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FRAUNHOFER INSTITUTE FOR MOLECULAR BIOLOGY AND APPLIED ECOLOGY IME

WELCOME

Contamination of food by chemical substances results from transfer processes. We explore such processes by carrying out a large variety of studies. To elucidate the transfer of fluorinated alkyl substances via feeding material into animal products we develop methods aiming at their total detection. For the prediction of metabolites originating from pesticides and reaching farmed fish via feedstuff we have developed a method to replace animals. In addition, we describe how we improve the determination of the degrading behaviour of chemicals in soil by investigating the reliability of recording experimental conditions.We also introduce Dr. Kerstin Derz, Deputy Head of the Department Ecological Chemistry.

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Prof. Dr. Christoph Schäfers

Photo: MEV-Verlag

In this issue you can read:

FROM FEED TO EGG

Better estimation of overall burdens of humans, animals and the environmment by perfluoroalkyl- and polyfluoroalkyl substances (PFAS)

Due to their unique properties perfluoroalkyl- and polyfluoroalkyl substances are widely utilised in specialty chemistry as well as in consumer products of any kind. The extreme stability of PFAS leads to their accumulation in the environment and organisms.

Thanks to their water, oil and dirt-repellent effect and extreme chemical stability PFAS are not only applied for example in galvanic and polymer industries and in fire-fighting foams, but also in outdoor-clothes, carpets and food packaging materials. Due to their extreme stability, their bioaccumulation potential and often toxic properties they pose a potential risk for humans and the environment. ▶

In a feeding study conducted by the German Federal Institute for Risk Assessment (BfR) with laying hens it was observed that the hens excreted more of perfluoroheptane sulfonic acid and the banned perfluorooctane sulfonic acid solely through the egg yolk than they had ingested through the PFAS-contaminated feed. It is assumed that the high excretion rates result from the degradation of precursor substances present in the feed material.

In their master theses conducted in the Environmental and Food Analysis Department Maria Eichhorn and Bernd Göckener in cooperation with the BfR identified and quantified PFAA (perfluoroalkyl acid)-precursors in poultry feed and in hen's eggs. They also adapted a total oxidizable precursor (TOP) method, as proposed by Houtz and Sedlak to determine PFAA precursors in soil, to determine PFAA precursors in both poultry feed and hen's eggs.

The influence of precursors on the overall burden by PFAS

Oxidation of the precursors caused an increase in concentrations of all perfluorinated carboxylic acids (PFCAs) with chain lenghts of C4 to C9. This indicated a wide spectrum of precursors. Upon oxidation of the perfluorooctanoic acid precursors in poultry feed and egg yolk the concentration rose by a factor of approximately 8 and 6.5, respectively. The spectrum of precursors in poultry feed material and egg yolk differed considerably.

Mark Bücking (Head of Department): "The results of this study depict the potential influence of PFAA precursors on the overall PFAS-burden of humans, animals and the environment. They also underline that routinely conducted determinations of PFAS may only provide a partial insight into overall burdens." ■

IDENTIFICATION OF METABOLITES IN THE TEST TUBE

The use of primary fish hepatocytes as a screening tool

In the application process for the authorization of plant protection products (PPP) in the EU, information about the composition of PPP residues in farmed fish which have ingested contaminated feed may be required. An in vitro – in vivo comparative study provides insight into the principle suitability of assays with primary fish hepatocytes (liver cells) for a fast and cost-effective identification of metabolite patterns in fish.

Fish farm.
Photo: iStock/Milen Dobrev

In her doctoral thesis, Ina Bischof from the department of Bioaccumulation and Animal Metabolism examined whether primary fish hepatocytes are suitable as an in vitro tool for identifying PPP metabolites in fish. For this purpose, Bischof carried out in vitro metabolism tests with primary hepatocytes from trout and carp and compared the metabolite patterns she found with in vivo studies conducted in parallel. The test substance was the pesticide Methoxychlor (MXC) radiolabeled with ^{14}C.

In vitro assays with primary fish hepatocytes are suitable as a screening tool for assessing the profile of PPP residues in fish.

The in vitro – in vivo comparison demonstrated that the metabolite patterns found in the assay with the primary fish hepatocytes are comparable to the ones detected in the liver under in vivo conditions. In comparative studies with carp and trout hepatocytes species-specific differences were evident. Bischof: "Our tests, using the example of MXC, show that primary fish hepatocytes in

principal are suitable for the detection of metabolite patterns. They can render valid information about the metabolites occurring in the fish liver." In vitro data therefore is suitable to identify metabolite pathways as part of the authorization procedure. The potential of primary hepatocytes lies, above all, in their use as a screening tool.

