Photo: Hannes Reinwald

Fraunhofer IME

NEWS | NOV24

Fraunhofer Institut for Molecular Biology and Applied Ecology IME

Welcome

At 2024 SETAC Annual Meeting in Seville, the IME made a visible contribution: as session chairs in the organising committee and as authors and co-authors of over 45 oral presentations, posters and presentations. Read about our SETAC topics of polymer degradation, molecular effects and assessment concepts, as well as the portrait of our doctoral student Marie Winter, who was presenting in Seville.

Yours sincerely

Prof. Dr. Christoph Schäfers

Transcriptomic Point-of-Departure: Integrating OMICs Data into Regulation

For substances to be approved for entry into the market, they must undergo an environmental hazard assessment, depending on their use and production volume. This includes the determination of effect thresholds for adverse effects in fish. The associated ecotoxicological tests are time-consuming and costly and involve animal testing. Furthermore, these tests examine a limited number of endpoints, such as mortality, growth or reproduction, which do not always allow conclusions to be drawn about the exact mechanism of action of the test substance. For these reasons, the European Chemicals Agency (ECHA) and the European Food Safety Authority (EFSA) encourage the use of OMICs data in 3R-compliant *New Approach Methodologies* (NAMs) for (eco)toxicological testing and prioritization of chemicals in current position papers.
At SETAC 2024 in Seville, Fraunhofer IME presented a case study on the use of the *transcriptomic point of departure* (tPOD) in ecotoxicology to derive quantitative endpoints from RNA-Seq studies. The use of

In this issue you can read:

transcriptomic data in the zebrafish embryo model as a NAM is particularly attractive for such a derivation, since it is recognized as an alternative to animal testing in accordance with EU Directive 2010/63/EU. In this model, gene expression changes are recorded and evaluated as a function of the concentration of the test substance, so that threshold concentrations can be determined for responsive genes. The distribution of these threshold concentrations for all responsive genes allows the calculation of a tPOD for the test substance, and the identity of the genes allows statements to be made about the primary mechanism of action of the test substance. The Ecotoxicogenomics Department of Fraunhofer IME, headed by Sebastian Eilebrecht, offers clients the methodology and know-how to capture and interpret tPODs for test substances. The literature available to date and studies conducted at Fraunhofer IME indicate that the tPOD value from fish embryo tests is protective, i.e. it is of a comparable magnitude but tends to be lower than the *No Observed Effect Concentration* (NOEC) derived from chronic fish tests.

As more data pairs from NOECs from chronic fish studies and tPOD values from NAMs become available, the predictive power of this new approach will increase, enabling the tPOD approach to be used for regulatory purposes in the medium term. ■

Link to publication: doi.org/10.1016/j.scitotenv.2024.176026

Performance of a Modified Screening Method for Degradation of Polymers

The fate of polymers has been in the focus of the new restriction (Commission Regulation (EU) 2023/2055) issued by the European Commission on intentionally added microplastics to products used for specific purposes. The restriction is based on the biodegradation of polymers, which is determined according to OECD test guidelines (TG), for example TG 301 (sewage sludge matrix) and TG 307 (soil matrix). It only considers synthetic polymers. Therefore, producers strive to move to natural polymers, which by default are considered to be degradable, and those synthetic polymers which show proof of fast biodegradation. The biodegradation potential of polymers is very often unknown. During product development a fast screening is needed to obtain a projection of the polymer's biodegradation potential to decide if the polymer can be considered for use in the product.

In order to address the degradation of polymers in her PhD project, Julia Peters developed a modified screening method, based on the OECD TG 301 B guideline. The included modifications were the reduction of the test volume, the duration of the experiment was shortened and a different measurement endpoint was chosen, all of which led to a streamlined experimental set-up (Fig. 1).

