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FRAUNHOFER INSTITUTE FOR MOLECULAR BIOLOGY
AND APPLIED ECOLOGY IME

WELCOME

Research dedicates itself to explore the unknown – and there is plenty of it in regulatory substance evaluation. Whether you look at chemical substances of unknown or variable composition (UVCB substances), unknown products formed during food processing or unknown effects on possibly unknown influence factors on fish populations – we show how our detecting and systematic approaches contribute to scientific understanding and evaluation. Furthermore, we introduce Verena Kosfeld as one of our doctoral candidates, who in her work merges the two research fields namely bioaccumulation and monitoring.

Yours sincerely

Prof. Dr. Christoph Schäfers

Photo: Monitoring with the help of eDNA |
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In this issue you can read:

MONITORING FISH POPULATIONS WITH THE HELP OF eDNA

Novel genomic tools address problems of traditional biomonitoring

The use of environmental DNA (eDNA) for monitoring fish populations, allows the non-invasive species determination and measurement of their DNA abundance. Traditionally, the response of biotic communities to human-induced stressors have been assessed using taxonomic approaches, however those approaches are limited by scalability, restricted temporal and spatial resolution, invasiveness, and the high level of expertise required to identify the species.

Novel genomic tools can avoid many of the above-mentioned problems and could complement traditional bioassessment and biomonitoring strategies. Suspended particulate matter (SPM) is the third important structural and functional element in aquatic ecosystems next to the water phase and the sediment. SPM is a transport vehicle and source ▶

of particle-bound substances or biological material. The use of SPM for monitoring fish communities has therefore a high value for assessing the effect of contaminants in aquatic communities (surface waters). The low temporal resolution of sedimentary eDNA may be appropriate for monitoring programs that can use information about current-or-past occupancy, such as retrospective genetic monitoring.

"In this study, we focused on the novel use of eDNA extracted from SPM from different riverine points in Germany", says Dr. Cecilia Díaz, manager of the laboratory for bio-analytics of the Department Ecotoxicology. The samples were obtained from the archive of the German Specimen Bank for monitoring fish communities.

Environmental DNA was extracted using different extraction techniques and subsequently sequenced using high throughput technology (next generation sequencing; NGS). The results showed that it is possible to obtain high quality DNA for metabarcoding analysis. In total, 28 species in the analyzed samples were identified showing clear differences between the sampling points.

For the first time, the applicability of NGS for eDNA analysis extracted from suspended particulate matter is confirmed.

"However, it has been observed that the chosen sampling volume, sampling size and extraction method have an impact on the recovered diversity found", says Dr. Díaz. Therefore, rigorous methodological investigation are needed for defining a suitable sampling and extraction strategy for monitoring. ■

ICON PROJECT *HEALTH KITCHEN* – HEALTH EFFECTS OF NUTRIENTS IN FOOD

Novel approach to evaluate the function and fate of food ingredients in cooperation with *Monash University*

The internal funding program 'ICON – International Cooperation and Networking' of Fraunhofer-Gesellschaft supports bilateral cooperation projects with international centers of excellence.

Processed food | photo: PantherMedia / jahina

While the direct relationship between food ingredients and health is well known, further research is needed to investigate which conversion products - with which kind of health impact – are formed during digestion and food processing. "As a consequence, in addition to consumer insecurity, there are the extremely high costs for the industry, which have to develop cost-intensive avoidance strategies without precise knowledge of their origins", says Professor Mark Bücking, Head of the Department Environmental and Food Analysis. Due to the complementary expertise of Fraunhofer IME and Monash University in Melbourne, a longer-term cooperation will be established within the framework of an ICON project.

The aim of this first joint project will be an exemplary assessment and elucidation of the fate of nutrients during food processing and digestion, which will allow the partners to use these procedures for industrial customers and public authorities as a tool for their food safety and quality issues.

"Our competence, to apply radioactively labeled carbon nutrients (14C-studies) for the chemical analysis of structure and metabolism during food processing and digestion as well as our ability to identify process metabolites during food preparation through the research approach of the radio kitchen is well complemented by the pharmacological competences of the Monash University", explains Professor Bücking.

Moreover, Monash University has great expertise in the field of chemical synthesis (synthesis of previously unknown metabolites) and in nutrition research, whereby studies at molecular level, as well as animal and human studies are implemented. In the areas relevant for this project, the current worldwide ranking of the university in the field of chemistry is 35, medicine 29 and pharmacy 2.

