

NEWS | NOV23

Fraunhofer Institute for Molecular Biology and Applied Ecology IME

Welcome

The changed perception of risks requires new assessment procedures and test methods, such as the inclusion of behavioural changes in substance assessment, the determination of molecular stress responses in environmental samples and the use of groundwater pollution models to determine the new mobility criterion. The portrait is dedicated to our long-standing method developer Uwe Boshof.

Yours sincerely



Prof. Dr. Christoph Schäfers

When water fleas perceive fish: Fish kairomones initiate a protective reaction in the water flea *Daphnia magna*

► In the course of evolution, species have developed different strategies to protect them from predators. An example is the well-known model organism *Daphnia magna* (water flea), which lives in the open water of lentic water bodies filter feeding on algae. To avoid being eaten by fish, the daphnids have developed a special behavior: daily vertical migration. During the day they stay in the lower part of the water to avoid being seen by predators, while at night they move to the upper layers of the water to filter algae. However, this behavior is only observed in waters where fish live.

Kirsten Germing, Dr. Lena Kosak and Dr. Elke Eilebrecht from the Department of Ecotoxicology were able to confirm this observation in the laboratory using a new tracking video system. Daphnids in normal culture medium were randomly distributed in the water column when exposed to light. However, daphnids which were transferred to a medium obtained from a zebrafish culture were observed almost entirely in the lower part of the water column. There are indications in the literature that fish messenger substances, so-called kairomones, which trigger a defence reaction, are responsible for the behavioral

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change of the daphnids. These kairomones are probably bile acids of the fish. This raises some exciting questions for us.

In cooperation with Prof. Dr. Bodo Philipp, Head of the Department of Environmental Microbiology, we want to investigate to which of the various fish bile acids the daphnia react and, by means of analytical chemistry (Department of Trace Analysis and Environmental Monitoring), prove in which concentration range of the kairomones the effects occur. In addition, we want to know whether bile acids of other animals as well as of humans induce such a reaction, since these are released into the environment e. g. by sewage treatment plants or agriculture. We are also curious to find out how long the daphnids show the reaction and whether they react similarly to degradation products of the bile acids.

In the context of an ongoing research project (master's thesis of our employee Vanessa Saalman) we could already demonstrate that the response of daphnids can be influenced by previous exposure to insecticides. Daphnids pre-exposed to imidacloprid or fipronil were found to be distributed in the water column after kairomone exposure. This is similar to the behavior in kairomone-free medium, compared to control animals. Therefore, we would like to investigate the molecular mechanisms of the inhibition of the defence reaction and whether a blocking of specific receptors is responsible for the perception of kairomones or whether processes required for the defence reaction response are disturbed by the various substances. ■

WildOMICs: Unravelling the biological potential of the German Environmental Specimen Bank

▶ Almost 40 years ago, the German Environmental Specimen Bank (ESB) was conceived as a long-term archive for describing and monitoring chemical pollution of the German environment. Today, more than 400,000 samples from various ecosystems in Germany are stored over liquid nitrogen. Storage at ultra-low temperatures preserves not only the chemical composition of the samples, but also environmental DNA (eDNA) and RNA (eRNA).

Recent advances in genomic analysis technologies - so-called OMICs methods - have proven to be powerful tools to decipher the differential biological information hidden in environmental samples. A group of experts from the Universities of Trier, Duisburg-Essen, Frankfurt, the Senckenberg institute and Fraunhofer IME, has come together to exploit the potential of the stored material with the aim of integrating novel molecular techniques into environmental monitoring. The first approach is being made in the TrendDNA project, studying eDNA for biodiversity assessment. Aiming at expanding the application of OMICs techniques in environmental monitoring the same working group launched the "WildOMICs" initiative.

"The integration of eRNA analysis opens up a range of new possibilities to explore ecosystem responses to dynamic environmental conditions (e.g. climate change, chemicals released into the environment, etc.)", says Dr. Sebastian Eilebrecht from IME who is member of the initiative.

