Hyalella azteca. Photo: © Fraunhofer IME

Fraunhofer IME

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Fraunhofer Institute for Molecular Biology and Applied Ecology IME

Welcome

The European chemicals regulation REACH requires the (further) development of guidelines for special issues. Read about our work on the detection and evaluation of non-extractable residues, endocrine disruptors and the bioaccumulation of nanomaterials. Portrait: Dr. Karsten Schlich, who is dedicated to UVCBs.

Yours sincerely

Prof. Dr. Christoph Schäfers

Bioaccumulation assessment of nanomaterials

Using aquatic invertebrate species in the risk assessment process for engineered nanomaterials

The volume of engineered nanomaterials (ENMs) produced annually and used in many products is about several million tons. However, this also means that large quantities of these ENMs find their way into the environment where they can be ingested, enriched and passed on through the food chain by aquatic organisms. Therefore, ENMs, like other chemical industrial products, are subject to regulation and risk assessment including investigations on their potential to accumulate in the environment. Within the regulation of chemicals in several jurisdictions, such as the European regulation REACH, the bioconcentration factor (BCF) is the standard endpoint. The BCF is mostly determined by flow-through fish tests. However, nanomaterials tend to agglomerate, which may lead to sedimentation in aquatic environments. Therefore, the bioavailability of the tested nanomaterials may be impaired for pelagic species, including fish, in comparison to benthic or filtrating species says Prof. Dr. Christian Schlechtriem, Head of Department Bioaccumulation and Animal Metabolism.

In this issue you can read:

Several risk assessment regulations allow the usage of data gained during tests using invertebrates and such data may allow a waiver of further tests using vertebrates. Dr. Sebastian Kühr (NIVA, Norway) identified benthic amphipods in his PhD studies at Fraunhofer IME as the most promising species for ENM testing. He showed that the results from bioconcentration and biomagnification tests with the freshwater amphipod *Hyalella azteca* have the potential to be included in a tiered assessment allowing a clear grading of the tested nanomaterials as »bioaccumulative« or »non bioaccumulative«. In the worst-case scenario of the amphipod test, the approach may allow a further waiver of vertebrate tests if there are no indications of bioaccumulation of ENMs.
The assessment scheme for bioaccumulation assessment of ENMs was recently published in the journal *Environmental Sciences Europe* (doi.org/10.1186/s12302-020-00442-2).

Experimental characterization of NER for persistence assessment

Non-extractable residues (NER) are formed during transformation tests of substances in soils or water / sediment systems (e.g. OECD 307, 308). How many NERs are formed depends on the substance properties and the properties of the solid matrix (soil, sediment). The extraction methods chosen also affect the NER, but are difficult to standardize. Thus, the boundary between the extractable portion and the NER is currently not precisely defined, says Dr. Dieter Hennecke, Head of Department Ecological Chemistry.

Laboratory set-up for the determination of NER Type I (Silylation).
Photo: © Fraunhofer IME | Klaus-Peter Kappest

The ECHA guideline R.11 (2017) emphasizes the importance of NER for the persistence assessment. It is assumed that parts of the NER are only weakly bound and can potentially be released over time. In a worst-case scenario, the entire NER is therefore assessed as a potentially releasable test substance, provided no other information is presented. A classification of different NER types according to their binding status is proposed for characterization: Type I NER as weakly bound and potentially releasable, Type II NER as irreversibly bound (safe sink) and Type III NER or bioNER as molecular components that have been broken down into biomolecules via microbial metabolism.

Together with the Federal Environment Agency and partners from DTU Copenhagen, RWTH Aachen University and UFZ Leipzig, the Fraunhofer IME has proposed an extraction scheme for the experimental determination of these NER types in laboratory tests. The study brings together the work of the BfG (Eschenbach and Oing, 2013) and the ECHA discussion paper (Kästner et al., 2018) and gives an idea of the possible application in the persistence assessment of NER-forming substances.

One of the main goals of the study was the development of procedures that can also be used in routine laboratory work. The result is a stepped process in which an NER definition is initially proposed with a final ASE™ extraction. This is followed by a stepped process with harsh, matrix-destroying extractions in order to release NER and, if necessary, to identify it as parent test substance,

or known metabolites, by means of chemical analysis. The experimental procedure is supported by the MTB Modeling Tool (Brock et al., 2017; Trapp et al., 2018), with which the BioNER can be estimated based on the measured mineralization. The project results were presented in an international workshop in February 2021 and are available at: https://www.umweltbundesamt.de/themen/chemikalien/chemikalien-reach/nicht-extrahierbare-rueckstaende-ner.

