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60 YEARS FRAUNHOFER IME

FRAUNHOFER INSTITUTE FOR MOLECULAR BIOLOGY
AND APPLIED ECOLOGY IME

WELCOME

This year Fraunhofer IME celebrates its 60th anniversary – this means 60 years of research into the environmental risk assessment of substances and consumer exposure. For almost 20 years we have been dealing with the effect of nanomaterials on the environment – by practical investigations and participation in the elaboration of international guidelines. Based on our comprehensive knowledge we develop solution approaches and provide answers to current problems. In this newsletter you will find examples as well as a portrait of Dr. Kerstin Hund-Rinke who very successfully promotes our research in the ecotoxicology of nanomaterials from the beginning to the present day.

Yours sincerely

Prof. Dr. Christoph Schäfers

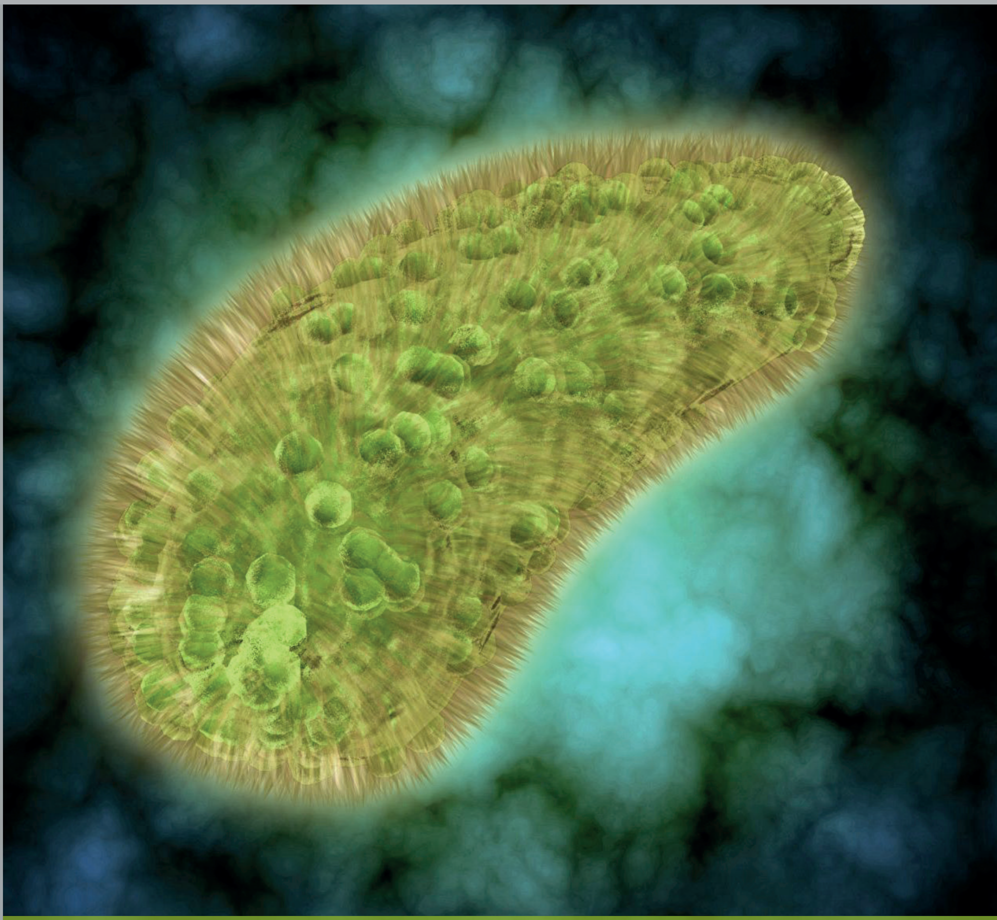


Photo: Green alga | © Panthermedia.net: Ryan Rossotto

In this special issue on nanomaterials you can read:

