Abstracts overview - Posters

Research Theme: Environmental and Human Health

1) Optimizing cross-experiment reproducibility in zebrafish-microbiome studies using selectively enriched zebrafish associated microbiota

Siraz Kader, Chloe Wray, Tim Jonat, Nicole Schweiger, Renee Owen, Greta Kleinert, Martin Zimmermann, Tamara Tal

Ecotoxicology

Zebrafish have emerged as a key model for studying microbiome-host interactions. This is because modification of colonization status is relatively straightforward and microbiome function on host neurodevelopment can be easily probed using automated behavior testing. While axenic (microbe-free) zebrafish protocols exist, microbiome research is hindered by inconsistent colonization due to inter-lab variability in rearing and husbandry strategies. To generate a reproducible inoculant, selectively enriched zebrafish microbiota (SEZAM) from the UFZ zebrafish facility were cryopreserved, reactivated, and used to conventionalize axenic zebrafish. This resulted in 60 SEZAM cultures isolated from four media types including blood broth (BB), lysogeny broth (LB), brain-heart-infusion broth (BHIB) and sabouraud dextrose broth (SBD).
Previous work showed that axenic zebrafish exhibit increased locomotor activity relative to conventionally colonized or conventionalized controls. We hypothesized that association with SEZAM would block axenic hyperactivity in 5-day post fertilization (dpf) zebrafish. Association of axenic zebrafish with two microbiome mixtures, selected in lysogeny broth and brain-heart-infusion broth, restored control-like behavior. Additionally, short-chain fatty acids, predominantly acetate were identified in ex-vivo SEZAMs, indicating a potential mechanism by which SEZAMs might influence host behavior.
SEZAM provides a reproducible platform for studying microbiome dysbiosis induced by disease or chemicals, bridging human, animal and environmental health to advance research in ecosystem health. More broadly, and aligned with the One Health concept, this standardized approach enhances reproducible, cross-disciplinary research on microbiome-related health, fostering insights into disease mechanisms, environmental influences, and potential therapeutic strategies.

2) Leaching of Plastic Additives from Custom-Made Polymers into Artificial Gut Fluids

Karla Schmitz, Mara Römerscheid, Alexander Böhme, Zheng Chen, Nico Jehmlich, Ulrike E. Rolle-Kampczyk, Annika Jahnke

Exposure Science

Microplastics are pervasive in the environment, including their presence in the food web. Humans can ingest microplastics through beverages and food, and are exposed not only to the microplastic particles themselves, but also to the plastic additives. These additives can leach into human intestinal fluids during passage of the gastrointestinal tract, where they may be transformed and bioaccumulate, thus posing a risk to human health.
As the composition and concentration of additives in plastic products are generally not disclosed, this study used custom-made polymers with known and realistic additive mixtures. The additives were

chosen to cover different categories such as UV stabilizers, plasticizers, and flame retardants. We used UV-weathered plastic as UV-light may cause surface alteration, degradation and structural modification of the plastic.
The study investigated material-specific leaching across different polymers and additives, comparing weathered and unweathered plastics and varying incubation times. By utilizing plastics with known additive concentrations, the extent of leaching was determined, providing critical insights into plastics with unknown compositions.
To simulate human digestion, microplastics were exposed to artificial gut fluids representing saliva, gastric juice, duodenal juice, and bile, with conditions reflecting physiological temperature, pH, and residence time in each digestive phase. To provide a more holistic understanding, a simplified human intestinal microbiota (SIHUMIx) was applied followed by metabolome and proteome analysis. Finally, the additives were extracted using hexane, and their concentration in the solution was measured.
These findings aim to improve risk assessments and inform regulatory agencies regarding plastic additives, particularly with respect to human gastrointestinal exposure.

