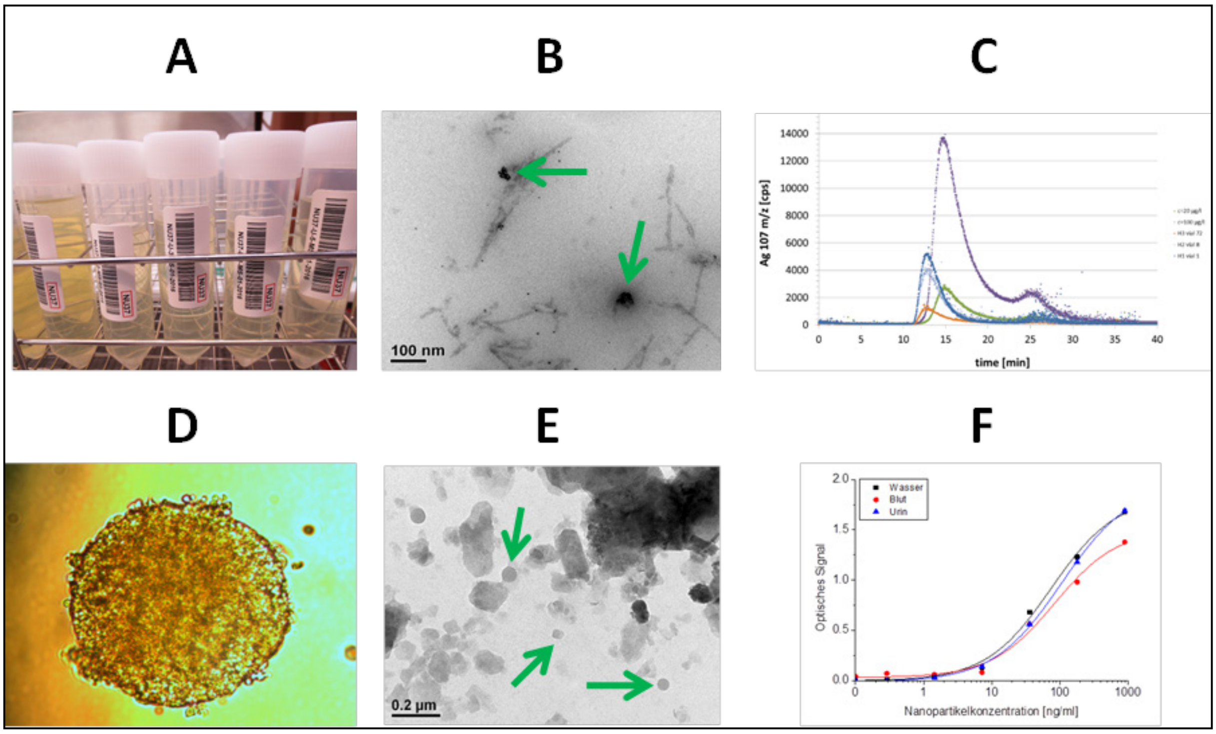
Against this background, the scope of the project NanoUmwelt is to quantify the entry of ENM in various environmental compartments, to characterize their form of appearance and to gain scientifically sound statements on the human- and ecotoxicological impact.

This presentation comprises an overview of the main achievements reached within the frame of NanoUmwelt.

This includes:

- The development of standardized sample preparation protocols for the characterization of ENM in complex human and biological matrices
- The characterization of ENM in complex human and biological matrices at trace level concentrations using field-flow fractionation and electron microscopy
- The development and validation of biological model systems for the investigation of ENM-transport across human, biological barriers and their application in human toxicological studies
- Eco-toxicological studies on the impact of ENM on the soil community at environmentally relevant concentrations

With the herein presented novel methodologies, NanoUmwelt offers promising tools and guidelines for a more comprehensive risk assessment eventually protecting human life and the environment from potential risks associated with ENM.



Some of the achieved results within NanoUmwelt at a glance.

A: Stored 24 h human urine; **B**: TEM image of AgNP in human urine; **C**: AF4-ICP-MS fractogram of AgNP in soil eluate; **D**: embryoid bodies from pluripotent stem cells; **E**: TEM image of polystyrene NP in loamy soil; **F**: Detection of polystyrene NP in human urine and whole blood via immunofiltration (ABICAP).

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NanoUmwelt

4.1.6 Fate and effect of manufactured nanomaterials in the aquatic food chain *(Christian Schlechtriem, Fraunhofer IME, GER)*

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Manufactured nanomaterials (MNMs) are increasingly used as additives or active components in various applications and commercial products, e.g. textiles, sunscreens, paints, drug delivery systems, cosmetics, medical devices for diagnostic, textiles, etc. The commercialization of these products implies the possibility of exposure of humans, either via direct contact to MNMs and indirectly via the environment, respectively. Since MNMs exhibit altered physical and chemical properties compared to bulk materials, such as higher reactivity or higher specific surface area, and since they may show enhanced uptake due to their size, potentially hazardous biological outcomes are a concern with obvious relevance not only for regulatory and legislative bodies. A well-founded risk assessment thus plays a central role for the use of MNMs. To date, however, crucial information and adequate understanding on the fate of MNMs in the environment and possible adverse effects both in humans and in other biota are lacking. The project FENOMENO funded by FP7 ERA-NET (SIINN) is an integrative project aiming at an understanding of the impact of end-of-life manufactured nanomaterials (MNMs) on the environment. Even though MNMs are mostly removed during wastewater treatment (WWT), the remaining MNM levels in the effluents are significant and MNMs may show an increased toxicity for aquatic organisms due to their modification during the WWT. The biological impact of wastewater-borne MNMs (TiO_2 and Ag MNMs) on different trophic levels and their bioaccumulation within a relevant food chain (algae-Daphnia-fish) have been investigated with innovative analytical and experimental approaches. In a further project funded by German Environment Agency (FKZ 3716 66 410 0) investigations are underway to elucidate the bioaccumulation of MNMs (e.g. Ag MNMs) in freshwater bivalve mollusks (*Corbicula fluminea*) using a new test method. Based on the results achieved, the bioaccumulation potential and possible adverse effects of MNMs in the aquatic food chain are discussed.

4.2 Session 4: Fate and Exposure in different environmental compartments

4.2.1 Getting our feet back on the ground: How the environment controls nanoparticle fate

Frank von der Kammer

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Research efforts into nanomaterials (NMs) environmental behavior and fate as well as their toxicological potentials have been triggered by the hypothesis that they bare unique properties, different from the bulk material, predominantly owed to their small size and high surface to volume ratio. In terms of their environmental transport and fate it has however become evident that many of these NMs behave very similar to natural colloids and nanoparticles already present in the environment. In fact most publications dealing with the behavior of NMs in natural waters confirm the general principles known for natural and synthetic, near-natural colloids. General rules for colloid behavior in natural waters, soil and groundwater can therefore be derived from existing studies dealing with natural systems or with colloids mimicking natural particles. However, NMs triggered also studies with much wider scope and more detailed investigations. Examples are NM transformation reactions, influence of NM coatings, relevance of complex aquatic chemistry and the investigation of heteroaggregation processes with natural particles. While many of these investigations revealed a typical to-be-expected behavior of NMs according to their intrinsic properties and hydrochemical conditions as e.g. sulfidation of NMs composed of chalcophile elements as silver and copper. Quantitative data as dissolution/transformation rates, (hetero-)aggregation rates and attachment efficiencies are still scarce. Additionally there is a strong demand to determine the behavior and fate of NMs in the environment in as realistic scenarios as possible. Not surprisingly investigations on natural colloid behavior and fate have revealed that working in systems which resemble natural waters, sediments and soils very realistically tend to render the obtained results as being mostly descriptive. Detailed quantitative information on specific processes are more likely to be gained in systems of reduced complexity and under well controlled conditions. This brings NM research in environmental media back to the well-known conflict: that when working in the real environment the complexity of environmental systems hinders the quantitative understanding of processes while working under reduced complexity produces data with little environmental relevance.

Combining the experiences from hydrochemistry, environmental colloid chemistry, colloid and NM analysis and the recent findings on NM fate and behavior could enable us to focus our environmental models to the relevant characteristics and parameters while still gaining a realism that serves our needs regarding NM transport, transformation and environmental exposure. This will be illustrated by the development of TG318 on dispersion stability and agglomeration behavior of NMs, one of the first NM-related OECD test guidelines.

4.2.2 The fate of nanoparticles in the subsurface - a risk for drinking water resources?

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With increasing production and application of engineered inorganic nanoparticles (EINP) their release into the environment may also increase. Nanoparticles may enter soils and water through various pathways. Once they enter these environmental compartments, they are subject to various processes, which further determine their colloidal stability and their interaction with solid surfaces. These include the adsorption of solutes such as dissolved organic matter and inorganic ions, homoaggregation as well as heteroaggregation with natural colloids and suspended matter. Together with the flow regime in the subsurface, these processes will determine the transport and retention potential of EINP in porous media. Understanding the fate and transport of EINP is essential to assess the potential occurrence of EINP in groundwater and riverbank filtrates, both serving as potential resources for drinking water supplies. The first part of the talk will give an overview of the current state of the literature.

In the second part of the talk, two examples of our own research conducted in saturated sand filter columns will be presented. They will address (i) the role of natural (natural organic matter from a drinking water reservoir (NOM) and synthetic (polyacrylic acid, PAA) coatings on the transport of CeO_2-NP in laboratory columns and (ii) the effect of environmental transformation ('aging') of Ag NP in soil extract and river water on their transport and remobilization potential under near-natural conditions. (i) Results demonstrated enhanced transport of CeO_2-NP coated with NOM and PAA over uncoated CeO_2-NP, with NOM and PAA preventing EINP aggregation through charge and steric effects. (ii) In general, the retention of Ag NP in the sand filter was high. Ag NP 'aged' in soil-extract showed higher mobility than Ag NP 'aged' in river water. Similarly, remobilization as determined from batch experiments was highest for soil-aged Ag NP. Dissolved organic matter, low ionic strength and the presence of physical forces enhanced the remobilization potential, whereas Ca ions in solution decreased it. These findings suggest that changing environmental conditions may lead to a remobilization of previously retained Ag NP.

Our results demonstrate that fate and transport of EINP may vary significantly. Hence, a case-specific assessment of EINP in the subsurface seems necessary to determine the vulnerability of potential drinking water resources.

4.2.3 Behavior of engineered nanoparticles in the pathway wastewater - sewage sludge - plant using the examples TiO_2, CeO_2, MWCNT and quantum dots

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The Project NanoSuppe is concerned with the behavior of nanoparticles along the pathway wastewater – sewage sludge – plant as this is one of the main expected paths in the life cycle of manufactured nanomaterials (MNMs) and marks the possible preliminary end of life of a MNM when it is removed from contaminated wastewater, as well as the potential reintroduction into the human food chain by the use of contaminated sewage sludge as fertilizer. Hence, work was conducted concerning the fate of MNMs in wastewater treatment plants by means of model WWTPs in the lab. Furthermore, environmental mobility studies concerning colloidal stability of MNMs in natural waters and their interaction with soil and sewage sludge were conducted by means of batch and column experiments. As a last step the uptake of MNMs by plants was investigated from hydroponic media as well as soil/sewage sludge matrices.

As a rather unique feature the used MNMs (TiO_2, CeO_2, MWCNTs and quantum dots) were radiolabeled to enable their sensitive detection at environmentally relevant concentrations in the complex matrices involved.

4.2.4 Investigation on possible risks - Are ENMs released during the combustion of nanoparticle-containing waste?

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Currently, a multitude of products is available on the market containing so-called ENMs (engineered nanomaterials). In the upcoming years, a further augmentation of ENM is predicted, which results at the end of their life-cycle in a higher amount of nanomaterials in the waste. However, a lack of knowledge exists in the environmental fate of nanomaterials during its waste disposal. These circumstances were taken into consideration during two research projects, coordinated by Fraunhofer UMSICHT.

First project called “Assessment of the emission behavior of nanomaterial-containing waste in thermal treatment plants” was commissioned by the German Federal Environment Agency. The project was determined to examine in detail the emission pathways during thermal waste treatment. Firstly, the knowledge level was depicted concerning the amount and distribution of nanomaterial-containing waste. Secondly, the principal mechanism and processes of nanomaterials during thermal waste treatment process were examined by systematic experiments with nanostructured titanium dioxide in small heating systems. Based on these results, measurements in real waste incineration plants and sewage sludge incinerators were completed examining the behavior of nanomaterial-containing waste during combustion. It was indicated that most of the used reference material was located in the solid residues (i.e., bottom ash) while a smaller part was detected in the products of the flue gas treatment. In the clean gas before the stack, the concentration was negligible; therefore, an emission to the environment is not expected. The examined incineration and flue gas purification plants comply with the requirements of the best available techniques. The results of the experiments cannot be transferred to plants with lower standards.

In another cooperation project "Investigation of the Emission Behavior of Nanoparticles in Waste Incineration"coordinated by Fraunhofer UMSICHT and funded by the Federal Ministry of Education and Research the behavior and the deposition of nanoparticles (barium sulfate) during thermal treatment have been investigated for nanomaterial-containing waste. The entire route from the residue to combustion, filtering of the exhaust gas, the release into the environment as well as the toxicological evaluation of the effect on humans and the environment was considered.

4.2.5 Mobility of different nanomaterials in unsaturated soils

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Nanomaterials are commonly used in many everyday life products. Depending on their application they may directly or indirectly enter the environment during their life cycle. An example for a direct exposure pathway to soil ecosystems is the application of pesticides containing nanomaterials and an indirect via the application of sewage sludge containing nanomaterials. Nanomaterials may reach the waste water and afterwards the sewage treatment plant during or after their usage for example in textiles, cosmetics or paints.

In the presented study laboratory experiments were performed to investigate the mobility of different types of nanomaterials in unsaturated sand and soil systems, based on the OECD test guideline 312. The mobility has been studied in dependence of material characteristics (functionalisation, hydrophobicity, size, zetapotential) as well as the effect of different soil characteristics like clay content, pH-value, organic matter or cation exchange capacity on the adsorption and mobility behaviour. Furthermore the carrier effect of P25 (TiO2) to copper or the pollutant triclocarban was investigated, as some studies have shown the ability of nanomaterials to operate as trojan horse for other substances. This behaviour can be important if the nanomaterials will get in contact with other substances e.g. in sewage treatment system, which may be harmful for the environment.