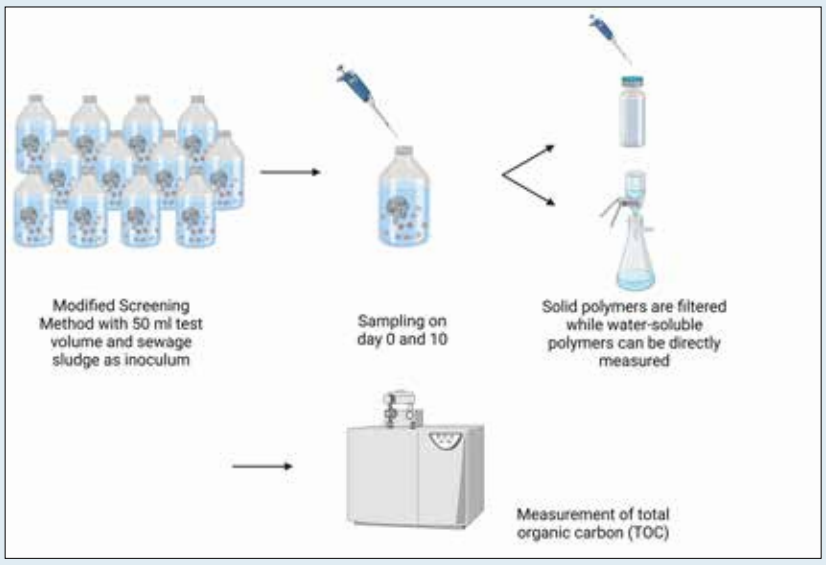

Fig.: Modified Screening Method to determine degradation potential for polymers. *Created by Biorender.com.*

Instead of the continuous measurement of mineralisation, the recovery of the total organic carbon content is determined at the end of incubation. As water-soluble polymers sodium alginate, carboxymethyl cellulose and gum arabica were tested. The modified screening method was also adapted to be able to evaluate the degradation of water-insoluble polymers, which was performed with Indian psyllium husks. This method was evaluated by comparing the measured degradation to the degradation results obtained by applying the OECD TG 301 B and F. It showed comparable results to OECD TG 301 B and F with a significantly reduced effort. Furthermore, the developed method was examined with respect to its limit of detection by determining the lowest applicable polymer concentration and reproducibility between replicates and differently sourced sewage sludges. The results showed that the source of the sewage sludge does not influence

the degradation significantly. In conclusion, the modified screening method saves time and space while allowing a higher sample throughput. Therefore, the method can be used to quickly determine the biodegradation potential of a polymer in order to decide if the polymer is further relevant during product development.
Although market approval for the final product can only be granted by fulfilling the degradation criteria according to the OECD TG 301 guideline or higher tiered studies (degradation in soil or water-sediment systems), the screening delivers reliable estimates of biodegradation behaviour. ■

Crop Protection by RNA Interference – a Horizon Scanning of Approaches to Inform Risk Assessment

Since the discovery of RNA interference (RNAi), for which researchers Andrew Z. Fire and Craig C. Mello received the Nobel Prize in Medicine in 2006, research has been conducted into numerous possible applications of the mechanism. RNAi-based applications aim to inhibit the expression of specific essential genes in target organisms by taking up and processing double-stranded RNA and subsequent silencing of the mRNA of the target gene. In addition to its use in medical therapies, RNAi is of great importance in the development of crop protection methods. In particular, the development of exogenous applications such as RNAi sprays has increased in recent years.

In the USA, the first RNAi spray has already been approved in 2023 and European authorities also expect an increasing number of approval applications for such pesticides in the coming years. Due to the particular mode of action of RNAi applications compared to conventional pesticides, this poses a major challenge for the authorities. As part of a literature project funded by the German Federal Agency for Nature Conservation, colleagues from Fraunhofer IME-sites Schmallenberg and Aachen have compiled and evaluated the current state of knowledge on research into RNAi plant protection methods and studies on the environmental compatibility of these applications. Parts of the project were presented as a poster spotlight by Elke Eilebrecht at SETAC Europe 2024 in Seville. In a workshop in October 2024, current studies on the environmental risk assessment of RNAi pesticides were presented and assessment strategies were discussed with national and international experts in this field and representatives of regulatory authorities.