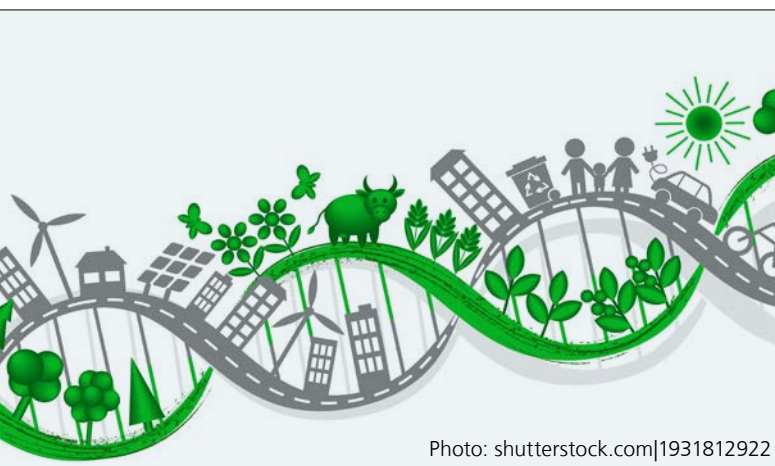


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Transcriptomic and proteomic analyses of ESB samples allow insights into changes in gene expression, impairment of biochemical pathways or metabolic functions in response to anthropogenic and other environmental stressors. These insights are expected to generate added value for monitoring substance-derived stress in the German environment. The future use of meta-transcriptomics represents the culmination of this ongoing revolution. This technique promises a deep understanding of the active functional genes within a community and enables the observation of dynamic responses to environmental changes.

WildOMICS aims to initiate an ongoing and dynamic discussion to provide the basis for the integration of these techniques and to further develop research by applying for public funding projects. The medium and long-term goal is not only to deepen

our understanding of the natural environment, but also to create a foundation for effective conservation strategies that ensure the resilience of our environment. ■

Leachability as a Measure for Substance Mobility in the classification as PMT/vPvM

▶ A new hazard classification concerning PMT/vPvM (persistent, mobile, toxic/very persistent, very mobile) will be introduced in the CLP and REACH regulations. This classification intends to encompass substances that pose a risk of reaching and accumulating in drinking water sources.

While persistence (P) and toxicity (T) are already covered in guidance documents, mobility (M) of chemicals is not conclusively defined. In the CLP regulation the classification criterion for mobility is primarily the sorption constant $\log K_{oc}$ which defines the adsorption of a substance to the organic carbon content of a soil or sediment. However, assessment of mobility solely based on the sorption constant is considered inaccurate, since (among other reasons) transport through soil and sediment also depends strongly on degradation in the transport matrix.

Dimitrios Skodras, Dr. Judith Klein and Dr. Michael Klein - Department Modelling and Bioinformatics at Fraunhofer IME - propose an approach using the EU FOCUS framework which considers degradation, climate, soil and crop effects. Specifically, FOCUS-PELMO is used to provide a measure of mobility in terms of leachability. We define leachability as the percentage of substance leached at a soil depth of 1 m related to the exposure at the soil surface. We varied the DegT50 between 1 day and 1 year while K_{oc} values between 0 and 10,000 mL/g

were used. This yields a two-dimensional grid of leaching predictions. Proposed thresholds for mobility are 1 % and 10 % leaching. Using this approach, a substance of which more than 1 % can be transported through a 1 m soil or sediment layer is considered mobile. In the case where more than 10 % is transported then it is considered to be very mobile. The proposed thresholds of 1 % and 10 % widely agree with the exposure thresholds of 0.1 $\mu\text{g/L}$ and 10 $\mu\text{g/L}$ in drinking water for plant protection products. Note that the concentration depends on the loading whereas the leachability does not.

It is recommended that the leachability concept becomes part of a Weight-of-Evidence approach in cases where the initial screening based on $\log K_{oc}$ indicates a risk of mobility.