In our study the tested nanomaterials showed at most a low mobility in all soil types, so probably the main part of the nanomaterial is absorbed effectively by the soils. Only for the hydrophobic, soluble and coated nanomaterials a transport was indicated. Regarding the carrier effect a tendency to lower mobility of the other substances was observed in the presence of P25. We hypothesize that the tendency to lower mobility of copper as well as triclocarban can be explained by their adsorption onto the surface of P25. The low mobility of P25 therefore leads to low mobility of the substance and in consequence to a higher exposure concentration for plants and animals living in the upper soil layers.

4.2.6 Measurement of manufactured nanomaterials in environmental samples

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Detection and quantification of nanomaterials of anthropogenic origin in environmentally relevant samples is a major challenge. Complex matrices, interference by (non-particulate) species of similar or identical composition and low concentrations of (transformed) manufactured nanomaterials in environmental samples are some of the challenges, which need sophisticated analytical solutions, which ideally should be applicable by many users to allow for an effective environmental monitoring.

Especially in the case of metallic nanoparticles cloud point extraction (CPE) is a very helpful tool as it allows for the selective extraction of nanoparticles from complex matrices. CPE is a simple and fast operation, which can be performed in virtually every laboratory. High enrichment factors around 100 from initial volumes of 40 ml are possible and the extracted particles are not modified by the CPE procedure with respect to their shape and composition. Element specific chemical adaptions during sample preparation allow the selective extraction of different nanoparticles.

Silver-based nanoparticles (Ag-b-NPs) for example, which are suspected to pose a particular risk to aquatic microorganisms can be selectively enriched by CPE and detected with electrothermal atomic absorption spectrometry (ETAAS) or ICP-MS with detection limits as low as 0.2 ng/L. In combination with single particle ICP-MS (spICP-MS), size-selective measurements with a detection limit of 2 ng/L for particles with a size down to 10 nm can also be performed paving a way for a more comprehensive understanding of the formation and distribution of Ag-b-NPs in the environment. CPE also works with other environmentally relevant elements e.g. palladium, platinum and rhodium, which are emitted in nanoparticulate form by car exhaust catalysts. CPE-ICP-MS and CPE-spICP-MS hereby also allow for low detection limits in the subng/L range and fast size selective measurements down to particle sizes of 3 nm (rhodium).

Applying CPE-ETAAS we were able to measure the concentration of Ag-b-NPs in all process steps of representative waste water treatment plants (WWTPs) and the extent to which WWTP effluent influences the Ag-b-NP concentration along a river from its source to its estuary. Moreover, the natural formation of Ag-b-NPs from geogenic silver sources in pre-alpine lakes was studied. The concentration of the naturally formed Ag-b-NPs (up to 2.5 ng/L) is very similar to the final Ag-b-NP concentration in the river Isar (2 ng/L) caused by WWTP effluent.

4.3 Session 5: Applications and Assessment

4.3.1 Nanopesticides: state of knowledge and implications for regulatory exposure assessment

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Pesticides and fertilisers are intensively used in agriculture and much effort is expended to manage and reduce possible deleterious effects on the environment. Nanoformulations are already used in the pharmaceutical or personal care products sector. In comparison, applications in the agrochemical sector are only just emerging and many predict a rapid growth in coming years. Deliberate application of nanoparticles within agricultural practices could result in one of the rare intentional diffuse inputs of engineered nanoparticles into the environment, and may thus be a topic of great concern. The anticipated new or enhanced activity of nanoagrochemicals will inevitably result in both new risks and new benefits to human and environmental health. After many years of research and development, nanoagrochemicals are starting to make their way onto the market. The introduction of this technology raises a number of issues, and it is essential that the regulatory framework is ready for the evaluation of these new products. The aim of the presentation will be to (i) review the current state of knowledge on the application of nanotechnology for developing new agrochemicals, and (ii) analyse the suitability of current exposure assessment procedures to account for the new risks and benefits, and discuss options for refinements.

We carried out extensive analyses of the literature and combined relevant information from published literature, company websites, patent databases, reports from governmental and non-governmental institutions [1,2]. The term "nanopesticide" encompasses a great variety of products and should not be considered as a single category. While some authors have expressed concerns regarding altered risk profile of the new products, many others foresee a great potential to reduce the impact that modern agriculture has on human and environmental health. The majority of nanoagrochemicals suggested up to now appears to be the reformulation of conventional active substances. Targeted delivery and/or increased efficacy are two strategies that could lead to reduced application rates, and thus reduced potential impact relative to conventional agrochemicals.

The adequate exposure assessment of nanopesticides is essential to identify the potential new risks and benefits relative to currently used agrochemicals [3], but data on the environmental fate of nanopesticides are very scarce. The standard regulatory protocols to determine fate parameters (OECD tests for sorption, degradation and leaching) were applied to series of nanopesticides covering a wide range of properties [4,5]. Experimental data indicate that nanoformulations (i) can significantly influence the environmental fate of conventional active substances, and that (ii) discrepancies relative to the pure active ingredient strongly depend on the soil-nanopesticide combination. Exposure modelling exercises carried out with models used for pesticide assessment in the EU indicate that adaptations are possible but that predicted environmental concentrations should be interpreted with great care [5].

[1] Kah et al. (2013) Crit. Rev. Env. Sci. Tech. 43, 1823-1867 [2] Kah and Hofmann, T. (2014) Env. Intern. 63, 224-235 [3] Kookana et al. (2014) J.Agri. Food Chem. 62, 4227-4240 [4] Kah et al. (2014) *Env. Sci. Pol. Res.*21, 11699-11707 [5] Kah et al. (2016) ES&T 50, 10960-10967

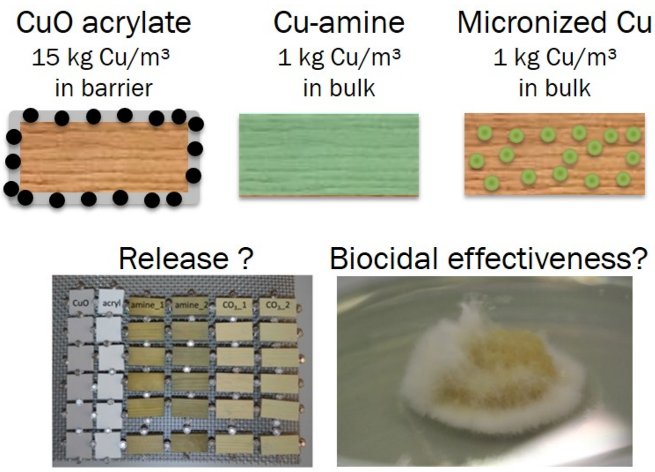
4.3.2 Comparing nano-enabled copper formulations govern release, hazards, antifungal effectiveness and sustainability throughout the wood protection lifecycle

W. Wohlleben*, BASF SE and numerous SUN FP7 partners*

Copper (Cu) is the most widely used fungicide for treating wood in contact with the soil, with no satisfactory alternative available since copper is the only biocide that shows significant effects against soft rot fungi and other soil borne fungi. The partners of the SUN project compared the conventional European benchmark of wood treatment by molecularly dissolved Cu-amine against two nano-enabled formulations: CuO NPs in an acrylic paint to concentrate Cu as a barrier on the wood surface, and a suspension on micronized basic copper carbonate ($CuCO_3 \cdot Cu(OH)_2$) for wood pressure treatment.

After characterizing the properties of the (nano)materials and their formulations, we assessed the pristine material *in vitro* effects against three species of wood decay fungi – the brown rot copper tolerant *C. puteana*, the white rot copper tolerant *T. versicolor* and the brown rot copper sensitive *G. trabeum*. Without the integration into the wood, antifungal effects were significantly different between formulation and were mediated only partially by ionic transformation. To assess the use phase, several protocols quantified release *rate* and *form*. Cu leaching rates for the two types of impregnated wood are not significantly different at 120 mg/m^2, with Cu being released predominantly in ionic form. Synergistic stresses with release sampling by run-off, during condensation, by different levels of shear, all resulted in comparable *form* and *rate* of release from the nano-enabled or the molecular impregnated woods. In contrast, Cu release from wood coated with the CuO acrylate contained particulate release *forms* but the *rate* was at least 100-fold lower. In the exact same ranking, the effectiveness to protect against the wood-decaying basidiomycete *Coniophora puteana* was good by both impregnation technologies, but remained insignificant compared to untreated wood by the CuO acrylic coating.

Overall, it appears that the transformation of the Cu-containing compounds from synthesis of the pristine materials via their formulation to release during the use phase are dramatic. The micronized form of Cu that was initially synthesized as nanoform, releases during the use phase the same form (Cu ions) as conventional (molecular, non-nano) forms of Cu. Both nanoform and conventional form thus appear equally effective against fungus during the use phase, but not if tested as formulations.



The behavior during the use phase determines both user benefit and environmental risk. The formulation determines human occupational risk. To further reduce the uncertainty in the estimates and assumptions, materials were tested in vivo by short term oral screening (STOS) and Short-Term Inhalation Study (STIS) and were subjected to soil ecotoxicity tests on *Enchytraeus crypticus*, soil microorganisms and fish cell lines. A "safer by design" surface-modified CuO was generated but was not compatible with product integration. Overall, the LICARA nanoscan indicates that the CuO acrylic coating is less sustainable than the technological alternatives, and should not be developed into a commercial product.

4.3.3 Volumes and Life Cycle of CeO2, SiO2 and Ag Nanomaterials - Knowns and Unknowns

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The precautionary principle demands a preferably prospective analysis of risks that are connected to technical processes and products. In order to conduct a risk assessment for engineered nanomaterials

(ENM), data on ENM-volumes released into natural and technical compartments and information about their toxicological relevance is required. Given the increasing number of applications of engineered nanomaterials (ENM) (cp. The Royal Society & The Royal Academy of Engineering 2004; Sun et al. 2017), a constant strong growth of ENM production is expected (Ricardo Energy & Environment 2016). Most probably, release of ENM into natural compartments will increase along with the intensified use of ENM. As recent toxicological findings for TiO_2-ENM show, an accepted use of a substance does not imply innocuousness (Bettini et al. 2017).

Apart from uncertainties regarding ENM-behavior in natural compartments, a critical aspect in modeling the use and fate of ENM and ENM-containing products is the lack of data on production and application due to i) a restrictive information policy of the involved companies and ii) insufficient analysis. One category of unknowns comprises information on the amount of produced ENM, its use and concentration in products and potential variations of its molecular structure or surface. Whereas initial volumes and concentrations finally affect the probabilistic environmental concentration (PEC), chemical modifications may have a profound influence on the environmental fate of ENM. Uncertainties of a second category are related to the release of ENM in specific applications, the respective product lifetime, the form of released ENM (as single particles, as aggregates, agglomerates or as a composite with other particles and matrix material). Beyond the use stage, lack of data also comprises relevant information on the EOL as e.g., the export fraction, the volume of recycled material and the share of ENM containing waste in the different technical compartments (waste incineration plants, landfills and sewage treatment plants).

In a comprehensive analysis we gathered the currently available knowledge on ENM-production, products and releases of ENM corresponding to their use in products and processes to estimate the time-dependent exposure to nanosilver (Ag-ENM), synthetic amorphous silica (SiO_2-ENM) and CeO_2-ENM. The said ENM where chosen on the one hand because of their meanwhile manifold use and on the other hand due to their high production volumes. Nanosilver has the longest tradition in use for medical purposes since the beginning of the last century due to its antimicrobial properties (Fung and Bowen 1996). Forms of synthetic amorphous silica (SAS) are produced in large amounts and incorporated in a variety of products and processes since the middle of the last century (JACC 2006). And also CeO_2 is used in a growing number of applications (Dahle and Arai 2015).

Numbers for the produced volume of ENM are hard to obtain. In the present study, estimates on global production volumes from previous publications where updated by a survey among producers, dealers and research institutions in the field of nanomaterials. To develop a categorization for the application of Ag-, SiO_2- and CeO_2-ENM in products and processes, databases for products containing ENM as well as scientific literature and product brochures where analyzed. Our investigation was hindered by the fact that most well-known databases are rather focused on household articles. Products with higher relevance with regard to the used volume of ENM seem to be underrepresented as for example tires or industrial processes e.g., chemical mechanical planarization (CMP), where the largest share of CeO_2-ENM is used.

For the identified product categories, release-specific information about the use phase as well as end of life (EOL) in combination with respective masses and trends enabled the reconstruction of a model[1] for the time dependent past, present and future mass flows and environmental releases of SAS, CeO_2-ENM

[1] The probabilistic model incl. a detailed presentation of results is given in the contribution of F. Gottschalk and B. Giese “Environmental release modelling in contexts of high uncertainty for engineered nano-CeO_2, -SiO_2 and –Ag in Germany” (see abstract in scientific key note 2 of this report).

and nanosilver. SAS can be differentiated into pyrogenic or precipitated silica, silica gel or sol with different physical and chemical properties that may lead to different (toxicological) effects. In contrast to colloidal silica for which only minimal overall toxicity was shown (Zhang et al. 2012), pyrogenic silica induced toxic effects and showed signs of inflammatory reactions in cell culture assays (Sandberg et al. 2012; Irfan et al. 2014). We investigated applications and respective mass flows separately for each type of SAS to establish a basis for a continuing differentiated analysis of SAS-types. For precipitated silica – the largest quantity of SAS – rubber products as tires and shoe soles represent important sources of diffuse ENM-release. Besides the use of CeO_2-ENM in batteries and for CMP, a diffuse source of CeO_2-ENM emission is release from exhaust systems due to its use in automobile catalytic converters and diesel fuel additives. With regard to nanosilver, the major share of released ENM potentially stems from textiles. Further relevant products containing nanosilver with the same release path via wastewater are cleaning agents and cosmetics.

However, due to knowledge gaps and the sheer variety of applications the assessment of ENM release, exposure and risk still relies on simplifying assumptions. But in the future to an increasing degree we will have the opportunity to check the validity of modeling outputs because a growing number of measurements of environmental nanomaterial concentrations is available[2].