The three-year project started on August 1st, 2018, with an overall budget of € 1.2 million. ■

CUSTOMIZED TESTING OF UVCB FOR REACH

Testing is faced with great challenges due to the wide range of substance properties

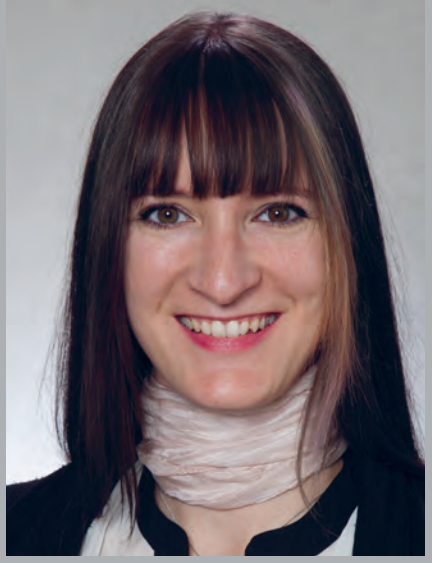
In the EU regulation for chemicals known as REACH, not only are single substances tested but also mixtures of chemicals, which are marketed as such. This is a great challenge for testing, especially in the field of so-called UVCB-Substances.

The spectrum of UVCB-substances ranges from mixtures of three single substances different in composition up to natural material (e.g. tree resin) and reaction mixtures with thousands of unknown components. Due to the great range of possible substance properties, a general test strategy for UVCB is not available. In most cases, it is from an analytical viewpoint not economically feasible to identify all substances with a share of up to 0,1%, as requested by authorities in a first step. Still, a way must be found to characterize and quantify mixtures sufficiently, even in complex matrices like for example soil.

"In addition, when testing the ecotoxicological properties of UVCB substances, water-soluble substances are often mixed with poorly soluble substances", says Dr. Dieter Hennecke, head of the Deparment Ecological Chemistry. "Thus, aqueous solutions do not in any way represent the composition of the mixture." Even the European Chemicals Agency (ECHA) has at present no coherent strategy as to which techniques offer feasible processes to produce the test medium. In aquatic tests, critical differences might occur sufficient to affect the assessment of the chemicals. More than that, many guidelines covering the environmental performance of chemicals are not at all intended for chemical mixtures and therefore have methodological loopholes. "At the end of the day, every testing of UVCB is an individual case, which needs a close coordination with the client for an agreed approach", says Dr. Hennecke. "Besides the necessary technical equipment and expertise with regard to chemical analysis, an in depth knowledge and practical experience with the guideline underlying the test is necessary."

Fraunhofer IME meets these requirements through its experience in developing guidelines, its interdisciplinary approach and its technical equipment, the latter being state of the art. ■

Tree resin as an example of UVCB | photo: PantherMedia / izanbar



"Cutting edge research projects offer me the possibility to take an active part in modern environmental protection – this makes the work on my doctoral thesis especially exciting.

Verena Kosfeld is a doctoral candidate at Fraunhofer IME since 2017. Previously, she studied molecular cell biology at the Philipps-University Marburg and did research at the Max Planck Institute for Terrestrial Microbiology on the suitability of fluorescence microscopy-techniques to monitor the gene expression of E. coli bacteria during biofilm formation. She gained her Master's degree in molecular and cellular biology in 2016. Before, she completed an apprenticeship (Biological-Technical Assistant, BTA) and subsequently studied molecular biology in Bielefeld. For her Bachelor thesis, she tested a proteomic-based approach to enhance the evaluation of bioaccumulation studies.

Verena Kosfeld...

… has been working at Fraunhofer IME since 2011 in the context of student research projects, and, since 2017, supports a project to investigate bioaccumulation of chemicals in an aquatic food web. For her, testing novel research approaches in chemical assessment is of special interest.

In terms of the assessment of chemicals, the bioaccumulation potential is a critical characteristic. Different experimentally derived metrics are based on experimental data, namely bioconcentration, bioaccumulation, and biomagnification factors (BCF, BAF, BMF). A still relatively new approach are so called trophic magnification factors (TMF), which integrate accumulation processes along a food web. Up to now, data is scarce and only available for a restricted number of chemicals. "Furthermore, there is a lack of sufficient practical experiences to standardize the method for TMF derivation to be suitable for chemical assessment", says Dr. Heinz Rüdel, head of the Department Environmental Specimen Bank & Elemental Analysis. "TMF data could potentially be used in the assessment of industrial chemicals under the REACH regulation, or in the context of biota monitoring according to the Water Framework Directive".

THE TMF APPROACH HAS BEEN REALIZED FOR THE FIRST TIME IN A GERMAN WATER BODY, NAMELY IN LAKE TEMPLIN NEAR POTSDAM.

Utilizing benchmark chemicals, such as PCBs, PBDEs, or mercury it will be investigated whether accumulation processes can be observed. In the following, the results obtained are compared to already published results from other countries. Thereafter, TMF will be derived for a number of substances for which further data on food web enrichment are required in the context of chemicals' regulation. All samples are prepared and stored using liquid nitrogen-cooling and standardized methods also applied for the sample preparation of the German Environmental Specimen Bank. The aquatic biota samples are now readily available for upcoming questions – a food web on ice. This project is funded by the German Environmental Agency.

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

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Fraunhofer Institute for Molecular Biology and Applied Ecology IME

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, Design and Layout
Dr. Kristina Bette-Gaußmann
Public Relations

Translation
Dr. Kristina Bette-Gaußmann
Dr. Terry Clark

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