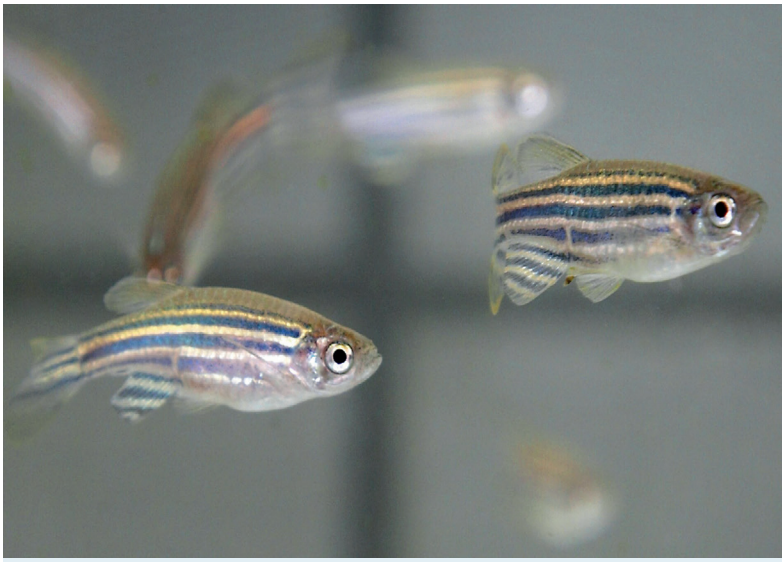
Endocrine disruptors as a threat to fish populations

Fraunhofer IME develops a new fish multigeneration test for the detection of endocrine disruptors

The research group »Fish Toxicology and Biomarkers« of the Fraunhofer IME led by Dr. Matthias Teigeler, Joint Head of Department Ecotoxicology, has many years of experience in the quality-assured and complex technical implementation of long-term fish studies for the detection of endocrine disruptors. The Fraunhofer IME is using its broad expertise and knowledge of regulatory requirements to develop a new fish test guideline.

The problem of exposure of aquatic life to substances that can affect the endocrine system of organisms has gained great importance in the regulation of substances by authorities in recent years.
This concerns plant protection products, industrial chemicals and biocides, as well as veterinary and human medicinal products.
Since the end of 2018, for example, the provision of data on the potential endocrine effect of an active substance has been stipulated for plant protection products as part of the authorisation process. The pressure on the manufacturing companies to generate this data is correspondingly great.
The definition of an endocrine effect by the European Union requires reliable identification of the connection of a physiological effect, which shows the characteristics of a hormone-like mechanism of action, with the significant impairment of a population-relevant parameter.

Together with the Federal Environment Agency, Fraunhofer IME is developing a fish test guideline that meets the requirements: In the *Zebrafish Extended One Generation Reproduction Test* (ZEOGRT), a complete life cycle of the test fish is investigated: The deposition of fertilised eggs by the exposed parental fish, the early life stage, juvenile adolescents as well as the reproductive performance of adult groups and the survivability of the eggs produced.



Zebrafish.
Photo: © Fraunhofer IME | Studio 95 | Frank Peinemann

The project is part of the OECD's work plan for the development of ecotoxicological guidelines. Four ZEOGRT studies were conducted at the Fraunhofer IME to validate the study type, and external laboratories will also be involved in the next step.

With this project, the Fraunhofer IME further underlines its claim to make significant contributions to the development of guidelines.

»Especially when studying the effect of UVCB substances on aquatic organisms of different trophic levels, there are no limits to the complexity of the test design.«

Karsten Schlich completed his engineering studies in the field of environmental protection at the Technical University in Bingen and subsequently earned his doctorate at the Free University of Berlin. As part of his doctoral thesis, he investigated issues related to the fate and ecotoxicological effects of nanomaterials in the aquatic and terrestrial environments. Since 2008, Karsten Schlich has been working on a wide variety of research projects investigating environmentally problematic substances at Fraunhofer IME. These include the effect of pesticides on aquatic plants and algae. Regarding industrial chemicals, in particular the so-called UVCBs, have become a focus of substance regulation. The varied properties of substances require adapted test and evaluation strategies. Karsten Schlich contributes his expertise to the optimization of these tests and strategies.

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Dr. Karsten Schlich...

…has been leading the working group »Aquatic Primary Producers and Microbial Diversity« at Fraunhofer IME since 2019. »The wide range of diverse methods coupled with ecological understanding offers the possibility to find the best possible approach for our customers' questions.«

In substance regulation, the requirements are increasing to investigate substances with particularly difficult properties. The challenge is to modify the existing test guidelines to be able to determine the effects of these substances. Here, both the aquatic and the terrestrial environment must be taken into account. Due to his experience with the investigation of nanomaterials, Karsten Schlich can also apply his knowledge of methods to other substances and transform ideas. This involves questions of the application of the test substance, its stability during the test and the selection of a suitable test design (static, semi-static or flow-through). Also, in discussion with colleagues from the chemical department he exchanges ideas about the selection of suitable analytical procedures. Relevant OECD guidelines exist, but only ecotoxicological expertise and practical experience ensure that in the end results are meaningful.

Through training events for users and authorities, Fraunhofer IME underlines its position as a scientific mediator between production and regulation.

In January 2022, Karsten Schlich will lead a workshop on »Aquatic toxicity testing with a focus on UVCBs/ multicomponents« for the European Chemicals Agency (ECHA). The difficulties in testing will be explained to the agency's review board and possible solutions will be identified.

In a new area of work, he is involved in advancing the regulatory assessment of microbial performance in soils by supplementing the state of the art with alternative methods and test systems for ecological assessment of microbial diversity and bioavailability of pesticides.

Karsten Schlich is a member of the DIN working group Marine Biotests. Since 2019, he has a teaching position in the master's program »Soil, Waters, Contaminated Sites« at the University of Applied Sciences in Osnabrück and is a member of the scientific advisory board.

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