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4.3.4 Prosafe's Review on Reliability of Methods and Data for Regulatory Assessment of Nanomaterial Risks – Part Environment

***Klaus Steinhäuser**, Germany*

Within the EU funded project Prosafe a review on the regulatory relevance of the results of several EU and US funded nanosafety research projects was conducted. The objective was to identify those methods, data and protocols that are appropriate for regulatory risk assessment of manufactured nanomaterials. A task force with nine experienced experts was established which examined 23 research programmes as well as peer reviewed open literature. They looked at reliability and regulatory relevance as the main criteria to identify which research products are most useful to regulators. Not all of the research results examined are reliable or even relevant for regulatory purposes. Some studies have significant deficiencies, e.g. the nanomaterials were not characterised appropriately. However, the review demonstrates that a number of tools to enable regulatory risk assessment of nanomaterials are now available or near completion. It will be necessary to increase the efforts to validate and standardize the identified suitable methods, e.g. within the OECD.

Adoption of the regulatory-ready procedures could lead to an improved regulatory review process for nanomaterials, despite the complexities related to their physicochemical properties such as size, shape, and surface chemistry. These properties create concern as functionality plays as important a role as chemistry. Nanomaterials of the same chemical composition may occur in different nanoforms with different size, surface characteristics etc. and may exhibit different hazard profiles. Many parameters can vary from nanoform to nanoform or even within the same nanoform, depending on the medium they exist in. Whereas the methods and tools for conventional chemicals are in principle suitable for manufactured nanomaterials, the unique properties of nanomaterials require that harmonized methods and tools have to be adapted, as well as new ones developed. Tiered testing and assessment schemes, functional assays, modelling tools and *in vitro* tests gain importance in order to reduce complexity and to make risk assessment workable.

The presentation will highlight some selected results of the review that are relevant to environmental risk assessment. Important knowledge gaps are identified and future needs for regulatory research are presented.

Nanomaterial structures are becoming increasingly complex. Moreover, nanotechnology will converge with bio- and information technologies. It will be a challenge for researchers and regulators in nanosafety to keep pace with scientific and technical progress.

4.3.5 Grouping/Read Across approaches to reduce the testing of nanomaterials regarding their environmental hazard

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Nanomaterials (NM) offer a great innovation potential and are assumed to be beneficial to mankind and the environment by e.g. reducing material usage and energy consumption. However, the large variety of synthetic nanomaterials represents a major challenge for scientists and regulators in terms of measuring and assessing the potential hazard caused by the materials and the products over the whole life-cycle. Currently, the assessment of potential hazards posed by NM towards environmental organisms is performed on a case-by-case basis, which is considered as not practicable for the many different variations of NM, which involve differences in composition, size, shape, crystalline structure and surface modifications.

In order to overcome the need for extensive testing, grouping and read across approaches for NM are considered helpful. The prediction of NM hazard based on existing knowledge requires the identification of relationships between nanomaterials' physicochemical properties and their ecotoxicological behavior. To gain knowledge on this relationship, we used several approaches involving extensive literature research, the formulation of grouping hypotheses based on physical-chemical parameters, as well as systematic toxicity testing. For the latter purpose, a test set of NM was compiled, involving in most cases several subtypes of a given nanoscaled substance, which differed in the parameter considered as driver for a specific effect. The NM test set underwent testing in the ecotoxicological relevant aquatic (algae, daphnia and zebrafish embryo) and terrestrial organisms (earth worm, microorganisms). All organism tests were conducted according to OECD guidelines under consideration of NM-specific modifications. The NM selected for testing were extensively characterized regarding their properties and their behavior in the respective ecotoxicological test media.

The talk will provide an overview on the most crucial results obtained so far. Basically it became obvious that an identification of groups consisting of NM with comparable ecotoxicity based on literature data is of limited value, because of fragmented reporting on nanomaterials' physical-chemical properties, the application of different methods to characterize the NM as well as a high variability in the applied test procedures. Further, as the systematic testing showed, there are no mono-causal relationships between one physical-chemical parameter and an ecotoxic effect. Our current proposal for a qualitative grouping scheme therefore considers various parameters. The reliability of the grouping scheme was tested by applying it to a further data set not used for the development of the scheme. It became obvious that not all NM exerting a comparable ecotoxicity grouped into the same group and accordingly, further parameters beyond the ones already considered in the grouping scheme need to be taken into account. Hence, in order to facilitate grouping with focus on environmental effects, further steps need to be taken.

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5 Abstracts of Poster Presentations

P01: Supporting the environmental risk assessment of nanomaterials with quality-approved information - the DaNa Literature Criteria Checklist

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Nanotechnology is of increasing significance for many sectors of industry opening the market for numerous new applications, ranging from electronics to health care and environmental remediation techniques. Nanomaterials offer a great innovative potential and they are assumed to be beneficial to mankind and the environment by e.g. reducing material usage and energy consumption. However, the large variety of synthetic nanomaterials developed in the last decade together with all current and future new (nano)materials represent a major challenge for scientists and regulators in terms of measuring and assessing the potential hazard caused by the materials and the products over the whole life-cycle.

This diversity is reflected in a considerable need for reliable and understandable information on nanomaterials, nanotechnology as well as nanosafety by scientist and non-scientists (e.g. consumers) alike. In order to facilitate the knowledge communication in these fields, the website www.nanoobjects.info was set up in 2009. Since then, the international DaNa (*Data and knowledge on nanomaterials*) expert team built a comprehensive web platform. It offers easy-to-understand, up-to-date and quality-approved information on 26 market-relevant nanomaterials concerning their effects on safety of humans and the environment, making an important contribution to science communication in the field of nanosafety.

Besides wrapping up the results of current research on nanomaterials regarding their influence on humans and the environment in an understandable way, the DaNa-project acts as an umbrella project for current projects funded by the BMBF (German Federal Ministry of Education and Research) with regards to safety of nanotechnology as well as their ecological benefits. Our presentation of complex scientific data as well as our methodology to evaluate the quality of scientific publications is acknowledged worldwide as evidenced by around 13.000 visitors to our page every month.

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P02: Tools for ENM safety assessment - OECD Test Guidelines and Guidance Documents and how to develop them

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The OECD test guidelines (TGs) for testing chemicals have been widely used for regulatory purposes all over the world since the establishment of the Mutual Acceptance of Data (MAD) principle in 1984. This MAD principle ensures that, if a chemical is tested under the Good Laboratory Practice (GLP) conditions accordingly to an OECD TG, the data should be accepted in all OECD countries. The TGs have been developed, harmonized, internationally validated (ring-tests) and adopted by OECD countries to be used for the hazard identification and risk assessment of various chemicals. OECD also publishes Guidance Documents (GDs), which usually give guidance how to use TGs and how to interpret the results. These GDs do not have to be fully experimentally validated, and hence they are not under MAD, but they are based on the latest published scientific research.

But are the TGs with the GDs applicable and adequate for the regulatory testing of nanomaterials? In principle, most of the "endpoints" or more precisely measurement variables are applicable. However, for some endpoints new TGs are needed. In addition, more precise advice on the test performance, e.g. including sample preparation and dosage of the test material, the characterization of the exposure and understanding the results need specific guidance, in order to gain regulatory relevant data.

The poster will illustrate the way from the idea for a new TG and new GD to an accepted OECD TG/GD guideline and which issues have to be considered during that procedure. In addition, it will list the ongoing activities of OECD to amend the TG for testing of chemicals in order to address the requirements for an adequate hazard identification and risk assessment of nanomaterials.

P03: Grouping of nanomaterials regarding their ecotoxicity – the ECOTOX tool

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Given the numerous manufactured nanomaterials (NM) already on the market and expected in future, the effort for the individual investigation and assessment would be enormous. Therefore, it is necessary to develop approaches that allow an adequate hazard assessment of NM while avoiding individual testing of a large number of the different forms. Approaches to meet data requirements in deviation from performing standard test requirements are already established for chemical substances. One of these is the grouping and read-across/analogue approach. In comparison to soluble organic substances, nanomaterials of the same chemical substance differ in their physicochemical parameters (e.g. surface chemistry, size, shape) and can potentially differ in their hazard profiles. But also fate related physicochemical properties which are influenced by surrounding conditions, like dispersion stability or dissolution rate, influence bioavailability and effects of NM. Annotation of NM to specific hazard groups or read-across based on their physical-chemical (PC) properties will be a great advantage to reduce the amount of testing necessary for an adequate risk assessment.

To identify PC properties which are relevant predictors for ecotoxicity, a literature research was performed. It was found that sparsely or non-soluble, and ion-releasing NM need to be considered differently. We have found that ecotoxicity correlates with more than one PC characteristic. Based on this finding the tool ECOTOX BOND was developed. ECOTOX BOND was tested for robustness with experimental data on eight NM in 23 modifications. The predicted and measured ecotoxicity of the NM tested mostly fit, but the ecotoxicity of green algae for TiO_2, CeO_2 and Fe_2O_3 is higher than predicted. This effect could be caused by shading and is investigated in more detail.

P04: Investigating the Trojan horse effect of nanoparticles on an aquatic community – An outdoor mesocosm study

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Carbon based manufactured nanomaterials (C-MNMs) are promising materials in nanotechnology. Although both fullerenes and carbon nanotubes have been detected in aquatic organisms, there is a lack of data on their bioaccumulation, potential toxic effects, transfer in the food chain, and interaction with other anthropogenic pollutants.

Most effect studies performed until now dealt with waterborne exposure of single species for short time periods in the laboratory. Here, we present a long-term experiment under environmentally relevant conditions. In particular, the Trojan horse effect has been investigated in this study, in order to obtain more data on the interaction between nanoparticles, other pollutants and biota. In principle, pollutants can become more bioavailable by adsorption to carbon-based nanomaterials. In addition, a spatial transfer of contaminated nanoparticles from the water phase to the sediment could increase the exposure to benthic macroinvertebrates but might also reduce the effect on the planktonic organisms.

An outdoor freshwater mesocosm study was conducted with C60 fullerenes and the biocide triclocarban (TCC) using twelve ponds with a water volume of 3 m^3. In addition to uncontaminated controls, both substances were tested alone and in combination. The aim of this mesocosm study is to investigate long-term effects of C60 fullerenes on the community level and to assess their potential to affect the toxicity of TCC.

In this outdoor mesocosm study direct and indirect effects on single species as well as on community level endpoints like diversity were evaluated. The taxonomic groups of interest are phytoplankton, zooplankton (e.g. *Daphnia* species), and macroinvertebrate species (e.g. chironomids, mayflies, oligochaetes, leeches). Different sampling techniques were used in order to include macroinvertebrates living on and within the sediment as well as hatching insects.

In this presentation, first results of the mesocosm study will be presented.

This work has been supported by the German Federal Ministry of Education and Research (BMBF) as part of the NANO-transfer project.

P05: Freshwater outdoor mesocosms: An approach for detection of long-term effects of ENM under natural conditions

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Through the continuous increase in production volume and use of engineered nanomaterials (ENM) their release to the aquatic environment has substantially increased. Engineered copper oxide nanoparticles (CuONP), for example, are often used as biocides in ship paint or as a cheaper alternative to silver nanoparticles in health care products. A drawback of this innovative and dynamic technology is the use and disposal of newly designed products and particles without adequate knowledge of their properties and behaviour in the environment. In addition the long-term impact of the ENM on the environment is currently insufficiently understood, and the few studies existing so far do raise concern.

Aim of the study is to create a manageable test system for testing ENM under environmentally relevant conditions. Each experimental unit is a plastic container (0.6m diameter; 0.3m depth) recessed into the ground. For measurements and to prevent contamination of the surroundings they are equipped with various installations. Before the CuONP were applied it was ensured, that equilibrium conditions in the ponds exist. For a realistic representation of the environment, natural sediment is used; moreover, to mimic a realistic exposure scenario, a repeated application at low concentration takes place. Commercially available uncoated CuONP were selected for the application. In order to distinguish nano-specific and toxic effects of the copper ions, an ionic control ($CuSO_4$) is additionally installed. The model ecosystems study are currently run for 12 months. Consequently long-term effects of the engineered CuONP under realistic conditions can be characterized and analysed. Using different sampling methods and endpoints it is possible to monitor physicochemical parameters, the organism groups and their diverse habitats such as sediment, water and water surface area during the observation period.

This approach also gives the possibility to observe life cycles of nanomaterials in complex systems including aging effects of the particles and interactions with the ecosystem.

Key words: CuONP, long-term effects, outdoor mesocosms; environmentally relevant conditions

P06: Human lung cells and Bariumsulfate nanoparticles from incineration processes - data from the project NanoEmission

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More and more products marketed all over the world contain nanomaterials. A considerable number of these products ends up in waste-incineration at the end of their life-cycle. The aim of the project NanoEmission, funded by the German Federal Ministry of Education and Research (BMBF), was to assess whether nanoparticles in the exhaust air emitted from waste-incineration plants pose a risk to humans and the environment. When comparing the cytotoxicities (MTT assay, Resazurin assay, LDH assay) of nano- and micro-scale $BaSO_4$-particles before and after incineration in a technical center-scale incinerator and particles from the exhaust gas of combustion without added nanoparticles a significant difference between the effects of the various $BaSO_4$-particles on cultures of normal human bronchial epithelial cells (NHBEC) could not be detected. There were also no effects of the barium particles on Glutathion (GSH) level. Nevertheless particles from combustion without barium particles induced GSH in lung cells after 24 hours. In line with these findings fresh $BaSO_4$ particles did not induce reactive oxygen species (ROS) in human lung cells. Also there were no significant differences between the cytotoxic effects of fresh $BaSO_4$-nanoparticles and particles up to 200 nm from the exhaust gas of a large-scale waste incineration plant resulting from combustion of household waste with or without added $BaSO_4$-nanoparticles. Moreover these particles did not have effects on the cellular GSH-level after 24 or 72 h and they did not induce ROS up to 0.0526 mg/cm^2. In line with these findings no significant differences in the effects on cell cultures were detected between fresh and annealed $BaSO_4$-nanoparticles. Taken together these results suggest that thermal treatment does not have a significant effect on the toxicological profiles of $BaSO_4$-nanoparticles. We also conducted Ecotoxicity tests with fresh $BaSO_4$-nanoparticles *on Chlamydomonas reinhardtii.* The results showed no effects on growth, but induction of GSH up to 140 % (1 mg/ml).

However this study is not a complete risk assessment and its results cannot be transferred directly to other nanomaterials. The mechanism behind the toxic effects of $BaSO_4$-nanoparticles could not be resolved in this project completely. Nevertheless oxidative stress does not seem to be the major driving force behind it. For completion of the risk assessment of these particles further studies are necessary.