3) Ensemble-Based Spatial Risk Assessment of Major Wheat Pathogens in Iran Using Biomod2: A Decision Support Framework for Sustainable Production"

Shirin Mahmoodi, Mostafa Aghaee Sarbarzeh

Community Ecology

Wheat pathogen dynamics significantly impact food security, yet spatial risk assessments for key diseases (yellow rust, black rust, brown rust, powdery mildew, Septoria tritici blotch) remain limited. This study employs the biomd2 package to model epidemic-prone regions and identify environmental drivers of pathogen distribution in Iran. We integrated 55 climatic parameters (monthly temperature extremes, precipitation totals, bioclimatic variables) with disease occurrence data through biomd2's ensemble modeling framework. Model performance was validated using AUC-ROC (95% ± 2.1 SD) and TSS (0.89 ± 0.03), demonstrating high predictive accuracy for multi-pathogen risk mapping.
Biomod2's variable importance analysis revealed maximum temperature (BIO5: 34.7% contribution) and precipitation during wettest months (BIO13: 28.1%) as primary determinants of pathogen spread. The ensemble approach optimized through biomd2's calibration tools identified high-risk zones where ≥3 pathogens show 87% spatial overlap probability.
Our multi-threat risk stratification enables:
Early-warning prioritization for regions with concurrent pathogen suitability.
Climate-resilient cultivar deployment based on pathogen assemblages.
Targeted surveillance in transitional ecotones showing rapid suitability shifts.
This mechanistic modeling approach advances sustainable wheat management by bridging spatial epidemiology with agronomic decision-making. The biomd2 implementation provides adaptable framework for dynamic disease risk forecasting under climate change scenarios.

4) A Self-Organizing Map of the Zebrafish Embryo Transcriptome for Toxicogenomic Assessment

Paul Michaelis

Ecotoxicology

An ever-growing market of chemicals for industrial, agricultural or consumer purposes is at odds with chemical risk assessment in which the testing of chemicals is so time- and cost intensive that risks for human and environmental health are often not adequately studied or understood. New approaches are needed in toxicology to cope with these novel compounds and accelerate chemical risk assessment. One possibility is to make use of the large amount of data generated by the scientific community in the recent years and integrate them into computational models to aid in the classification and assessment of novel compounds via mechanistic understanding of their potential modes of action (MoA) or similarity of effects with those of well-known chemicals.

A self-organising map (SOM) of the zebrafish embryo (ZFE) transcriptome trained with toxicogenomic data obtained with ZFE is a tool to visualise and compare effects of different chemicals (Schüttler et al. 2019, doi:10.1093/gigascience/giz057). Visualising novel data on this map, we have been able to show that clusters of genes perturbed by chemical exposure are associated with general chemical stress but also specific MoAs.

Now, we extend this assessment tool by adding a second map, based on developmental ZFE transcriptomic data. While the existing map is focused on an arrangement of genes of direct toxicological interest, the new SOM will contain clusters of genes related to developmental processes and physiological structures. This map will aid in understanding temporal aspects of chemical perturbation in the developing ZFE as well as link transcriptomic changes to phenotypic effects. Preliminary results show a clustering of relevant functions in specific regions of the map, however its use in the assessment of toxicogenomic data remains to be validated.

5) Multi-omics Pathway Enrichment for Dose-Response Modeling in Systems Toxicology

Sebastian Canzler, Julienne Lehmann, Jörg Hackermüller

Computational Biology & Chemistry

Integrating multi-omics data is pivotal for comprehending cellular or organismal responses to chemical exposures on the molecular level.
The application of multi-omics data in toxicology has become increasingly relevant, as echoed by researchers and regulators alike, aiming to reduce live animal testing, increase the substances tested, enhance reproducibility, and significantly decrease costs.

Existing pathway enrichment methods face challenges that limit their efficacy in computational toxicology.
These include inadequate enrichment techniques, lack of support for time- and concentration-resolved data, and limited pathway source availability.
Moreover, many methods do not consider the interconnectedness of different biomolecule types, often integrating data sequentially rather than simultaneously.

