

Hyalella azteca © Fraunhofer IME | Klaus-Peter Kappest

NEWS | NOV26

Fraunhofer Institut for Molecular Biology and Applied Ecology IME

Welcome

This issue focuses on our developments of new alternative methods to animal testing for chemical assessment in the fields of bioaccumulation and ecotoxicology, which are in line with the **"European Commission Roadmap."** Data collection yields legally admissible results only when conducted within the framework of quality-assured studies: In our profile, we introduce Dr. Ursula Wahle, our deputy head of quality assurance, who is leaving us to enjoy her well-deserved retirement after 40 years of service.

Yours sincerely,

Prof. Dr. Christoph Schäfers

Focus on Polymers: Using nanoHYBIT to Obtain Reliable Bioavailability Data

Developing Non-Vertebrate Test Methods for Innovative Environmental Assessment

Due to their composition and varying molecular weight fractions, polymers pose significant regulatory challenges when it comes to assessment. How bioavailable are they? And how can their bioaccumulation potential be investigated without conducting vertebrate animal testing?
In its "Key Areas of Regulatory Challenge" (KARCs), the European Chemicals Agency (ECHA) identifies a need for further research: There is a lack of suitable methods to better understand the bioavailability of polymers and the associated hazards. At the same time, there is a need for validated, non-vertebrate methods to assess the bioaccumulation potential of nanomaterials, as identified by the European Roadmap for phasing out animal testing in chemical safety assessment.
This is where the work of Fraunhofer IME comes in. The HYBIT approach (OECD Test

In this issue you can read:

- nanoHYBIT
- FET in a Flow-Through System
- E-FET
- Portrait: Dr. Ursula Wahle

Guideline 321), developed as an alternative to the fish test under OECD Test Guideline 305, uses the copepod *Hyalella azteca* to investigate the bioaccumulation potential of substances. Building on this, the "nanoHYBIT" was developed to investigate the uptake of nanomaterials from water and food. The method supports the 3Rs principle by replacing vertebrates with invertebrate test organisms.
As part of a project funded by the BMFTR, the "nanoHYBIT" method was optimized. An OECD ring test, using ^{14}C-labeled nanopolystyrene, is now investigating, how reproducible and transferable the method is between laboratories. This is an important step toward the validation and potential establishment of a new OECD test guideline or an extension of OECD Test Guideline 321.
The relevance of polymers in substance regulation is continuously growing. Our goal is to support industry in the assessment of polymers and other complex materials using a scientifically sound, non-vertebrate method. We support our clients throughout the entire process — from planning the investigations and characterizing the test materials to conducting the laboratory studies. In doing so, we also draw upon the technical expertise and analytical capabilities in the field of "Physical Metrology" at our location on the Soest campus of the South Westphalia University of Applied Sciences: meaningful bioavailability data requires that the properties of the test material be described in a traceable manner.

With "nanoHYBIT", our customers gain access to an innovative and practical method for reliably assessing the bioaccumulation potential of complex materials: From scientific conception through methodological refinement and international testing to subsequent application in industrial studies, the conditions for practical knowledge transfer are established. ■

Contact: Dr. Sebastian Kühr
sebastian.kuehr@ime.fraunhofer.de

Fish Embryo Test in a Flow-Through System

The fish embryo test (FET, OECD Test Guideline 236) using zebrafish is designed to be conducted in a small volume of test medium. The test is set up in multiwell plates to allow for optimal assessment of embryo development. The biological parameters measured in this test are robust and can be reliably observed. A greater challenge lies in the consistent application of test chemicals. However, this is necessary to enable a good assessment of a chemical's toxicity. Challenging properties of a substance—such as high lipophilicity—become very apparent in this test system.

Furthermore, the small volumes of test medium often make meaningful chemical analysis difficult, particularly when sample preparation including enrichment is required. Added to this are guideline-based requirements for the quality of analytical measurements, such as validation and reproducibility, as well as the ability to analyze reference samples.

Fraunhofer IME has many years of experience conducting ecotoxicological tests using flow-through systems. These are typically designed for fish studies and accommodate larger groups of fish, and thus relatively large volumes.

The challenge lies in scaling these systems down to a small scale. In a master's thesis, a flow-through system was adapted to meet the requirements of a FET. Through collaboration with a project involving the European Chemicals Agency, which proposes the FET as an alternative to animal testing, it was possible to conduct the tests under both semi-static and flow-through conditions and to compare the results.


Zebrafisch Embryo © AdobeStock_#2038996331

Preliminary tests without a test substance demonstrated that the embryos develop well even under flow-through conditions. For a stable test substance, consistent exposure was confirmed in both the semi-static and flow-through systems. The second test substance was not stable in the semi-static system but remained detectable in the flow-through system until the end of the test.

The results show an improvement in data quality by steady substance application. ■

Contact: Dr. Matthias Teigeler
matthias.teigeler@ime.fraunhofer.de

Fish Embryos Instead of Long-Term Tests: Transcriptomics Provides Thresholds for Chronic Effects

Fraunhofer IME's E-FET Supports the EU Roadmap for Phasing Out Animal Testing in Chemical Assessment

In June 2026, the European Commission presented its roadmap for the phased phase-out of animal testing in the safety assessment of chemicals. For chronic fish toxicity, it aims to make better use of information from non-animal testing approaches and, in the long term, to establish assessment strategies that are entirely free of animal testing. Under REACH, the Fish Early-Life Stage Test (FELS, OECD Test Guideline 210) has been the primary method to date - a complex test lasting several weeks and involving many animals. In this test, the *No Observed Effect Concentration* (NOEC) - the concentration above which chronic effects begin to appear - is the key parameter.