P07: Impact of CeO_2 nano powder applied on the leaf surface on photosynthesis

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Cerium dioxide (CeO_2) has become ecologically relevant as an air pollutant forming prominent nanoparticles (CeO_2-NP) upon use in diesel fuel. Concentrations of NP on the leaf surfaces of different species vary with their surface characteristics to intercept, retain and/or absorb particles. In case of airborne CeO_2-NP, phytotoxicity is associated with the induction of reactive oxygen species (ROS) while its impact appears independent of the concentration in the tissues. The source of ROS may be either the surface of CeO_2-NP or the plant itself via defense-related metabolism (DRM).

We hypothesize (H1) dry CeO_2-NP to reduce PSII activity and (H2) the intensity of this effect to be independent of the concentration. Furthermore, we expect the effect to dependent on the leaf surface characteristics of the study species (H3). CeO_2-NP (25 nm) were applied by means of a brush on leaves of corn (*Zea mays*), basil (*Ocimum basilicum*), cabbage (*Brassica oleracea*), Rudbeckia (*Rudbeckia hirta*), clary (*Salvia officinalis*), sunflower (*Helianthus annuus*) and oak (*Quercus robur*). Effects of CeO_2-NP on leaf photosynthesis were analyzed by chlorophyll fluorescence and varied with time and species. Rudbeckia and sunflower displayed increased oxidation rates of the primary electron acceptor (q_L) upon NP application. Basil and corn reduced the non-photochemical fluorescence quenching by heat dissipation (NPQ) and increased their maximum photosynthetic efficiency (F_v'/F_m'). These results reflect characteristic patterns of DRM response. Conversely, an increase of NPQ and reduction of F_v'/F_m' were found in oak as an immediate responses. Overall, the concentration of CeO_2 in the leaf tissue varied with the species and was negatively correlated with F_v'/F_m'. Although common in the study species, the increase of NPQ was independent of the CeO_2 concentration.

In conclusion, CeO_2-NP tended to reduce the PSII functionality through the increase of NPQ and reduction of F_v'/F_m'. The increase of q_L presented in some species indicates that the reduction in F_v'/F_m' is not correlated with the inability to conduct electrons, but with the increase of NPQ.

P08: Effects of Ag NM-300K on *Folsomia candida* (Collembola) in different standard soils and in long-term aged sludge-treated soils

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Soils are considered a major sink of AgNP because AgNP enter these by application of sewage sludge from waste water treatment plants to agricultural soils. Due to the rising use of AgNP, long-term emission to soils and long-term accumulation are expected.

In this study, the effect of AgNP (NM-300K) and $AgNO_3$, as a metal salt reference, in three standard soils (OECD, RefeSol 01-A, Lufa 2.2) on *F. candida* reproduction at low (µg Ag kg^{-1} range) and medium (mg Ag kg^{-1} range) concentrations was determined. In addition, to simulate realistic exposure pathways, effects on *F. candida* reproduction after AgNP application to soil via sewage sludge and after aging this treatment in the soil for up to 140 days were studied using environmentally relevant concentrations of AgNP.

The generated data demonstrate that the presence of AgNP in the soil in the low mg Ag kg^{-1} concentration range results in significant, but concentration independent inhibition of *F. candida* reproduction. Toxic effects were found in RefeSol and Lufa soil but not in OECD soil. The toxicity did not differ between RefeSol and Lufa in this concentration range. Significant inhibition of *F. candida* reproduction due to NM-300K and $AgNO_3$ was also observed for soil amended with AgNP treated sludge. An increase in inhibition with aging of the AgNP and $AgNO_3$ in the soil was evident. This effect was more pronounced and more consistent for AgNP than for $AgNO_3$ and was detected only after aging of 60 days and longer. In conclusion, long-term experiments reveal an increase in the toxicity of NM-300K on *F. candida* with aging at environmentally relevant concentrations and realistic exposure scenarios. Consequently, the consideration of transformations and the implementation of long-term tests when performing the environmental risk assessment of AgNP (and other nanoparticles) are essential.

P09: Grouping and Read Across of Nanomaterials: Objective and Needs for Environmental Hazard Assessment

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Nanomaterials find a very wide range of applications which are not limited with regards to their specific properties. Some nanomaterials, for example TiO_2, silicon dioxide (SiO_2) or carbon black, are produced in large tonnages and have already been used for decades, and indeed some nanomaterials were already used in ancient times. These nanomaterials have found new applications as technology has developed. Other nanomaterials such as quantum dots or carbon nano tubes (CNTs) are relatively recent developments that have yet to establish themselves on the markets. Furthermore, nanomaterials of the same chemical substance are available in numerous modifications varying for instance in size, shape, or surface modification.

Given the numerous manufactured nanomaterials already on the market and expected in future, the effort for the individual investigation and assessment would be enormous. Therefore, it is necessary to develop approaches that allow an adequate hazard assessment of nanomaterials while avoiding individual testing of a large number of the different forms.

The project nanoGRAVUR (nanoGRAVUR: nanostructured materials – grouping for occupational health, consumer and environmental protection and risk mitigation) made its central objective to develop different criteria catalogues for a grouping of nanomaterials according to the respective potentials for exposure, hazard and risk. As part of the project, the developed approaches and project outcomes will be evaluated for its regulatory applicability in the area of environment, consumer and work place protection.

The poster will present how grouping and read across is defined and utilized in the context of the hazard assessment for EU chemical legislations, which guidance are available and what kind of needs and challenges exist regarding grouping of nanomaterials with respect to environmentally relevant aspects.

P10: The impact of multiwalled carbon nanotubes on toxicity and bioaccumulation of triclocarban in the freshwater algae *Desmodesmus subspicatus*

POSTER AWARD WINNER

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Carbon based manufactured nanomaterials like carbon nanotubes (CNTs) are promising materials in nanotechnology. Although CNTs have been detected in aquatic organisms, there is a lack of data on their bioaccumulation, potential chronic effects and distribution within organisms. To begin with closing these lacks, multiwalled carbon nanotubes (MWCNTs) were studied in its pristine state. Additionally, triclocarban (TCC) is widely used as an active ingredient in antibacterial bar soaps, a commonly element of domestic wastewater, and the subject of academic researchers. Equally to MWCNTs, TCC is a high production volume chemical, and also is continually released into the aquatic environment. In the present study the behavior of both, MWCNTs and TCC, were investigated as a function of another. Therefore, MWCNTs were studied for the adsorption of TCC as a representative type of pharmaceutical and personal care products (PPCPs) in an algal medium, to predict behavior of these nanomaterials in the environment. To understand the effect of these chemicals on biota, the freshwater green algae *Desmodesmus subspicatus* as testorganism was chosen. Sorption to MWCNTs was assessed by using solid phase extraction as a partitioning method. In this study radiolabeled TCC (^{14}C - TCC) was used. ^{14}C - TCC log K_{MWCNT} value for OECD - medium was found to be 7.60 ± 0.29 and for Kuhl - medium 7.55 ± 0.31. The effect of algal growth inhibition of TCC was investigated as endpoint in presence and absence of MWCNTs. Growth of *D. subspicatus* was inhibited with effect concentration of 50 % (EC_{50}) value of 20.6 µg TCC/L. The test with co-exposure of MWCNTs (1 mg/L) indicate that no effect of toxicity of TCC was found. High sorption of TCC to MWCNTs might be the reason for the low bioavailability of TCC and hence no adverse effect on algae. Furthermore, the uptake of ^{14}C - TCC (log K_{OW} 4.9) in presence and absence of MWCNTs was studied. The determined bioconcentration factor (log BCF) of TCC was 4.30 ± 0.06. Whereas it was shown that uptake of ^{14}C - TCC was significantly reduced in presence of MWCNTs compared to uptake without MWCNTs. Probably, strong adsorption behavior of MWCNTs on hydrophobic organic chemicals like TCC was responsible for this observation. During uptake in both studies it could be observed that BCF decreased with increasing density of algae.

The production of carbon based manufactured nanomaterials (C-MNMs) is expected to increase in the future, and hence, entrance of these materials in the environment should be considered. For this purpose, the European project "NANO-Transfer" will further look on bioaccumulation and chronic effects of dispersed and weathered (via "simulated solar radiation") carbon nanotubes and fullerenes in different test species. To determinate fate and behavior of C-MNMs in the environment, realistic scenarios of exposure will be defined, e.g. partitioning of MWCNTs in a water-sediment system. Radioanalytical methods will be used to quantify the amounts of ^{14}C-MWCNTs in organisms, tissues and media over time. Due to radiolabeled MWCNTs organisms will be exposed to relevant environmental concentrations.

P11: Engineered silver nanoparticles in the environment: an overview of ecotoxicological effects and the mobility in natural soil

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We studied ecotoxicological effects of silver nanoparticles (AgNPs) on aquatic and terrestrial organisms and in addition the mobility of AgNPs in soil under various conditions. Since the fate and toxicology of AgNPs are strongly influenced by various factors in particular the environmental medium (e.g. soil type) all studies which will be presented were performed with natural soil (a non-polluted silty soil).

For the investigation of the ecotoxicological effects on terrestrial organisms 14 day lethality tests with earthworms (*Lumbricus terrestris* and *Eisenia fetida*) and a reproduction test with collembola were carried out with AgNO3 and AgNPs (tenside stabilized, 15 nm diameter). AgNO3 was about twice as toxic as AgNPs and *L. terrestris* was more sensitive to both substances than *E. fetida* (AgNO3 *L. terrestris* LC50 170 mg Ag/kg soil, NOEC 88 mg Ag/kg soil; *E. fetida* LC50 315 mg Ag/kg soil, NOEC 198 mg Ag/kg soil; AgNPs *L. terrestris* LC50 334 mg Ag/kg soil, NOEC 187 mg Ag/kg soil; *E. fetida* LC50 649 mg Ag/kg soil, NOEC 324 mg Ag/kg soil). The results of the collembolan study and of additional experiments with the aquatic organism *Gammarus pulex* are currently evaluated and will be presented in the meeting.

The mobility of silver nanoparticles in natural soil was studied by evaluating the influence of earthworms on the transport. For this purpose artificial columns (diameter 10 cm and length 40 cm) were filled with natural soil and either *Lumbricus terrestris* or *Eisenia fetida,* which differ significantly in their burrowing behavior, were inserted in the system. After an acclimatization time of 6 days, silver nanoparticles (tenside stabilized, 15 nm) were applied (262 mg Ag/kg soil). Experiments with AgNPs in absence of organisms served as controls. After 14 days the soil column was dissected into 1 cm and 5 cm layers and the layers and earthworms were analysed regarding the total silver content(ICP-MS). In all treatments at least 50% of the introduced silver nanoparticles were retained in the upper soil layers (0-3 cm). However, the presence of the different earthworm species had a clear effect on the mobility of AgNPs in soil. *L. terrestris* contributed to leaching of AgNPs into deeper soil layers (ca. 20 cm) whereas most of the silver nanoparticles in presence of *E. fetida* remained in the upper part of the column. Additionally, leachate studies with artificial soil columns (with and without earthworms) and AgNPs (tenside stabilized, 15 nm; PVP coated, 20 nm) were performed. After continuous irrigation (10 mm/d) for 14 days the leachate was sampled and the total silver content was measured (ICP-MS). In general the measured Ag concentrations in leachates were low (≤ 5 µg/L) and no significant differences in the amount of Ag in the leachates in presence and absence of earthworms were detected. Unlike the PVP coated AgNPs, the tenside stabilized AgNPs were measured in all leachate samples indicating that the tenside enhances the transport of AgNPs.

P12: Influence of aging history and physico-chemical changes on the remobilization of Ag NP from sediments of an artificial riverbank filtration system

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Engineered nanoparticles (ENP) can be released into aquatic systems via direct discharge or after application of sewage sludge on agricultural fields. Once they enter the aquatic environment they may impair the quality of drinking water reservoirs. Riverbank filtration systems can prevent contaminants from reaching such resources. Their retention capacity and the remobilization potential are therefore important aspects for risk assessment.

In a previous experiment, silver nanoparticles (Ag NP) aged in different media (soil solution, river water, ultrapure water (UPW)) were applied to water-saturated columns (length 1.5 m) filled with medium quartz sand, simulating a riverbank filtration scenario. The NP were mainly retained in the top 10 cm of the columns, ranging from 50 % for Ag NP aged in soil extract to almost 100 % for Ag NP aged in UPW and river water. However, it is unclear under which conditions the retained Ag NP can be remobilized. Therefore, the aim of this study was to determine the effect of physical forces and hydrochemical changes on the remobilization potential of Ag NP.

In batch experiments, Ag NP were redispersed from column sediments using short manual shaking, 24 h of horizontal shaking, or ultrasonic treatment in the presence of different remobilization media (UPW, soil extract, Suwannee River Natural Organic Matter solution, NH_4NO_3 solution). Upon down-centrifugation of the sediments the total amount of remobilized Ag in the supernatant was measured by ICP-MS following acid microwave digestion. The size and composition of remobilized particles were characterized by single particle SP-ICP-MS, SEM-EDX and GFAAS.

Up to 50 % of the total mass of retained Ag is potentially remobilizable. Increasing physical forces, the presence of organic matter and the reduction of ionic strength enhance the remobilization potential, while the presence of Ca^{2+} decreases NP remobilization. Remobilization seems to take place in the form of heteroaggregates. GFAAS indicates that Ag NP are still present in the size of initial NP. Nevertheless, a strong chelating agent can mobilize Ag^+ from NP or sediment surfaces.

We conclude from our results that although the retention of Ag NP in sand filter systems is high, changing environmental conditions may lead to remobilization of nanoparticles (Figure 1). Therefore, a potential risk for drinking water reservoirs such as groundwater cannot be excluded and should be further studied.