- Grouping instead of testing – This is how it works
- Do nanomaterials bioaccumulate in mussels?
- Tracking down nanomaterials
- Portrait: Dr. Kerstin Hund-Rinke

GROUPING INSTEAD OF TESTING – THIS IS HOW IT WORKS

Grouping nanomaterials – a tool for reducing the amount of ecotoxicological testing

Nanomaterials (NM) are produced in a wide variety of variations. Not all of them can be comprehensively tested for their ecotoxicological effects. The European Regulation on Chemicals (REACH) makes it possible to transfer data from a tested nanomaterial to another, untested NM (Read-Across). A prerequisite for the data transfer is that both NMs behave similarly regarding the target endpoint, i.e. their relevant properties need to be verifiably similar. Based on a systematic testing approach we have identified relevant properties for metals and metal oxide nanomaterials.

Due to the significant variability of the test designs literature data have proven to be insufficient in identifying relevant properties. Systematic testing of 25 metals and metal oxide nanomaterials which have been ▶

comprehensively characterized in the respective test medium turned out to be considerably more effective. For these substance classes the properties "release of toxic ions" (e.g. Ag, Zn, Cu), "reactivity" and "form" became evident. The toxicity to daphnia, for example, is significantly influenced especially by the form of the NM. Algal toxicity additionally is influenced considerably by the attachment behavior of the NM. Thus, modifications of nanoparticular CeO_2 or Fe_2O_3 can affect the docking potential and accordingly influence algal toxicity. Modifications which increase the tendency to attach also result in an enhanced toxicity. Our newly developed testing approach allows a rapid microscopical determination of the tendency to attach.
Based on the systematic tests we could group the investigated nanomaterials with respect to their aquatic ecotoxicity using the parameters "release of toxic ions", "reactivity", "form" and "tendency to attach".

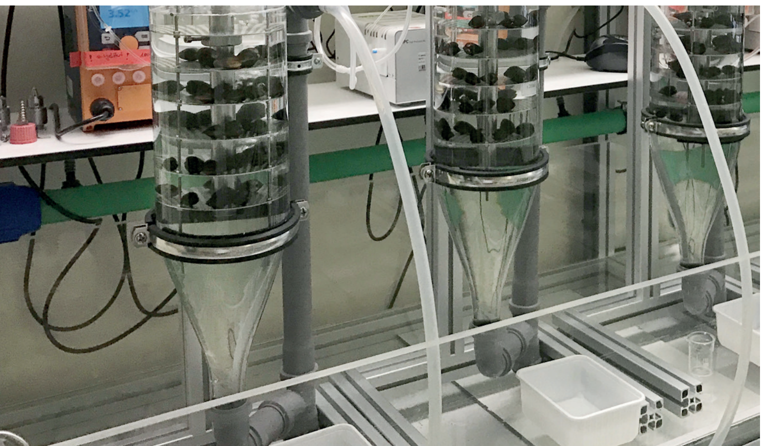
Grouping allows the transfer of aquatic ecotoxicological data of a tested nanomaterial from one to another.

Within the clusters the toxicity for the most sensitive organism differs by no more than a factor of 10. Moreover, the sequence of the test organisms algae, daphnia and fish embryo is identical with respect to their sensitivity towards the NM. The clusters themselves differ in these two criteria. In soil, many differences between toxic effects of nanomaterials are less pronounced. So far, we can distinguish between two clusters: non toxic NMs such as TiO_2, CeO_2, Fe_2O_3 and SiO_2 as well as NMs which release toxic ions and are toxic for soil organisms. With this a cornerstone is laid for reasonably transferring ecotoxicological data from tested NMs to other NMs. ■

DO NANOMATERIALS BIOACCUMULATE IN MUSSELS?

Solving the problem of testing the bioaccumulation of nanomaterials in aquatic systems

The classical method to elucidate the potential accumulation of chemicals in organisms and finally in the food chain (bioaccumulation) is the flow-through approach with fish. Since this test system is used for investigating dissolved substances, it is not suitable for particles.