We introduce SiMToPa, a novel approach towards a consistent and simultaneous multi-omics-based pathway enrichment that overcomes these obstacles while incorporating pathway topology explicitly.
This methodology utilizes node importance, which iis assessed via various graph metrics, and considers the influence of each node based on the effect of its outgoing edges.
We combine the pathway representation with multi-omics measurements to calculate a topology-based pathway fold change, enhancing the consistency of observed molecular responses.

Using time- and concentration-resolved multi-omics datasets, we optimized the representation of pathway topologies and the integration of different omics layers while also assessing the impact of individual and combined layers on the enrichment analyses.

SiMToPa is a robust multi-omics data integration method suitable for dose-response modeling at the pathway level.
This facilitates the detection of molecular responses to toxicants and deepens our understanding of mechanisms of action, thereby enhancing the predictive capabilities of toxicological assessments.

6) **CoModHaz: Co-Creation process for the modernization of chemical hazard indicators**

Eunhye Bae, Stefan Saretz, Vanessa Srebny, Sarah Stevens, Luise Henneberger, Beate Escher

Cell Toxicology

Chemical hazard assessment is a key element for the safe management of chemicals. In a recent concept paper, a group of Helmholtz researchers proposed scientific concepts for modern hazard indicators beyond existing persistence, bioaccumulation and toxicity (PBT) assessment. The conventional indicators for PBT are recommended to be replaced by two innovative hazard indicators only, termed cumulative toxicity equivalents (CTE) and persistent toxicity equivalents (PTE). CoModHaz sets out to bring this novel concept into practice and test it on challenging use cases developed in a co-creation process with five practice partners from regulation (national authorities), NGOs and industry.
We aim to develop and realize case studies on UFZ's research platform CITEPro for high-throughput bioassays using cellular in vitro systems and modern imaging technology. One of the greatest experimental challenges of in vitro assays are (super)hydrophobic persistent organic compounds, where also classical animal tests often failed to produce results. The first case study deals with hydroxyphenyl-benzotriazoles and the second tackles a set of polyhalogenated flame retardants. We explore if cyclodextrins and other dosing techniques can facilitate and accelerate their uptake into cells. Among cyclodextrin derivatives, gamma-cyclodextrin with hydroxypropyl substituents was found to be the least toxic in the RTgill-W1 assay. In a third case study, we focus on plastic chemicals and propose an in vitro test battery covering clusters of noncommunicable diseases prevalent in humans. Relevant endpoints are selected using pathway-based information from AOP networks and integrating the key characteristics concept. We found oxidative stress, cytotoxicity and mitochondrial dysfunction to be central to most disease clusters. The fourth case study tackles the challenging chemical group of cationic and anionic surfactants and the coupling of toxicity and persistency tests. Toxicity tests in the AREc32 and the RTgill-W1 assays suggest that the benzalkonium chlorides act as non-specific baseline toxicants, fitting well the model predictions for baseline toxicity.
By mapping stakeholder needs, investigating and addressing implementation barriers, the co-production of implementation pathways and the four case studies will pave the way for these novel hazard indicators.

7) Proteomic Insights into PFAS-Induced Alterations in THP-1 Macrophages

Anna Didio, Anna van Echten, Stefanie Raps, Qiuguo Fu, Tamara Tal, Kristin Schubert

Molecular Toxicology

Per- and polyfluoroalkyl substances (PFAS) are a broad group of man-made chemicals notable for their widespread environmental presence and remarkable resistance to degradation. Although a growing body of evidence suggests that PFAS can affect the immune system, studies on innate immune responses often yield conflicting results, leaving open questions about their underlying mechanisms of toxicity.

In this work, we aimed to characterize how PFAS influence the innate immune system by applying concentrations previously quantified in human blood (Berntsen et al. 2017) and a 1000-fold increase of those concentrations in a THP-1 macrophage cell culture model. Specifically, we exposed THP-1 macrophages to PFHxS, PFOS, PFOA, PFNA, PFDA, and PFUnDA—both individually and as a mixture—and employed untargeted proteomics along with iodoTMT to study cysteine oxidation (redoxome).