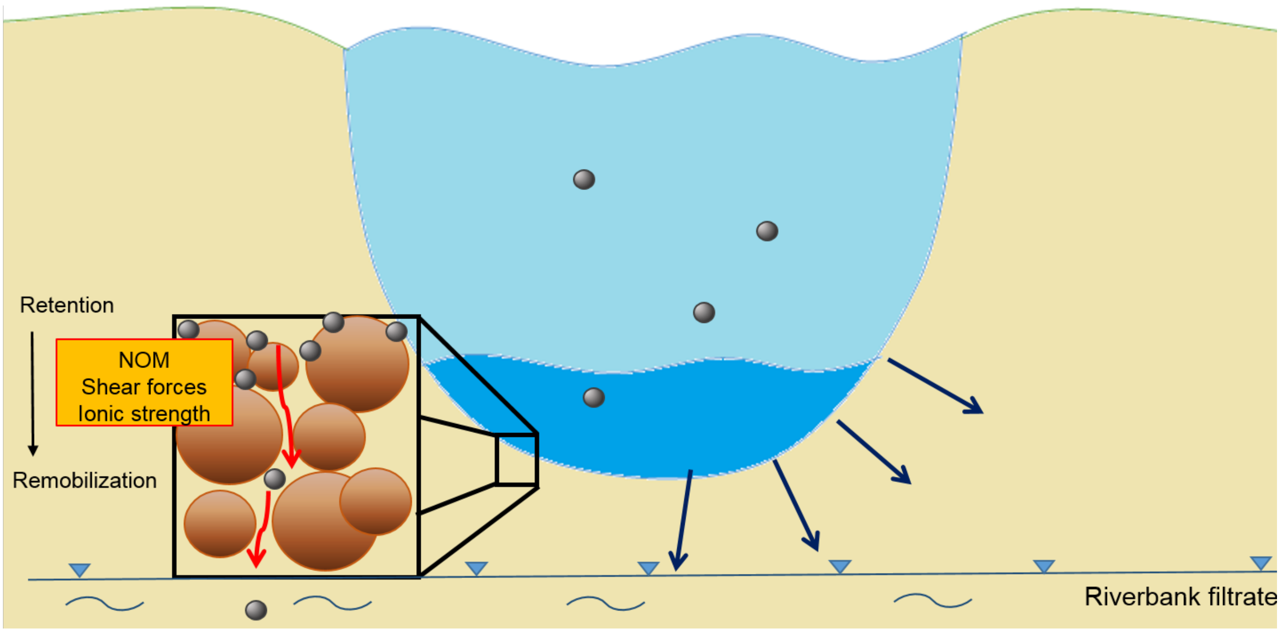

Figure 1: Potential remobilization of Ag NP (●) from riverbank filtration systems through changing environmental conditions.

P13: Aggregation kinetics of TiO_2 and Ag nanoparticles in soil solution

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The increasing use of nanoparticles (NP) during the last years results in a larger release of NP into soil via several pathways such as flooding of floodplains, application of fertilizers and biosolids. Therefore, process understanding about stability of NP in soil solution is important to determine possible adverse effects of NP on ecosystems.

Aggregation kinetics of NP were already studied depending on various chemical parameters such as ionic strength, pH and dissolved organic matter (DOM). Increasing ionic strength causes aggregation of NP by screening the surface charge and compressing the electric double layer. Furthermore, surface properties of NP can be modified through the adsorption of DOM, which can cause stabilizing or destabilizing of the NP suspension. However, the effect of differences in the primary particle size on aggregation kinetics under varying chemical conditions is not fully understood.

The aim of this work was to elucidate the effect of Ca and DOM concentration as well as pH on the aggregation kinetics of two different primary particle sizes of silver nanoparticles (Ag NP) and titanium dioxide nanoparticles (TiO_2 NP).

The following hypotheses were tested using batch experiments: 1) smaller NP need a lower Ca concentration to induce aggregation than larger ones; 2) the destabilising effect of Ca at the isoelectric point (IEP) is less pronounced than at higher pH values; 3) DOM in soil solution has a stronger stabilizing effect on smaller than on larger particles; 4) hydrophilic DOM stabilizes NP less than hydrophobic DOM.

Aggregation kinetics of TiO_2 NP (79 nm, 164 nm) and citrate-stabilized Ag NP (73 nm, 180 nm) were investigated in Ca solution (0, 0.2, 1, 2 mM) farmland- (containing 0.2 mM and 2 mM Ca) and floodplain soil solution (containing 2 mM Ca) using time-resolved dynamic light scattering (DLS) at a pH range of 7-8. DOM was characterized with Fourier Transform Infrared (FTIR). An empirical model was fitted to the data of aggregation kinetics to parameterize the rate constant (velocity until equilibrium of aggregation is reached) and maximum particle diameter.

Our results demonstrate that NP suspensions examined here were destabilized by Ca. For TiO_2 NP aggregation kinetics seem to be independent of primary particle size, while for Ag NP smaller particles were less stable than larger ones. At pH values higher than the IEP TiO_2 suspensions are sensitive to the addition of Ca, whereas at the IEP Ca has no effect, since nanoparticle suspensions are already destabilized. In the presence of Ca hydrophobic DOM stabilizes NP to a larger degree than hydrophilic DOM. The stabilization effect of DOM on NP is depending on composition of DOM, primary particle size, nanoparticle material and Ca concentration.

From an environmental point of view stabilization of NP in soil solution may increase their mobility and thus, may pose an ecological risk.

P14: Effect of natural organic matter and synthetic polymers on CeO_2-nanoparticle colloidal stability and their transport in saturated porous media

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With the development and increased usage of novel nanomaterials such as surface-functionalized engineered nanoparticles (ENPs), concerns on health risks and the environmental fate are rising. Understanding the transport of ENPs within and between environmental compartments such as surface water and groundwater is crucial for risk assessment and the protection of drinking water resources. The transport of ENPs is mainly controlled by i) their surface properties and ii) water chemistry. Therefore, functionalization of ENP surfaces by synthetic and natural organic coatings will change their behaviour in the environment.

Our aim is to assess the colloidal stability and mobility of CeO_2 ENPs with various surface coatings featuring different physico-chemical properties such as weakly anionic polyvinyl alcohol (PVA), strongly anionic poly acrylic acid (PAA) or complex natural organic matter (NOM) during slow sand filtration. Therefore, we conduct (i) batch experiments to assess colloidal stability, surface charge (zeta potential) and aggregation of coated CeO_2 ENPs in aqueous matrices and (ii) sand filter column experiments under various hydrochemical conditions (pH, ionic strength) to quantify CeO_2 ENP transport.

While uncoated CeO_2 ENPs aggregate in the range of pH 4-8 in 1 mM KCl electrolyte, our results show that PAA and PVA surface coatings as well as NOM sorbed to CeO_2 ENP surfaces can stabilize CeO_2 ENPs under neutral and alkaline pH conditions. With increasing concentrations of KCl, PVA coated particles aggregated first followed by the PAA coated ones. No aggregation was observed at up to 25 mM KCl for NOM coated CeO_2 ENPs. $CaCl_2$ had a stronger destabilizing effect on all ENP suspensions compared to KCl, inducing aggregation at $CaCl_2$ concentrations >2 mM for PVA and >3mM for PAA and NOM coated CeO_2 ENPs respectively. The influence of ionic strength on transport in sand columns was investigated using deionized water and natural freshwater with 2 and 4.5 mM $CaCl_2$, respectively. $CaCl_2$ concentration was the main parameter controlling CeO_2 ENP retention in column experiments. Further results on the effect of organic particle coatings on ENP transport will be presented.

P15: Transport behavior of engineered silver nanoparticles in natural aquifer material

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The widespread use of engineered silver nanoparticles (Ag-ENPs) in various consumer and industrial products increasingly contributes to Ag-ENP release into the environment. One major route for environmental release is through wastewater. Ag-ENPs are mostly retained in sewage sludge in wastewater treatment plants, which is then used as agricultural fertilizer or disposed of at landfills. From there, Ag-ENPs can enter the vadose zone and pertaining the environmental conditions also shallow aquifers. Therefore, it is essential to study Ag-ENP behavior in saturated aquifer materials where they can act as a potential contaminant.

Laboratory column experiments were conducted to investigate the transport and blocking behavior of surfactant and polymer-stabilized silver nanoparticles (Ag-ENPs) in natural silicate-dominated aquifer material with varying silt and clay content. For the experiments a mean grain size diameter of 0.7 mm was chosen with varying background solutions and ionic strengths. Particle concentration in the effluent was measured using ICP-MS and the numerical finite element code HYDRUS-1D was used to model the transport and retention processes using one or two kinetic sites with Langmuirian blocking.

The obtained results show that Ag-ENPs are more sensitive to Ca^{2+} than Na^{+} in the background solution. Higher attachment to the solid-water interface and blocking values were found to be associated with increasing Ca^{2+} concentrations which result in a larger delay in Ag-ENP breakthrough. The delay in breakthrough was more pronounced in the presence of silt and clay particles due to blocking. The lower zeta potential of the polymer stabilized Ag-ENPs decreased their mobility compared to surfactant stabilized Ag-ENPs.

P16: Oxidative potential of nanomaterials in the eluate of quartz sand and natural soils?

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Nanomaterials (NM) are commonly used in many everyday life products and in the end NM will enter the environmental compartments. Soils were identified as one important sink for NM with the potential of accumulation of biopersistent NM and/or passage into the groundwater. During this processes they will probably undergo certain environmental transformation processes which potentially affect the hazard potential of the NM. As one promising metric describing the potential hazard of NM the reactivity or oxidative potential (OP) of NM is nowadays discussed. However, whether the OP in soils can be a sensible intrinsic characterization parameter for NM hazard assessment within ecotoxicological questions has not been investigated so far.

Accordingly, it was investigated in this study whether it is possible at all to use the spin trap-based electron paramagnetic resonance spectroscopy (EPR) for the detection of OP in eluates from soil column tests and of diluted “natural” soils (sludge), and whether NM (when passing the soil column) change their OP.

Therefore, within the DENANA project in a first approach we performed soil column experiments with a silver and a copper NM and detected the OP in the eluate at three different timepoints. We additionally analysed six types of natural reference soils (sludge) to their OP by EPR.

The results show that the spin trap-based ESR analysis is in principle applicable in eluates as well as in natural soil samples. However, the often high OP background in soils does not allow a differentiation of additional NM elicit OP at the moment. Additionally a change of the reactivity of the NM is indicated after passing quartz sand filled soil columns.

P17: Development of a method to determine the bioaccumulation of manufactured nanomaterials in filtering organisms (Bivalvia)

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The determination of the bioaccumulation potential of substances plays a key role within the framework of the PBT/vPVB assessment. The bioaccumulation potential of a substance is currently determined decisively using the bioconcentration factor (BCF) estimated in flow-through studies with fish. Because of the sedimentation of the manufactured nanomaterials (MNMs) and the absence of suitable test systems that allow a permanent and constant exposition, the bioaccumulation potential of MNMs is difficult to investigate. In aquatic habitats MNMs are primarily taken up by benthic species. Different studies have shown that mussels are able to ingest and to incorporate MNMs from water. However, the standardised test methods to investigate the bioaccumulation of substances in mussels have been developed and optimized for non-particular substances. Therefore, the development of an alternative test concept to assess the bioaccumulation of MNMs in mussels is necessary. First bioaccumulation studies with silver MNMs were successfully carried out with a new test system. Analytic methods for the characterisation of MNMs (e.g. silver) in water and tissue samples, gained through the studies, were developed and validated. The quantification of the total content of the used chemical elements, e.g. silver, was carried out by ICP-OES or ICP-MS. The estimated tissue concentration were used to determinate the BCF. After an uptake of about 6 days an uptake and accumulation of silver from MNMs was measurable and steady state was reached. By using silver MNMs we were able to compare the accumulation and elimination of silver from MNMs with those from an ionic source ($AgNO_3$).

P18: Effects of Wastewater Borne Silver and Titanium dioxide Nanomaterials on the Growth, Bioaccumulation and Biochemical markers of *Onchorhynchus mykiss*

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The use of nanoscale silver (nano-Ag) and titanium dioxide (nano-TiO_2) has largely increased in the last decades due to their application in a variety of products, e.g. textiles, bandages, sunscreens and building facades. Their increasing use leads to the transfer of nano-Ag and nano-TiO_2 into wastewater treatment plants (WWTP) via industrial and urban sewage. During water treatment, these nanomaterials remain in the sewage sludge to a great extent. Only a small amount of mostly transformed nanomaterials is finally released into the environment. In this study, we investigated the toxic effect of pristine and transformed nano-Ag and nano-TiO_2 on the growth, bioaccumulation and biochemical markers of the rainbow trout *Onchorhynchus mykiss*.

Six model WWTPs were conducted according to OECD Guideline 303A. The collected effluents were used to perform Juvenile Growth Tests and Bioaccumulation Tests with *Onchorhynchus mykiss* according to OECD guidelines 215 and 305. Animals were exposed to (i) effluent from model WWTPs previously contaminated with nano-Ag or nano-TiO_2, (ii) uncontaminated effluent, manually spiked with nano-Ag or nano-TiO_2 and (iii) dilution water enriched with pristine nano-Ag or nano-TiO_2. In the end of the four-week exposure, gill, brain, liver and muscle tissue of the test animals were dissected and analysed for changes regarding the biochemical markers superoxide dismutase, glutathione S-transferase, thiobarbituric acid reactive substances to assess lipid peroxidation, lactate dehydrogenase, catalase and acetylcholinesterase. Effects of Wastewater Borne Silver and Titanium dioxide Nanomaterials on the Growth, Bioaccumulation and Biochemical markers of *Onchorhynchus mykiss* will be discussed.

P19: Detection of nanoparticles in rivers and industrial wastewaters

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Very little data is available on the occurrence of engineered nanoparticles in the environment. Therefore, it is difficult to assess whether these particles pose a risk to the environment. Current risk assessments are essentially based on model calculations. To get an overview on the occurrence of nanoparticles in the aquatic environment, samples were taken at 25 Bavarian river surveillance monitoring stations and analysed for metal nanoparticles. Asymmetric field-flow-field-fractionation (AF4) combined with inductively coupled plasma mass spectrometry (ICP-MS) resp. single particle ICP-MS was used for analysis.

With this analysis, data on the occurrence of 8 metal nanoparticles in Bavarian rivers was made available for the first time. The highest values (300 ng/l) were measured for nano iron, with the contents of nano zinc and nano aluminium being half as high. Nano forms of silver, titanium and nickel were found in concentrations < 20 ng/l. This data is used for the assessment of potential environmental risks.

About 40% of the Bavarian nanotechnology companies discharge their wastewater into river, whereby they must comply with official regulations. Overall, the wastewater of 27 companies that directly discharge their effluents was analysed for the contents of nanoparticles.

Nano forms of silver, zinc, copper, aluminium and iron were widely spread while the nano forms of titanium, nickel and cerium were found only in a few cases. Depending on the origin of the wastewater, characteristic differences in the nanoparticle patterns could be observed.