Professor Schlechtriem, supervisor of the thesis at Fraunhofer IME: "The in vitro test can deliver valuable information about the metabolism in fish, even in the early development stages of a plant production product. Furthermore, the in vitro test can help shine a light on the emergence of toxic metabolites." ■

SOILTESTS UNDER ANOXIC CONDITIONS - CHALLENGES FOR OECD-GUIDELINE 307

Tests with six different reference soils delivered information about the comparability of test results.

OECD-guideline 307 describes a method for elucidating the degradation of chemicals in soils under aerobic and anaerobic (oxygen-free) conditions. While requirements on test system, soil material and test substances are presented in detail, there is a lack of criteria regarding the measurement of the redox potential under anaerobic incubation conditions. Thus, the comparability of test findings is compromised.

In his master thesis in the Ecological Chemistry department, Thomas Hentzel determined the parameters pH, redox potential, oxygen saturation and temperature in oxygen-free incubated soil-water samples for six different reference soils to get insight into spatial and temporal dependences of the measurements as well as to capture further influencing factors. The soils were preincubated in ambient air for 14 days and then covered with demineralized water and incubated in an oxygen-free system. "The measurement of the redox potential is especially influenced by a great number of both sample internal and external factors, which are interrelated", says Hentzel. "This is why it is difficult to assign a single redox-value".

A consistent capture of the biodegradation of chemicals in soils requires an exhaustive description of the methods to determine the measurement parameters.

Applying standardized measurement methods e.g. definition of the number of redox electrodes, checking the functionality of the electrodes,

Soil incubator.
Photo: Fraunhofer IME

definition of measurement times and positioning of the electrodes, can improve the quality of the redox-measurement and enhances the comparability of measured values. Consequently, the use of at least two redox electrodes in redox testing solutions is recommended. The positioning of the electrodes in the aqueous phase of the sample should be specified precisely. However, even if applying these criteria, it is often not possible to determine whether a soil sample is anaerobic. This is why the oxygen concentration should be measured additionally. Depending on the soil type, the experiments further show a change in the redox potential during the anoxic incubation, even when no oxygen was traceable in any of the samples. An appropriate modification of the guideline should therefore be discussed. ■

Expanding the question "what happens?" to "why?" helps to better estimate potential risks by pollutants.

After completing her training as a biological technical assistant Kerstin Derz studied biology at the Institute for Ecology, Ecotoxicology and Ecological Chemistry at RWTH Aachen University. For her diploma thesis she was awarded the "Friedrich-Wilhelm-Preis". With her doctoral thesis on "Degradation of ^{14}C-marked pyrene and benzo[a]pyrene by a *Mycobacterium*-species described for the first time" the bioavailability of environmental chemicals came into her focus of interest. Today she develops methods for elucidating metabolism pathways and transfer processes of chemicals for regulatory bodies and industrial clients. She also applies these methods in the framework of studies required for the registration of pesticides, pharmaceuticals and chemicals.

Dr. Kerstin Derz…

… has been Deputy Head of the Department Ecological Chemistry since March 2011. Elucidating metabolism and transfer pathways of environmental chemicals and thus contributing to the protection of the environment and consumers is a real concern to her.

With regard to the environmental fate of pollutants, bioavailability becomes an increasingly greater focus of attention. Especially the bioavailability of a substance for soil-living micro-organisms is an important criterium for a chemical to be degraded or to accumulate, and thus will be potentially harmful for the environment. The bioavailability of a substance in soil can also be of importance for humans, for example when decisions have to be made on whether a contaminated site can be released for certain uses. In the context of various research projects Kerstin Derz has developed methodologies regarding both aspects of bioavailability, which finally had an influence on legislation on the assessment of soils and contaminated sites. Industrial clients also show increasing interest in taking bioavailability into account, for example through the award of research contracts relating to pesticide registration.

TRANSFERRING KNOWLEDGE THROUGH MEMBERSHIPS IN SCIENTIFIC COMMITTEES…

Her interest in getting a deeper insight into questions of bioavailability motivated Kerstin Derz to take over the position of a convenor in the ISO working group ISO/TC 190/SC 7/WG 4 "Human Exposure" which she will be leading as of 2019 for an initial period of two years. Further to developing ISO-standards the working group focuses on methods to determine bioavailability as well as on risk assessment methodologies.

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

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