Fraunhofer IME has developed an alternative in the form of the fish embryo test (E-FET), which incorporates transcriptomics. It is based on the established fish embryo test (OECD Test Guideline 236) using zebrafish embryos, which, at this early stage, are not subject to the EU Animal Testing Directive. After 96 hours of exposure to sublethal concentrations of the test substance, the entire transcriptome is sequenced. *Transcriptomic Points of Departure* (tPODs) - the concentration at which coordinated changes in gene expression begin - are derived from concentration-effect models of the responding genes.

In a study designed in collaboration with and funded by the European Chemicals Agency (ECHA), 16 industrial chemicals for which FELS data were available were examined. For eleven substances, tPODs could be derived; these were mostly at or below the chronic NOECs and ranked the substances in largely the same order as the FELS; on average, the NOECs were about a factor of four higher than the tPODs. Substances without chronic effects did not yield a tPOD. "The E-FET is thus protective without being overly conservative, and provides additional insights into the mechanism of action via the affected signaling pathways," says Dr. Sebastian Eilebrecht, head of the Department of Ecotoxicogenomics. The study also highlights areas where further development is needed: For unstable or poorly water-soluble substances, optimized exposure systems are crucial. Thus, the study provides exactly the kind of data foundation called for by the roadmap in the medium term: reference data that link the findings of new methods to chronic fish effects in vivo.

Our Services: We conduct the E-FET under standardized conditions - including analytical exposure verification, RNA sequencing, and reproducible bioinformatic tPOD derivation - for substances without prior data, with an upstream range-finding study. In addition, we provide consulting on the integration of transcriptomic data into regulatory *weight-of-evidence* approaches and support validation studies and case studies in line with the Roadmap. ■

Contact: Dr. Sebastian Eilebrecht
sebastian.eilebrecht@ime.fraunhofer.de

The European Commission's Roadmap

On 1 June 2026, the European Commission adopted its "Roadmap towards phasing out animal testing for chemical safety assessments." The roadmap outlines 22 actions across three pillars, covering 15 legislative domains - from industrial chemicals and pesticides to pharmaceuticals and food additives - supported by more than 30 recommendations to replace, reduce, or refine animal testing for each (eco)toxicological endpoint. It is a key framework for advancing the regulatory acceptance of new approach methodologies (NAMs) and animal-free testing strategies.



"The quality and data integrity of the studies conducted at the IME have always been a matter close to my heart. However, it remains a constant challenge to get all employees on board with this objective."

Dr. Ursula Wahle...

Dr. Ursula Wahle studied chemistry at the University of Cologne, where she completed her master's thesis at the Institute of Biochemistry. During her subsequent doctoral studies (1987–1990) under Prof. Werner Klein at what was then Fraunhofer IUCT in Schmallenberg, she worked on the development of a universal analytical framework for organic chemicals in soil—a pilot project of the then Federal Ministry of Research and Technology. Her work made an important contribution to the systematization of sample preparation in environmental analysis.

After successfully completing her doctoral degree, she served as laboratory director starting in 1990 and as deputy department head in the Division Soil and Water Protection under the direction of Dr. Werner Kördel starting in 1993. In this role, she was entrusted with directing studies conducted in accordance with OECD test guidelines for the registration of chemicals, as is still done today at Fraunhofer IME. The principles of Good Laboratory Practice (GLP) were already in effect at that time. The institute was among the first institutions to be certified under GLP.

Ms. Wahle conducted studies on the fate and metabolism of organic chemicals in soils and sediments, as well as investigations of volatilization behavior, photolysis, and hydrolysis. A wide variety of research projects focused largely on the behavior of chemicals in different environmental compartments, bioavailability and bioaccumulation, as well as the influence of DOC (dissolved organic carbon) on the mobility and ecotoxicity of poorly soluble substances. Consequently, she published extensively on these topics. In addition, she served on the works council for many years.

After taking parental leave, Ursula Wahle worked part-time in the Environmental Risk Assessment department, focusing on impact assessments for technical products and processes, life cycle assessments, and the evaluation of the environmental hazard potential of chemicals. In 2005, she moved to the Quality Assurance department at Fraunhofer IME Schmallenberg. There, she was able to contribute her extensive experience with research and regulatory studies to the review and qualitative optimization of work conducted under GLP and the implementation of new guidelines. In addition, from 2005 to 2025, Ursula Wahle worked as a quality manager in the context of accreditation according to DIN EN ISO/IEC 17025.

Over the past 40 years, the regulatory requirements for quality-assured work and data integrity have continuously evolved. For example, the OECD has published numerous new test guidelines and advisory documents to resolve inconsistencies and increase the efficiency of quality assurance monitoring by incorporating modern, risk-based, and digital requirements. ■

Read more about our research activities here:

www.ime.fraunhofer.de/en/ae

IMPRINT

Published by
Fraunhofer Institute for Molecular Biology and Applied Ecology IME

Division Applied Ecology
Auf dem Aberg 1
57392 Schmallenberg
Germany
Phone +49 2972 302-0
www.ime.fraunhofer.de/en/ae

Director
Prof. Dr. Christoph Schäfers

Editing, Design & Layout
Julia Karbon, Public Relation

Translation
Dr. Terry Clark

Production
Schäfers Druck GmbH, Schmallenberg
100% Recyclingpaper