P20: Development of a method for the analysis of nanoparticles in homogenized biota tissue from the German Environmental Specimen Bank

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Aim of this project is to adapt reported approaches to detect and characterize engineered nanoparticles (ENPs) in biota samples from the German Environmental Specimen Bank (ESB). These samples are already homogenized and stored in the ESB according to internal standard procedures for the German Environment Agency. For the analysis and characterization of nanoparticles in samples of blue mussel (*Mytilus edulis*) and zebra mussel (*Dreissena polymorpha*) two promising analytical methods were applied: (i) single particle inductively coupled plasma mass spectrometry (spICP-QQQ-MS) as well as (ii) Flow-Field-Flow-Fractionation (Flow-FFF) coupled to ICP-QQQ-MS. The spICP-QQQ-MS technique uses short acquisition times in the 0.1-10 ms range for the detection of individual particle events during transient analysis and enables the measurement of number-based particle size distributions. Flow-FFF is a separation technique based on the different diffusion coefficients of particles which separates them according to their hydrodynamic diameter without using a stationary phase.

Furthermore a tissue hydrolysis procedure was developed to transfer the particles into stable suspension which is a prerequisite of both analytical techniques. Special emphasis was placed on the development of a mild method for the release of ENPs from the mussel tissues. Two procedures were applied (i): enzymatic hydrolysis with Proteinase K and (ii): alkaline hydrolysis with tetramethylammonium hydroxide. Silver, cerium dioxide, and titanium dioxide nanoparticles were chosen as model particles.

Complete tissue hydrolysis of mussel samples was achieved with both procedures. For evaluation of the method, nanoparticles were measured in original samples as well as in spiked samples. First results showed minimal shifts in spiked samples compared to dispersions of the pristine particles and indicate that the original particle size distribution is not affected by the hydrolysate matrix according to spICP-

QQQ-MS measurement series. In unspiked mussel tissue samples we found cerium containing particle events in the spICP-QQQ-MS.

This project is funded by the German Environmental Specimen Bank.

P21: Asymmetrical Flow Field-Flow Fractionation hyphenated with ICP-MS for Trace Level Analysis of Engineered Silver Nanoparticles in River Water

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Asymmetrical Flow Field-Flow Fractionation (AF4) is a powerful tool for the separation of nanomaterials according to their hydrodynamic size. However, significant dilution within the separation channel usually limits its application towards trace level analysis. These limitations can be overcome by taking advantage of two unique features, which this separation technique can offer:

1) Slot outlet

Here, the channel outlet flow is split into two separate flows. Since the sample is usually located close to the semipermeable accumulation wall in the lower 5-10% of the channel, the upper, sample-free, void volume can be removed thereby reducing sample dilution.

2) High volume injection (HVI)

In AF4, a focusing or relaxation step is necessary prior to elution, where sample components arrange in different heights of the separation channel according to their different diffusion coefficients counteracting with an external force field. This is realized by a second flow, the so called cross flow, which acts perpendicular to the channel flow and pushes the sample components towards the acumulation wall. During sample introduction, a focus flow, which counteracts the sample injection flow, hereby enables virtually unlimited sample introduction and thus sample enrichment and cleaning directly on the separation channel.

A third mean to prepare AF4 for trace analysis is the coupling with a high-sensitivity detector:

3) Hyphenation with ICP-MS

ICP-MS is particularly powerful, when it comes to the chemical identification and quantification of metallic and metal oxidic sample components such as e.g. silver nanoparticles (AgNP). Hence, its hyphenation with AF4 (AF4-ICP-MS) enables the collection of data on elemental composition over the size distributions even at trace level concentrations.

In this presentation, we demonstrate the applicability of AF4-ICP-MS for the quantification of AgNP spiked in Rhine water at trace level concentrations. Using an injection volume of 8 mL together with a 60% removal of the upper channel flow, we were able to identify and quantify AgNP down to a LOQ of 14 ng/L (Fig. 1).

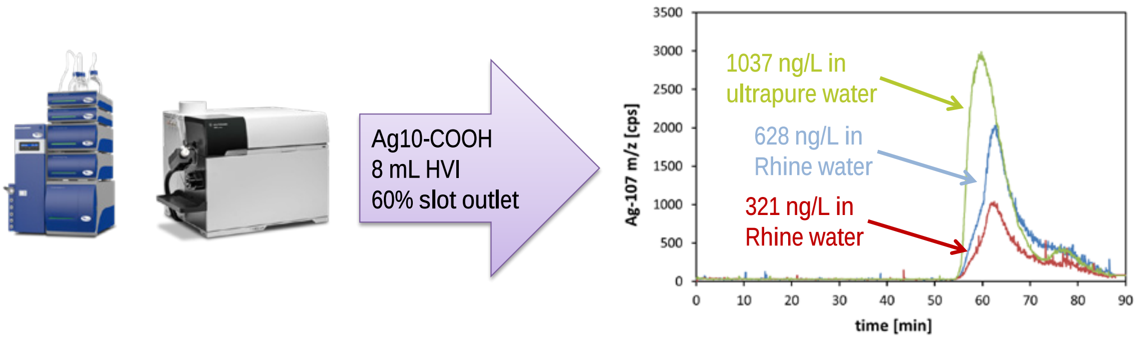

Fig 1.: Quantification of AgNP spiked in Rhine water at trace levels using AF4-ICP-MS with 8 mL injection volume and 60% slot outlet (LOQ: 14 ± 4 ng/L).

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P22: Predictability of silver nanoparticle speciation and toxicity in ecotoxicological media

POSTER AWARD WINNER

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The use of silver nanoparticles (AgNP) has increased significantly over the past decade due to their antimicrobial effect. Since the antimicrobial effect of AgNP is mediated by the release of silver ions (Ag^+), it was the purpose of this study to investigate the silver release of silver nanoparticles dispersed in a wide variety of (eco-)toxicological test media for aquatic organisms (algae, plants and crustaceans), terrestrial organisms (bacteria) and cells, to understand the contribution of the chemistry of the surrounding environment to the bioavailability and hence to the toxicity of AgNPs.

It was the second aim to predict silver speciation in aqueous solutions by equilibrium speciation calculation and validate the results by comparison with experimental speciation using ultracentrifugation and membrane filtration. Silver amounts were quantified using GF-AAS, ICP-OES/MS and UV/VIS. The release experiments were conducted in the test media without organisms in the time frame of the test periods.

The dissolved silver concentrations were controlled by the fast initial release of a limited amount of Ag^+. After this initial release, the media components chloride and proteins were controlling the available dissolved silver by precipitation and complexation. Further release of silver ions due to oxidation was not observed in the time frame of our experiments, except for media with very high chloride content.

The findings revealed that theoretical prediction of bioavailability of ions released from redox-active nanoparticles can be done without including kinetic information, when experimental speciation data of the initial conditions are available and the kinetics of ion release is slow compared to the time frame of the toxicological tests. This approach could circumvent the challenging intricacy of kinetic modelling in complex biological media for these kind of nanoparticles.

P23: Development of Microscopic and Spectroscopic Techniques for the Detection and Characterization of Manufactured Nanomaterials in Aquatic Samples

This work is part of the project FENOMENO.

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Due to their increased application in various fields, the environment is at least indirectly exposed to manufactured nanomaterials (MNMs). Among those, silver (Ag) and titanium dioxide (TiO_2) nanoparticles (NPs) represent two examples, which are used for commercial purposes in high volume outputs and are hence very widespread. However, their end-of-life impact on the environment remains, as for many other materials, unclear. MNMs are typically not found in their pristine state, but are subject to physico-chemical changes like oxidation or the adsorption of biomolecules, especially during wastewater treatment (WWT). This alteration in NP properties may lead to an increase in bioavailability and toxicity for water organisms.

In the framework of FENOMENO we investigate the fate of pristine as well as wastewater-borne Ag (NM-300K) and TiO_2 (NM-105) NPs after exposure to relevant aquatic species (algae, *Daphnia*, fish eggs, fish) in order to validate and optimize microscopic methods for the detection and characterization of MNMs. Among those methods, Darkfield Optical Microscopy has proven to be a valuable technique for rapid overview scans of thin tissue sections due to intense light scattering effects of small particles. This first and crucial step is followed by more detailed investigations including Scanning Electron Microscopy (SEM) in combination with Energy-dispersive X-ray Spectroscopy (EDX) to determine the elemental composition. These laboratory studies are compared with field samples from Lake Mondsee, Austria.

Additionally, artificial wastewater effluents containing Ag NPs are under investigation to study the chemical transformation during wastewater treatment. The major challenge here is to separate and concentrate nanoparticles from their corresponding matrix. A commonly used method in this context is the so-called Cloud Point Extraction in which Ag NPs are encapsulated in micelles and hence removed from their aqueous environment via phase separation.

P24: Measuring at relevant concentrations – radiolabeling as a versatile tool in nanosafety research

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The employment of radiotracers is a versatile tool for the detection of nano-particulate materials in complex systems such as environmental samples or organisms. With the increasing usage of nanoparticles in applications outside of research laboratories, a careful risk assessment of their release into the environment becomes mandatory. However, the monitoring of nanoparticles in such complex natural systems as soil, natural waters, plants, sewage sludge, etc. is nearly impossible using conventional methods, especially at environmentally relevant concentrations. This obstacle can be overcome by radiolabeling, which may be of crucial value in enabling such research.

We have developed various methods of introducing radiotracers into some of the most common nanoparticles, such as Ag, carbon, SiO_2, CeO_2 and TiO_2 nanoparticles. The labeling techniques are the synthesis of the nanoparticles using radioactive starting materials, the binding of the radiotracer to the nanoparticles, the activation of the nanoparticles using proton irradiation, the recoil labeling utilizing the recoil of a nuclear reaction to implant a radiotracer into the nanoparticle, and the in-diffusion of radiotracers into the nanoparticles at elevated temperatures. Using these methods we have produced $[^{105/110m}Ag]Ag$, $[^{124/125/131}I]$CNTs, $[^{48}V]TiO_2$, $[^{139}Ce]CeO_2$, $[^{7}Be]$MWCNT, $[^{7}Be]SiO_2$, $[^{44/45}Ti]TiO_2$, etc.. The methods are adaptable for a wide range of other nanoparticles. The so-labelled nanoparticles can be detected at minimal concentrations well in the ng/L range even with a background of the same element and without complicated sample preparations necessary.

Using our methods one can radiolabel commercial nanoparticle samples for sensitive detection in environmentally relevant trace concentrations. The labeled particles have been successfully used in release studies, environmental mobility studies, fate studies in waste water treatment and plant uptake studies.

P25: Designing a sustainable European Centre for Risk Management and Safe Innovation in Nanomaterials & Nanotechnologies (EC4SafeNano)

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A central challenge to ensure the sustainable production and use of nanotechnologies is to understand the risks for environment, health and safety associated with this technology and resulting materials and products, and to identify and implement practical strategies to minimize these risks. Knowledge about nanotechnology-enabled processes and products and related environment, health and safety issues is growing rapidly, achieved through numerous European or national R&D programs over the last decade, but effective use of this knowledge for risk management by market actors is lagging behind.

The EC4SafeNano initiative (www.EC4SafeNano.eu) is an ongoing effort to build a **European Centre for Risk Management and Safe Innovation in Nanomaterials and Nanotechnologies**. EC4SafeNano aims to bridge the gap between scientific knowledge on hazard and risk, and 'fit-for-purpose' risk management tools and strategies supported by measurement and control methods. The consortium comprises 15 partners (INERIS (coordinator), EU-VRi, TNO, BAM, FIOH, VITO, SP, DEMOKRITOS, TECNALIA, Health and Safety Executive, NRCWE, Paris Lodron University Salzburg, Université Libre de Bruxelles, University of Birmingham and ENEA) from 11 European Member States with significant expertise on risk assessment and management, who already provide knowledge and technical services to public and private organizations, to industry and to public authorities and regulatory bodies.

The overall objective of the EC4SafeNano project is to develop a distributed Centre of European Organisations offering services for Risk Management and Safe Innovation for Nanomaterials & Nanotechnologies. The Centre will be structured as a hub-based network of organizations managed by a core group of public-oriented bodies providing risk management and safe innovation support to all stakeholders. It will be operated with the support of Associated Partners so as to expand its capabilities, resources and services. It will interact with existing platforms and centres of excellence in nanosafety and foster the organization or development of national hubs mirroring the European hub. The Centre will seek financial support from stakeholders and service users to sustain the services in the longer term.

The operational objectives of the project involve **understanding and mapping the needs of the various stakeholders** (private and public). It will identify the **resources and capabilities and develop a range of harmonized services** required to meet these needs. The construction of the centre will include putting in place and implementing processes to deliver and update services, to test and benchmark services, to evaluate the governance of the Centre, and developing a business plan to ensure self-sufficiency of the Centre beyond the project lifetime.

A cornerstone of the project is to build a community for risk management and safe innovation for nanotechnology. Interested persons or organisations are invited to join this initiative as registered stakeholders or Associated Partners, to engage in focus networks and to help shape the future Centre. The poster will present the EC4SafeNano initiative and will detail the role of registered stakeholders and Associated Partners.

6 Knowledge Café

The participants of the stakeholder meeting were separated into 5 groups. Each group was invited to visit three tables at which different topics were suggested for discussion. Consequently, each table was visited by three different groups. The chairs of the tables introduced the groups to the specific topic and facilitated the group discussions. The chairs also reported the discussion results to the plenary.

The following sections introduce the topics and questions posed at the tables and summarise the discussion results.

6.1 Importance of, and methods for determining, intrinsic and extrinsic properties of nanomaterials for ERA

The table was chaired by Iseult Lynch, University of Birmingham, UK.

A list of proposed intrinsic and extrinsic properties of nanomaterials was provided as a poster[3] (c.f. Table 1). At the beginning of each discussion round, stakeholders marked the properties they evaluated most relevant for environmental risk assessment (ERA) with an adhesive dot[4]. The participants reflected on the ranking of importance of the various properties and how the properties are interlinked. Finally, some ideas were exchanged on the suitability of available methods for determining the properties and on possible methods that could be standardised in the future. Table 1 shows the results of the marking of properties. The higher the number in the column "Assigned relevance", the more participants evaluated the property as important.