In addition, the topic of RNAi crop protection is also being addressed in a practical way at Fraunhofer IME. Katharina Knapp, a master's student at the IME in Gießen, is currently working on the identification of a suitable test method for an RNAi spray in *Daphnia magna* and is investigating, among other things, effects in the transcriptome of the organisms.
In cooperation with the German Environment Agency, Hannah Dey's doctoral thesis is being carried out at Fraunhofer IME in Schmallenberg, where practical work is being carried out on the specific challenges of environmental risk assessment of RNAi pesticides. ■

Special SETAC 2024 Meeting: Regulatory Requirements for Scientific Development

At the initiative of the ECHA, a special meeting on the topic of "Regulatory requirements for scientific development" was held at SETAC in Seville in 2024, chaired by Wim de Coen, Blanca Serrano and Christoph Schäfers.

Read on our website which proposals were discussed in order to bring together the views of regulators, industry and academia on further research needs.

Link to website: www.ime.fraunhofer.de//SpecialSessionSETAC2024

"Reviewing the regulatory state of the art on the basis of new scientific findings on (bio-)polymers enables effective environmental protection."

Marie Winter...

...has been part of the Ecotoxicology department at Fraunhofer IME for student research projects since 2021. She deals with regulatory issues related to different substance groups that are relevant to agricultural management. Since 2022, she has been involved in the Fraunhofer PREPARE project SeedPlus, where a sustainable polymer-based seed coating is about to be developed. As part of her doctorate, she is developing an ecotoxicological assessment strategy for (bio-)polymers in the aquatic and terrestrial environments.

Marie Winter studied Ecosystem Management at the Georg-August-University Göttingen and completed her Master's degree in "Soil, Water, Contaminated Sites" at the University of Osnabrück in 2022. The focus was on soil protection, with a particular emphasis on the prevention of contamination and the subsequent care of affected areas. In her master's thesis at Fraunhofer IME, she investigated the sensitivity of various soil microbiological test systems in comparison to a method required in the authorisation process for plant protection products. Since 2022, she has been working on her doctorate on the development of an ecotoxicological evaluation strategy for (bio-)polymers in the agroecosystem.

To enhance crop growing conditions and a secure yield, commercial agricultural practice resorts to seed coatings or mulch foils. In these products, (bio-)polymeric substances are often included as their physico-chemical properties can have beneficial effects on the microclimate improving seed emergence. Even though the (bio-)polymers are directly exposed to the environment, they are not holistically regulated in Europe. As a result, the actual environmental impact of the different polymer groups remains unknown. As part of the doctoral thesis, a systematic evaluation of potential ecotoxicological effects of these non-regulated substances is therefore to be examined. Standardised short- and long-term exposure tests are used to investigate the impact on various aquatic and terrestrial organisms and ecosystem services at different trophic levels.

Therefore, natural (modified) polymers are considered, which are often evaluated as harmless due to their origin and are therefore categorized as relevant for the future. First results, which were presented at this years SETAC in Seville, indicate concentration-dependent ecotoxicological behaviour of the natural (modified) polymers. External phyisical factors could be responsible for the adverse effects on specific aquatic test organisms and soil functions. The ecological relevance of these findings and the derived assessment strategy is finally verified under realistic conditions by means of field exposure studies in small lysimeters.

IMPRINT

Published by
Fraunhofer Institute for Molecular Biology and Applied Ecology IME

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, Dorothea Weist
Public Relations

Translation
Dr. Terry Clark

Production
Schäfers Druck GmbH, Schmallenberg
100% Recyclingpaper

Read more about our activities at SETAC 2024 Sevilla on our website:

www.ime.fraunhofer.de/SETAC2024