Flow-through system to determine the bioaccumulation potential of mussels. Photo: Fraunhofer IME

A new test system allowed us to investigate the bioaccumulation potential of different NMs in freshwater mussels.

Due to their specific properties nanomaterials are widespread, and increasing amounts are released to the environment by production, use or disposal of NM containing products. Therefore, a potential environmental impact due to persistence (P), bioaccumulation (B) or toxicity (T) has to be excluded. The bioaccumulation test according to OECD guideline 305 (fish flow-through approach) is an established tool for assessing the bioaccumulation potential of chemicals. It is, however, not suitable for nanomaterials since it does not enable the maintenance of stable exposure conditions. The freshwater filter feeding bivalve *Corbicula fluminea* has previously been shown to ingest and accumulate NMs from water. To investigate the suitability of *C. fluminea* as a test organism for bioaccumulation studies we developed a new flow-through system to expose the mussels under constant exposure conditions.

C. fluminea is a suitable test organism for assessing the bioaccumulation of NMs.

Nanomaterials with different characteristics were applied to determine their bioaccumulation potential. The silver nanoparticle NM 300K was tested as a well dispersible and ion releasing NM. In addition, *C. fluminea* was exposed to $AgNO_3$ as a source of dissolved Ag^+ to compare the bioaccumulation of dissolved Ag^+ and Ag from nanoparticulate sources. The titanium dioxide NP NM 105 was used to investigate a non ion releasing NM, and the polystyrene nanoparticle Fluoro-Max™ containing a fluorescence dye was tested as a representative for NMs that are based on organic polymers. Bioaccumulation and organ specific distribution factors provided clear indications that the tested NMs were simply ingested rather than accumulated by the mussels.

The new test system may serve as a novel screening tool to be integrated in a tiered bioaccumulation testing strategy for NMs and could allow a waiver of further tests using vertebrates. ■

TRACKING DOWN NANOMATERIALS

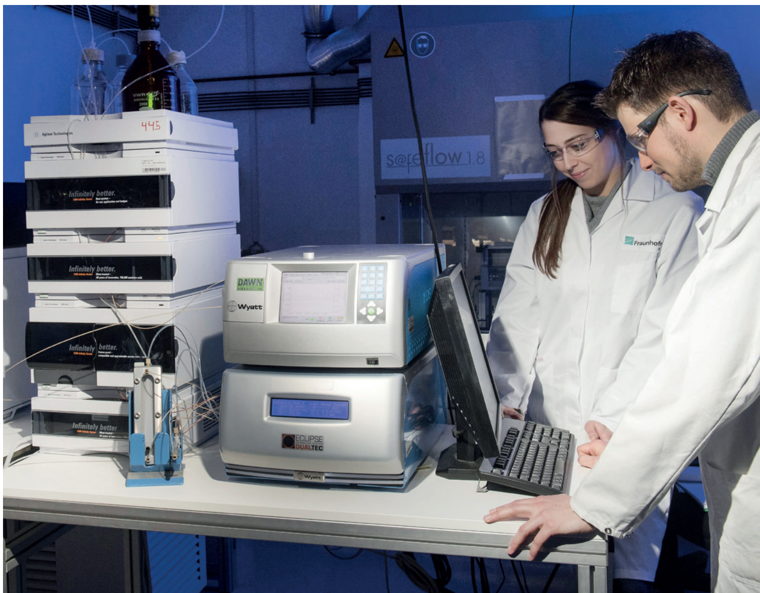
Detecting nanoparticles in environmental matrices using complex methods

Nanomaterials (NM) find their way into the environment via different pathways. They can end up in the aquatic environment via wastewater treatment plants and can be spread over the ground in sludge used for agricultural purposes. The Fraunhofer IME is developing complex methods for the detection and characterization of NMs in environmental samples.