Table 1: Overview of relevance for ERA stakeholders assigned to intrinsic and extrinsic properties

Intrinsic properties	Assigned relevance	Extrinsic properties	Assigned relevance
Surface characteristics, including coating chemistry, functionalisation, surface charge	24	Dispersibility/agglomeration and dispersion stability	26
Chemical composition	19	*Bioaccumulation*[3]	23
Solubility	13	Rate of dissolution (in environment)	23
Particle size distribution (number average)	8 + (3[5])	Biological (re)activity (or surface reactivity)	11
Hydrophobicity / Wettability	6	Surface affinity / *attachment efficacy*[4]	9
Redox potential / Band gap	3	Photo reactivity	4
Specific surface area	2 + (3[5])	Zeta potential	3
Impurities	2	Dustiness (depends on moisture)	1
Iso-electric point	1		
Particle shape (e.g. aspect ratio)	1		
Crystalline phase(s) and size	0		
Rigidity	0		
Total intrinsic	**82**	**Total extrinsic**	**100**

Overall, the stakeholders' opinions on the relevance of properties for ERA converged across the discussing groups; i.e. a similar few properties achieved the highest scores. The extrinsic properties evaluated as most important are dispersibility, bioaccumulation and rate of dissolution (extrinsic) while

[3] During the first discussion round, "attachment efficacy" and „bioaccumulation" were added to the list.

[4] Each participant had 6 stickers for intrinsic properties and 6 stickers for extrinsic properties and they could use one or more of them to rank a property as important.

[5] Three dots were glued on the line between particle size distribution and specific surface area, as the participants were indecisive.

the intrinsic properties evaluated as most important are surface characteristics and chemical composition. Furthermore, Table 1 illustrates that both intrinsic and extrinsic properties are regarded as important.

All groups attempted to distinguish properties that are unique and independent from properties, which are interrelated and could hence be predicted if information on the related, other properties was available. Stakeholders discussed among others:

- if and how, particle size and surface area can be related to each other and, if this is possible, in how far the relationship could also work for aggregates and agglomerates;
- that the surface affinity is a property that expresses the likelihood that particles attach to different surfaces, such as soil, membranes, biomolecules etc. This might also indicate the bioaccumulation potential of a nanoparticle;
- that the zeta potential can be used to deduce the surface charge of a particle.

The first discussion group added the "bioaccumulation potential", which it evaluated as an effect rather than a property, to the list. Also the further groups agreed that information on the bioaccumulation of nanomaterials is very important for environmental risk assessment.

Many stakeholders stated that the chemical composition is a major driver for various other properties. However, a particle's properties could not be fully deduced based on composition information alone.

Overall, stakeholders opined that few methods are available to identify intrinsic than extrinsic properties. They highlighted that different time scales and different test media should be used to take transformations and the effects of the environmental conditions on the particles' properties into account. This should be considered for example in determining "bio-persistence", attachment affinity, or the dissolution potential. The participants saw a lack of widely available methods to characterise a particle's surface chemistry.

Further issues mentioned in the discussions concerned the need for benchmarking materials (e.g. persistent and non-persistent reference materials), agreement on relevant timescales of testing to these, the need for accessible and validated testing methods and the urgency of standardising testing methods for the dissolution potential (rate of dissolution) and the attachment affinity, including a recommended set of media conditions (± natural organic matter) and timescales for assessment of stability, attachment and loss of coating.

6.2 Needs for test guideline development

The table was chaired by Jukka Ahtiainen, Drumsö Ecotox Consultancy, Finland.

The discussions should collect stakeholder opinions regarding which OECD test guidelines (TG) and guidance documents (GD) should most urgently be adapted to nanomaterials and for which properties new TGs/GDs should be developed. In addition, stakeholders were asked to point out methods that could be proposed for standardisation and to discuss, how the research community could be (more) involved in the OECD standardisation process.

The following ongoing OECD activities were presented in poster form to the groups:

- New TG on Dispersion Stability of Nanomaterials in Simulated Environmental Media
- Development of anew TG on Dissolution Rate of Nanomaterials in Aquatic Environment
- Development of a new GD on Agglomeration and Dissolution Behaviour of Nanomaterials in Aquatic Media
- Development of a new TG for Removal of Nanomaterials from Wastewater
- Development of a new GD to Assess the Apparent Accumulation Potential for Nanomaterials

- Development of a new GD Aquatic (and Sediment) Toxicity Testing of Nanomaterials
- Development of a new GD for TG 312 - Leaching in Soil Columns of Nanomaterials
- Development of a new TG/GD for particle size and size distribution of Nanomaterials in relation to TG 110

The debaters found all of the listed TGs/GDs useful. They pointed out that the TG on dissolution rate is very important and stressed that its finalisation should be pushed forward. Also the GD on agglomeration and dissolution behaviour in the aquatic environment as well as on aquatic and sediment toxicity testing were highlighted as important.

The participants identified a need for a TG/GD on soil/terrestrial toxicity of nanomaterials. Here, a starting point could be to describe the State of the Art in a guidance document and revise it regularly. Furthermore, the groups evaluated guidance on identifying the heteroaggregation potential and the eco-corona of nanoparticles as important, both for test systems and in environmental samples. The stakeholders also stated that due to the lack of a work list at OECD level easily accessible for public the ongoing activities are insufficiently transparent.

The participants of the groups named the following methods as potential candidates for standardisation at OECD level:

- The bivalve test (mussels) could have potential to assess aquatic bioaccumulation potential and possibly toxicity in the aquatic environment.
- Lysimeter-, microcosm-, mesocosm- and field studies could have also potential to assess fate and effects of nanomaterials.
- The attachment affinity of particles to sludge, which relates to surface affinity of nanoparticles, could have also potential for future standardisation.
- The assessment of heteroaggregation or adsorption of natural bio-compounds (e.g. “eco-corona”)in the environment and during the tests might also need harmonized approaches.

The groups stated that the needs for (new/adapted) TGs is triggered by regulatory demands, such as the REACH regulation, the Biocidal Products Regulation or the Plant Protection Products Regulation of the EU. Hence, the agreement on and publication of the REACH Annexes for testing of nanomaterials could further accelerate the OECD activities.

The debaters at the table agreed that researchers should be more involved in standardisation work. However, researchers strive for novel information and uniqueness of results, while standardisation, including test validation, focusses on test optimisation and reproducibility, involving repetitive conduction of the same work. Therefore, there is a fundamental conflict in the aims of work that needs to be overcome, which may hinder e.g. PhD students to involve in the OECD standardisation work.

The participants discussing at the table highlighted that awareness of the opportunities to contribute to the development and validation of testing methods at OECD level should be raised in the scientific community. In addition, the role of national coordinators should be better communicated. The example of Germany that organises OECD work also via the environmental research plan (UFOPLAN) was evaluated as efficient and target oriented.

6.3 Exposure assessment of nanomaterials

The table was chaired by Wiebke Galert, UBA, Germany.

The discussions at this table aimed to identify the data needs for assessing exposure concentrations of nanomaterials in the environment, e.g. on use and release. Furthermore, it was explored if and how existing guidance on exposure assessment and therefrom derived tools need adaptation to nanomaterials. Methods, defaults, etc. that cannot be applied for exposure assessment of nanomaterials should be identified and potential alternatives or adaption needs should be named.

The stakeholders stressed that information on the production amounts of nanomaterials as well as the amounts entering different uses and being released from them are the essential basis for exposure assessment. However, this type of market and product information would be largely missing. Therefore, they suggested, among others, collecting information on the emission potential of nanomaterials from manufacture and use in cadastres or on databases to make it available for exposure assessment. The groups did not conclude on whether or not and how exposure information from one particle could be used to another; i.e. how to take account of the varying chemistry, including surface coating and functionalization. Some participants expressed their expectation that the EU observatory on nanomaterials (EU ON) would be a chance to get market-related data.

The groups found it important that a legally binding definition of nanomaterials is necessary to decide on relevant emissions concerning environmental compartments.

The stakeholders saw a need to validate existing exposure models regarding their applicability for nanomaterials, including related guidance. Adaption needs concern in particular the integration of nano-specific fate and behaviour aspects. Information on the background concentration of nanomaterials in the environment have to be considered in estimating trigger values for several exposure assessments and considering exposure as a dynamic process.

In the regulatory context, the groups recommended a systematic screening of existing guidance documents for their adaptation needs. Furthermore, the parameters triggering (in-depth) exposure assessments should be reviewed, as the properties/thresholds applied to bulk materials are not always useful for nanomaterials (e.g. water solubility, LogKow).

The question, which exposure assessment methods stakeholders consider inappropriate for nanomaterials could not be answered at a general level, because of the physical and chemical individuality of each nanoform. Therefore, it was stated inappropriate to regulate “nanomaterials” as such. Consequently, the need for risk management would have to be determined case-by-case.

6.4 Short, mid and long term goals for risk assessment tools

The table was chaired by Anu Kapanen, ECHA, Finland.

The aim of the discussion was to identify the need for environmental risk assessment tools for nanomaterials and discuss how these tools could be developed. Furthermore, the justification of grouping approaches for nanomaterials in ECHA’s guidance document on QSARs (Appendix R.6-1)[6] was planned to be discussed. However due to time constraints this topic was not covered in detail.

The three group discussing the topic structured the need for risk assessment tools according to the different information needed to derive a risk characterisation ratio for the environment, i.e. on a nanomaterial’s hazards, emissions, environmental fate and the resulting exposure. Tools identified were (standard) methods and protocols, guidance documents, models, QSARs, new concepts for grouping and regulation.

The identification of a test material was considered as very important, as it is the basis for any further assessment. This characterisation should include the determination of intrinsic properties as well as

[6] ECHA, 2017: Guidance on information requirements and chemical safety assessment, Appendix R.6-1 for nanomaterials applicable to the Guidance on QSARs and Grouping of Chemcials: https://echa.europa.eu/documents/10162/23036412/appendix_r6_nanomaterials_en.pdf/71ad76f0-ab4c-fb04-acba-074cf045eaaa

any potential transformations a particle may undergo. The need for tools to identify the materials (intrinsic or transformed) was regarded a short term goal.

Tools needed for hazard identification

- Systematic testing approaches for the various hazard endpoints of nanomaterials are needed, including tools to compare and/or group nanomaterials (short term);
- Tools to identify ecotoxicity drivers within the groups and to depict a “worst case” would be useful as well as tools to assess if and how testing information in the laboratory correlates with the situation in the environment.
  As standardised approaches to test sediment and soil toxicity do not yet exist, at least an OECD guidance document should be developed, including spiking methods (short term).

Tools needed for the environmental fate assessment

- Methods to describe transformation and speciation changes in the environment are key to identify the hazards of nanomaterials comprehensively. It is expected that this approach would cover also “worst case” scenarios.
- Impact of the transformations on the mobility and uptake into the organisms should also be covered.
- Tools to consider the time-scale of and the conditions leading to transformations are needed; this could be translated into boundaries of environmental conditions that determine the applicability of the method/protocol/approach taken in the fate assessment.

Exposure assessment tools

- Exposure models exist but need to be validated, at least to specify for which nanomaterials they could be used e.g. specify the applicability boundaries.
- More information is necessary on the production volumes and uses of the nanomaterials, including in which form they are used, into which product types they are included and if/ how they are released.
- Tools for identifying mobility and monitor nanoparticles in the environment partly exist and are further developing, as has also been presented at the meeting e.g. cloud point extraction linked with different detection techniques. Also here, the applicability domain of the methods needs to be defined.
- In the area of waste incineration, tools are needed how to measure and control emissions.

Risk assessment

The stakeholders agreed that the risk assessment of transformed particles is difficult. They saw a need to develop a conceptual approach to identify, if and when transformation is relevant regarding a nanomaterials’ hazard and/or fate. How transformation can be adequately considered, e.g. in the derivation of PNECs was seen as an area that needs further discussion. In specific cases there might be a need for new concepts considering the role of transformation of nanomaterials in PEC and PNEC derivation. Considering the role of transformation was also seen as important for example in grouping approaches.

The participants discussed whether a tiered risk assessment should be preferred over a conservative one. A tiered approach should include, according to the discussion, among others:

- *in vitro* testing (mid-term);
- identification of dissolution rates (short-term);

- determination of the attachment affinity (mid-term) in relation to different environmental compartments. Methods require (further) validation and a definition of boundaries of applicability;
- considering the (stability of a) transformation of particles (long-term) in relation to the environmental conditions, which could be reflected by EC-values generated in laboratory and field studies/mesocosm experiments;
- assessing PEC/PNEC derivations for the transformed or aged particles.

Stakeholders pointed out that any grouping approach would have to have clearly defined boundaries for the group members, the environmental matrices and the time scales. The groups agreed that ECHA's grouping approach for nanomaterials in Appendix R6-1 is generally applicable; however, case studies would be needed to test and verify it.

Measuring the exposure was seen as a challenging task. There are advances in detection techniques in measuring the amount of nanoparticles in environmental samples for example results of cloud point extraction and radio-labelling presented during this meeting. In general using combination of different detection techniques was seen beneficial. Also here, the methods' applicability would have to be clearly defined.

Finally, the groups converged in that available information should be used (more) efficiently, i.e. more information exchange among different parties and also across different regulatory areas should be ensured. This would necessitate further collaboration between all involved parties.

6.5 Research on nanosafety

The table was chaired by Thomas Kuhlbusch, BAuA, Germany.

The stakeholders at this table discussed, which research topics should be prioritised in the field of environmental safety of nanomaterials and in relation to regulatory risk assessment and risk management. Two other questions were foreseen for discussion but could not be elaborated due to time constraints: how information that is not directly useful for regulation could help identifying information needs and how research results could more efficiently enter regulatory decision making and standardisation.

The opinions in all groups converged in that research needs exist along the entire line of risk assessment, i.e. on hazard and effect identification, exposure estimation and measurement as well as determining fate and uptake into organisms.

Overall, a need to conduct targeted testing of nanomaterials was expressed and research should support developing respective approaches (i.e. intelligent testing and assessment strategies). Such approaches would benefit from research on the interaction and interlinks between different hazard and fate properties of nanomaterials, which were stated to be unclear, although some related research exists. It would also be useful to enhance research on similarities/differences of nanomaterials that could enable grouping for risk assessment.

According to the discussions, a potential first step of an intelligent testing strategy would consist of an evaluation, which effects are specific for nanomaterials and whether they are relevant; i.e. if they could contribute to environmental risks. If so, these properties should be subject to further assessment and research: The uptake / attachment of nanomaterials and related bio-kinetics were highlighted as particularly important. Similarly, the participants - although having a different understanding of its meaning - pointed out bioavailability as an important issue for further research, in particular as it could vary for different species.