The result of the simulations is implemented in a user-friendly tool (Leaching Calculator) that is publicly accessible: [\[Leaching Calculator\]](#). ■

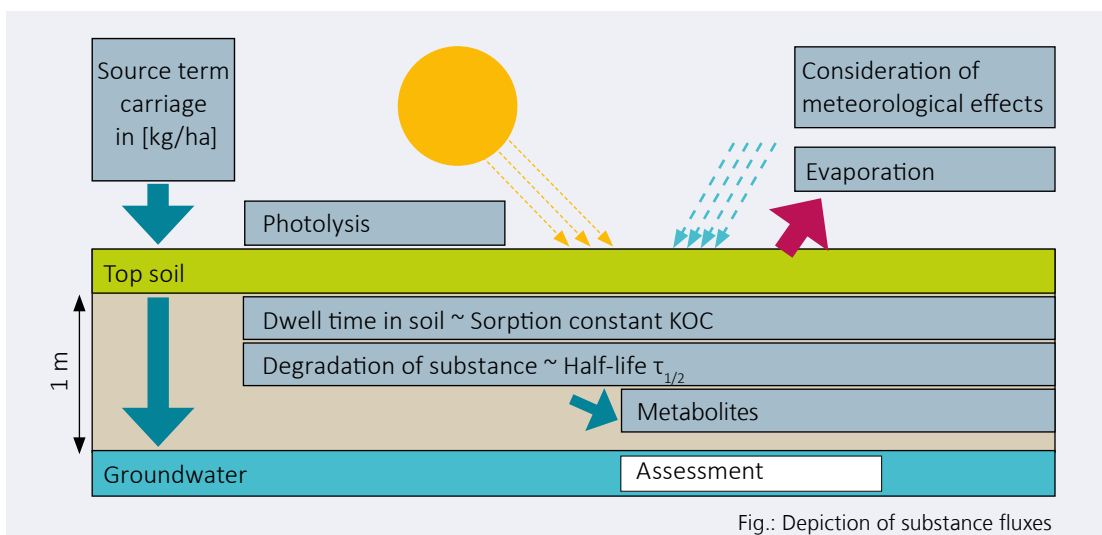


Fig.: Depiction of substance fluxes



Uwe Boshof: Serving ecotoxicological research for over 40 years

Core practical steps such as taking blood samples from small laboratory fish were developed by Mr Boshof more or less in passing. The technology and accuracy play a decisive role here and ensure that the data quality is correct. Furthermore, the test systems developed at the Fraunhofer IME, such as the fish test in the static system, are unique worldwide. Currently, a new multigeneration fish test guideline is being validated for the OECD and coordinated by IME. This also requires training of the international partner laboratories, for which Mr Boshof is prepared to travel away for several days.

► In April 1982, Uwe Boshof started his varied work as a medical-technical assistant at the Fraunhofer Institute for Toxicology and Aerosol Research ITA in Schmallenberg. In his early years the then topics concerning air quality shaped his range of tasks. This primarily included inhalation tests with mammals concerning the carcinogenicity of heavy metals. In elaborate, and sometimes highly unusual field studies, he got to the bottom of the exposure of the urban population to asbestos and wood preservatives. In order to investigate the effects of acid rain on forests, physical effort was also required to take samples.

Uwe Boshof's activities changed with the establishment of ecotoxicological topics at the Schmallenberg site. He was significantly involved in the establishment and further development of various test systems. In addition to invertebrates, the focus here was particularly on fish. His commitment and experience contributed to the practical implementation of successful test protocols such as the life cycle test with zebrafish and consequently these studies are associated with the Fraunhofer Institute in Schmallenberg in that research field today.

In addition to the fish studies, Mr Boshof was responsible for the technical implementation of microcosm studies, which measure effects on complete biotic communities in artificial pond systems. To investigate algae, plants and small invertebrate aquatic inhabitants, enormous technical effort is required to bring sediment and pond water to the facility. This includes the control of environmental conditions such as temperature, air and application of the test substance, take representative samples and determine the sampled species. Here, too, our employee has done pioneering work.

Uwe Boshof's commitment goes beyond conducting the studies: in addition to core tasks in occupational safety and radiation protection, he shows great dedication and takes great pleasure in training new colleagues. He is valued throughout the Institute as a contact point and problem solver, always true to the motto:

"There's no such thing as can't be done!"

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