To assess the potential risk of NMs, it is necessary to determine both, the concentration of the particles and their particle size distribution. In most cases, the particles must be transferred to an aqueous suspension in such a way that their particular properties are retained. For different matrices, as soil or tissue samples, we apply specific methods including colloidal extraction or the targeted enzymatic breakdown of the biological matrix. For inorganic materials a promising approach is the single-particle inductively-coupled plasma mass spectrometry. To distinguish and precisely quantify NMs we can also count and quantify individual particles in aqueous suspensions.

For less sensitive questions we apply gentle separation techniques such as asymmetric flow-field-flow fractionation (AF4) which allows particles to be separated according to their size in complex samples. Coupled to different detectors such as optical or element selective detectors (e.g. ICP-MS) the AF4 provides a significant amount of size-dependent data.

To determine the mass balance, element specific procedures such as ICP-MS or ICP-OES are required. A prerequisite for applying these procedures is a dissolution of the particles. As a consequence, different digestion methods have to be developed for different types of particles. This is normally performed in a microwave digestion unit with acids or acid mixtures. In a further step particles can be separated from the dissolved fraction by ultra filtration (e.g. 3 kDA filter) and quantified by ICP-MS or ICP-OES.



AF4 test system.
Photo: Fraunhofer IME

Dr. Kerstin Hund-Rinke...

… has been leading the work group Terrestrial Ecotoxicology, Soil Protection and Nanomaterials since 2002. "Developing pragmatic approaches to estimate the environmental risk posed by nanomaterials in all their diversity and thereby take into account the viewpoints of regulatory bodies, industry and science – this is a challenge I am happy to take on myself."

" Different views on the life cycle of substances result in synergies and creative solutions.

Kerstin Hund-Rinke studied biology at the University (LMU) of Munich and carried out her doctoral thesis on soil microbiological problems in ectoxicology at the TU Munich and the German Research Center for Environmental Health, Helmholtz Zentrum Munich. At Fraunhofer IME she has been involved in a wide variety of studies on current environmental problems since 1988. She has developed methods and test systems in the context of the ecological assessment of soil quality, microbial biodiversity and the bioavailability of pollutants. For many years, her research in the context of the risk assessment of substances was focused on the environmental toxicity of nanomaterials. On this issue she published the first paper that included regulatory aspects.

The regulation of chemicals requires standardized test procedures. But how can such test systems be adapted to the specificities of nanomaterials (NM)? Do we have to investigate all modifications of NMs or can we transfer data from one NM to another? Are studies in the laboratory suitable for longterm predictions of environmental behavior and effects? These are the questions Kerstin Hund-Rinke and her team are dealing with: "The simulation of processes occurring in sewage treatment plants, in the digestion tower, in residual material deposits, in soil or in surface waters help to find possibilities and limitations for assessing the ecotoxicity of nanomaterials – in the laboratory or on a technical scale. We could show that the sulfidation of silver nanomaterials in sewage treatment plants does not result in the expected detoxification, but that NMs applied to soils are bioavailable in the long term. We also found that the toxicity in the algae test depends on the attachment behavior of the NM – a criterium which is of particular importance for read-across methods."

NETWORKING – CONTRIBUTE AND DEEPEN KNOWLEDGE

Kerstin Hund-Rinke is a member of various national and international scientific committees. Among other things she is vice chair of the scientific advisory board on fertilizer issues (Wissenschaftlicher Beirat für Düngungsfragen), member of the German delegation at the "OECD Working Party for Manufactured Nanomaterials (WPMN)" and on the advisory board for the master program "Soils, Inland Waters, Contaminated Land" of the Hochschule Osnabrück.

Read more about our research activities here: www.ime.fraunhofer.de/en.html

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Applied Ecology
Auf dem Aberg 1
57392 Schmallenberg, Germany

Tel +49 2972 302-0
Fax +49 2972 302-319

Director
Prof. Dr. Christoph Schäfers
christoph.schaefers@ime.fraunhofer.de

Editing
Brigitte Peine
Public Relations

Translation
Brigitte Peine
Dr. Terry Clark

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