As in other groups, also at this discussion table it was stressed that information on release is scarce and imprecise and hence more data on production and use volumes as well as the types of uses and their release potentials for nanomaterials are needed. Data should be generated systematically and in a form for use in release and transport models. The distribution of nanomaterials, i.e. on which pathways they enter the environment and in which form (in a matrix or as 'free' particles) and in which environmental compartment they end up, were indicated as important.

In relation to the assessment of environmental exposures and risks, the relevance of transformation and potential changes in hazard from pristine to transformed materials were mentioned as a crucial research topic. This includes validating how transformation in laboratory testing corresponds to transformations in the environment. The debaters stressed that research should ensure relevant transformation processes are not overlooked.

Finally, analytical methods to detect and identify nanomaterials in different matrices are still needed. This includes methods and instruments for sampling, sample preparation and the analytics. The major focus lies here in environmental matrices which make an identification of the nanomaterials more difficult.

Anyhow, a recommendation for less research funding for the investigation of the knowns, accepting some uncertainty, were given beside the discussion on needs. For example, no further testing of the toxicity of silver or copper is needed. However, it would be valuable to check why assessments of the same issue do differ; i.e. to determine factors and conditions influencing related research results. Furthermore, the groups stressed that the OECD standards should be used in order to make results comparable and support their evaluation and validation or improvement.

7 Lessons learned

At the end of the meeting, four participants each representing a stakeholder group presented their learnings from the meeting with regard to:

- the need for tools and methods to conduct valid ERAs for nanomaterials;
- how scientific knowledge on environmental risks from nanomaterials could better enter regulatory processes and
- their individual take-home message of the meeting.

7.1 The industry perspective: Eva Lammer (BASF)

Eva Lammer concluded from the meeting that adequate tools are available to characterise and assess the hazards of nanomaterials, such as the OECD guidance documents and technical guidelines. These should be used and implemented by industry and regulators. However, she saw an urgent need to adapt methods and tools used to assess exposures in order to make them fit for nanomaterials. She mentioned exposure models and QSARs as not taking nano-specific kinetics into account, such as the dissolution or aggregation behaviour of nanomaterials. She stated that the existing exposure models allow a realistic worst-case exposure estimation for nanomaterials. However, nano-specific tools (for example the nano-Simple box developed by RIVM) need to be implemented.

The meeting provided a good overview of recent research, in particular on the fate and exposure pathways of nanomaterials. However, Eva Lammer mentioned that the discussion is still missing why the assessment of particle effects is necessary for nanomaterials but would not be relevant for insoluble conventional chemicals. According to her, more focus should be drawn to distinguish between "only physical effect" and effects of the nanomaterial itself. This question needs to be covered by using adequate controls in the test design. For example, bulk material and soluble material should always be used as comparison. In addition, studies should be conducted in a way that physical effects can be distinguished from substance-related effects. Only by adequate comparison it can be determined if the nanomaterial itself has a hazard, according to Eva Lammer.

Eva Lammer also stressed that there is a lot of information on metallic nanoparticles which are used for general assumptions on all nanoparticles in principle. However, information on other types of nanomaterials (e.g. organic nanomaterials) would be lacking and more work should be conducted on representatives of other types of nanomaterials. She also wished that more scientists publish studies that demonstrate a lack of adverse effects of nanomaterials to underpin that only some nanomaterials are hazardous while others are not.

Her take home message was “nano” does not mean “hazard” and this should always be kept in mind when conducting tests.

7.2 The environmental NGO perspective: Rolf Buschmann (Friends of the Earth Germany, BUND)

Rolf Buschmann stressed that from the NGO perspective the lack of data on the use of nanomaterials in products and the amounts released from them is one of the key issues that need improvement. The meeting confirmed that this lack of data not only hinders informed consumer choices but also creates considerable uncertainties in the exposure assessment. He therefore considers the implementation of a reporting requirement and the establishment of a database collecting use and emission information as a tool that is requested both by NGOs and the scientific community. Another commonality he mentioned is that both NGOs and researchers see a need for more research funding on nano safety to close the existing knowledge gaps, of which several were illustrated in the presentations.

According to Rolf Buschmann, the implementation of scientific knowledge in regulatory processes would firstly require the improvement of legislation, including the implementation of a consistent definition of nanomaterials across legislation. Furthermore, the information requirements posed under different legislation should be adapted: sufficient and appropriate data should be required to enable the characterisation of a nanomaterials as well as on those parameters that determine the risk. The further implementation would benefit from any grouping approaches developed by the scientific community.

As take-home message, Rolf Buschmann recommended NGOs and scientists teaming up in requesting better data to be made available on the uses and emissions of nanomaterials.

7.3 The academic perspective: Frank von der Kammer (University of Vienna)

Frank von der Kammer expressed that several types of new or improved tools would be necessary to improve environmental risk assessments of nanomaterials: Clear guidance is needed on which test systems are applicable to nanomaterials and on how particulate materials, including e.g. micro-plastics, should be applied to and handled in these test systems. This would improve the quality and reduce the span of testing results. However, as science is guided by hypotheses, deviations from standard operating procedures are necessary to gain knowledge on e.g. mechanisms, which causes a mismatch with the regulatory needs for standardised testing. Researchers could support standardisation processes by providing orientation on which aspects need TGs/GDs most, i.e. properties that control environmental fate or toxicity etc. Finally, he stressed that monitoring nanomaterials in products and in the environment is needed to improve exposure assessment.

Frank von der Kammer emphasised his wish that less work is conducted on “the obvious” and the “knowns”. He regarded existing experience on environmental colloid chemistry (OECD Guidance) sufficient to explain most observations and recommended assessing only those aspects which are actually new or nanomaterial-specific. In addition, he saw a need for more and better collaboration of scientists from different disciplines, such as material science, biology and chemistry, as various experts would be needed to resolve many of the research questions on nanomaterials. Furthermore, he pledged for more systematic studies, e.g. aiming at enquiring the mechanisms of effects and the relation between effect and (specific) properties of a nanomaterial. If the release of ions causes an eco-toxic effect, those aspects that determine the ion release from a nanomaterial should be subjected to research.

Frank von der Kammer's take-home message was that researchers should focus on the new and (currently) unexplainable. Furthermore, existing knowledge should be put together and utilized in a better way.

7.4 The authority perspective: Corinna Burkart (UBA)

Corinna Burkart concluded from the meeting that a lot of data is available and hazard assessments for nanomaterials are generally possible. Promising techniques for environmental monitoring and analytics would be developing, while more extensive efforts appeared necessary to improve exposure assessments. She also evaluated that there is some insight into the properties affecting fate and behaviour in the environment. However, Corinna Burkart mentioned that little work is ongoing on more complex nanomaterials and that the effect of "non-standard coatings" appears to be not yet well known. Another gap would exist in the area of long-term studies in soils, which she found important due to the findings that nanomaterials accumulate in soils and may persist for a very long time.

With regard to the implementation of scientific findings into regulatory processes, Corinna Burkart appreciated any step forward in the development of grouping and read-across approaches for hazard assessment and their extension to fate, behaviour and exposure assessments. She saw a need to identify the parameters that would most support regulatory decision making, as those used for conventional chemicals would not work for nanomaterials. Nano- and probably coating-specific trigger and cut-off values, for instance analogously to $logK_{OW}$ for estimation of bioaccumulation potential, would have to be defined in order to prevent unnecessary animal testing in hazard assessment. She saw a need to update the emission scenario documents for biocides, to develop guidelines for effect data evaluation and to harmonise all risk assessment guidance applicable to nanomaterials at EU level.

Corinna Burkart's take-home message is that there are still many open questions to be answered.

Annex 1: Agenda

Day 1

09:00-09:30	**Registration at UBA**
09:30-09:45	Welcome Remarks by the German Environment Agency *(Jutta Klasen, Division on Chemical Safety, UBA)*
09:45-10:00	Welcome Remarks by the Federal Ministry of the Environment, Nature Conservation, Building and Nuclear Safety *(Anke Jesse, IG II 6, BMUB)*
10:00-10:10	Overview on current funding activities of the BMBF in the area of nanosafety (*Rosita Cottone, BMBF represented by Eva Gerhard-Abozari, PT Jülich)*
10:10-10:15	Housekeeping Information *(UBA)*
Session 1	**Regulatory Key Note Talks**
10:15-10:35	OECD`s contribution to the safety of nanomaterials (*Mar Gonzalez*, OECD) [This presentation was cancelled due to unavailability of the speaker]
10:35-10:55	Regulatory activities in the area of environmental risk assessment of nanomaterials *(Anu Kapanen, ECHA)*
Session 2	**Scientific Key Note Talks**
10:55-11:25	Importance of physical-chemical properties of ENM (intrinsic/extrinsic) for environmental hazard and risk assessment *(Iseult Lynch, University of Birmingham, UK)*
11:25-11:55	Environmental release modelling in contexts of high uncertainty for engineered nano-CeO_2, -SiO_2 and –Ag in Germany *(Fadri Gottschalk, ETSS, CH)*
12:00-13:30	**Lunch and Poster Session**
Session 3	**Effects and Accumulation**
13:30-13:50	Adaptation of OECD test guidelines on ecotoxicity tests – experience gained from the EU project MARINA and further considerations *(Kerstin Hund-Rinke, Fraunhofer IME, GER)*
13:50-14:10	What is the meaning of pristine nanoparticles, their lifecycle and fate? An overview and forward look *(Marianne Matzke, NERC, UK represented by Iseult Lynch, University of Birmingham, UK)*
14:10-14:30	Ecotoxicity and Fate of nanomaterials in laboratory and outdoor lysimeter experiments – experiences from the BMBF project DENANA *(Karsten Schlich, Fraunhofer IME, GER)*

Day 1 (continued)

14:30-14:50	Investigating effects of silver nanoparticles on the soil community – An outdoor TME study *(Monika Hammers-Wirtz, gaiac, GER)*
14:50-15:10	NanoUmwelt - Risk analysis of engineered nanomaterials in the environment: identification, quantification and analysis of the human- and ecotoxicological effects *(Florian Meier, Postnova Analytics GmbH, GER)*
15:10-15:30	Fate and effect of manufactured nanomaterials in the aquatic food chain *(Christian Schlechtriem, Fh IME, GER)*
15:30-16:00	**Coffee and Poster Session**
Session 4	**Fate and Exposure in different environmental compartments**
16:00-16:20	Getting our feet back on the ground: How the environment controls nanoparticle fate *(Frank von der Kammer, University of Vienna, AT)*
16:20-16:40	The fate of nanoparticles in the subsurface - a risk for drinking water resources? *(Sondra Klitzke, UBA, GER)*
16:40-17:00	Behavior of engineered nanoparticles in the pathway wastewater - sewage sludge - plant using the examples TiO_2, CeO_2, MWCNT and quantum dots *(Karsten Franke, HZDR - Institute of Resource Ecology, GER)*
17:00-17:20	Investigation on possible risks - Are ENMs released during the combustion of nanoparticle-containing waste? *(Jürgen Oischinger, Fraunhofer UMSICHT, GER)*
17:20-17:40	Mobility of different nanomaterials in unsaturated soils *(Carmen Nickel, IUTA, GER)*
17:40-18:00	Measurement of manufactured nanomaterials in environmental samples (*Andreas Wimmer, Technical University of Munich, GER)*
18:00	**Get Together and Poster Session**

Day 2

<table>
<tr><th>Session 5</th><th colspan="6">Applications and Assessment</th></tr>
<tr><td>09:00-09:20</td><td colspan="6">Nanopesticides: state of knowledge and implications for regulatory exposure assessment (Web Conference: Melanie Kah, University of Vienna, AT and CSIRO, AUS)</td></tr>
<tr><td>09:20-09:40</td><td colspan="6">Comparing nano-enabled copper formulations govern release, hazards, anti-fungal effectiveness and sustainability throughout the wood protection lifecycle (Wendel Wohlleben, BASF, GER)</td></tr>
<tr><td>09:40-10:00</td><td colspan="6">Volumes and life cycle of CeO2, SiO2 and Ag nanomaterials - knowns and unknowns (Bernd Giese, BOKU Vienna, AT)</td></tr>
<tr><td>10:00-10:20</td><td colspan="6">Prosafe’s review on reliability of methods and data for regulatory assessment of nanomaterial risks – part environment (Klaus Steinhäuser, GER)</td></tr>
<tr><td>10:20-10:40</td><td colspan="6">Grouping/Read Across approaches to reduce the testing of nanomaterials regarding their environmental hazard (Dana Kühnel, UFZ, GER)</td></tr>
<tr><td>Session 6</td><td colspan="6">Knowledge Café</td></tr>
<tr><td>10:40-10:50</td><td colspan="6">Introduction to Knowledge Café</td></tr>
<tr><td rowspan="3">10:50-12:00</td><td colspan="6">Knowledge Café (including coffee supply): In depth discussion on selected topics</td></tr>
<tr><td colspan="2">Table 1:
Importance of & Methods for determining Intrinsic and Extrinsic Properties (chaired by Iseult Lynch, University of Birmingham, UK)</td><td colspan="2">Table 2:
Needs for Test Guideline Development (chaired by Jukka Ahtiainen, FIN)</td><td colspan="2">Table 3:
Exposure Assessment of Nanomaterials (chaired by Wiebke Galert, UBA, DE)</td></tr>
<tr><td colspan="3">Table 4:
Short, Mid and Long Term Goals for Risk Assessment Tools (chaired by Anu Kapanen, ECHA)</td><td colspan="3">Table 5:
Research on Nanosafety (chaired by Thomas Kuhlbusch, BAuA, DE)</td></tr>
<tr><td>12:00-13:00</td><td colspan="6">Lunch and Poster Session</td></tr>
<tr><td>Session 7</td><td colspan="6">Lessons Learned</td></tr>
<tr><td>13:00-14:00</td><td colspan="6">Summary of results of the Knowledge Café</td></tr>
<tr><td>14:00-14:30</td><td colspan="6">Lessons Learned with statements by different stakeholders and discussion with the plenary</td></tr>
<tr><td>14:30-14:45</td><td colspan="6">Conclusion and Closure of the Meeting</td></tr>
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Annex 2: Participants List

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