Overall, we observed no consistent functional impact on the THP-1 proteome across the various PFAS tested. Concentrations of 1× and 1000× often yielded contradictory effects, with PFDA and PFUnDA displaying the most similar proteomic changes. Cysteine oxidation was most prominent under LPS-stimulated conditions: PFDA, PFUnDA, and the PFAS mixture induced oxidation of proteins involved in translation, nuclear envelope integrity, the CCT/TRiC complex, autophagy, and mitosis. Notably, these effects were reversed (i.e., cysteine residues were restored) at the 1000× concentration for the same PFAS.

These findings indicate that PFAS can influence macrophage homeostasis at the proteomic level in a manner that is not strictly concentration-dependent, leading to divergent outcomes between individual and mixed PFAS. We identified PFAS-driven cysteine oxidation in key pathways, although the precise functional implications of these oxidative and reductive changes warrant further investigation.

8) Impact of PFAS mixtures and individual compounds on placental function during early gestation

Yu Xia, Tamara Tal, Oddvar Myhre, Birgitte Lindeman, Nicola Margareta Smith, Violeta Stojanovska, Ana Claudia Zenclussen

Environmental Immunology

Impact of PFAS mixtures and individual compounds on placental function during early gestation
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Background: Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants that are associated with several adverse effects on human health. In utero exposure to PFAS is linked to various pregnancy complications. The placenta serves as the critical interface between maternal and fetal systems, and it is thought to be a primary target of PFAS. Moreover, 3D in vitro models provide better organizational tissue structure and a more realistic response to chemicals. In that order, this study investigates the effects of a human blood-relevant PFAS mixture and individual PFAS

compounds on placenta morphology and phenotype during early gestation using 3D trophoblast spheroids.
Methods: PFAS mixture was designed based on human blood concentrations from the Norwegian Institute for Public Health, and contained 6 PFAS: PFOS, PFOA, PFHxS, PFNA, PFDA, and PFUnDA. Trophoblast spheroids were generated by culturing JEG-3 cell lines in ultra-low attachment plates and subsequently exposed to individual PFAS compounds and mixture for 48–96 hours at varying concentrations. Viability was evaluated using multiparametric live/dead staining, while placental functions, such as invasion and human chorionic gonadotropin (hCG) production, were assessed through a matrix invasion assay and ELISA.
Results: PFAS mixture impacted the viability of the trophoblast spheroids only at ultra-high concentrations of 10000× (where × means times higher than human blood levels) after 48 hours of exposure. Surprisingly, the invasive properties of trophoblast spheroids increased following exposure to individual PFAS compounds and PFAS mixture at 72 hours and 96 hours. Ultimately, PFAS compounds and mixtures didn't impact the hormone hCG levels.
Conclusion: This study offers valuable insights into the complex and concentration-dependent effects of individual PFAS compounds and mixtures, highlighting the importance of using relevant in vitro models to address the chemical risk assessment in pregnancy-related outcomes.

9) Leveraging zebrafish embryo phenotypic observations to advance data-driven analyses in toxicology

Paul Michaelis, Nils Klüver, Silke Aulhorn, Hannes Bohring, Jan Bumberger, Kristina Haase, Tobias Kuhnert, Eberhard Küster, Janet Krüger, Till Luckenbach, Riccardo Massei, Lukas Nerlich, Sven Petruschke, Thomas Schnicke, Anton Schnurpel, Stefan Scholz, Nicole Schweiger, Daniel Sielaff, Wibke Busch,

Ecotoxicology

Zebrafish have emerged as a central model organism in toxicological research. Zebrafish embryos are exempt from certain animal testing regulations which facilitates their use in toxicological testing. Next to the zebrafish embryo acute toxicity test (ZFET) according to the OECD TG 236, fish embryos are used in mechanistic investigations, chemical screenings, in ecotoxicology, and drug development. However, inconsistencies in the applied test protocols and the monitored endpoints in addition to a lack of standardized data formats, impede comprehensive meta-analyses and cross-study comparisons. To address these challenges, we developed the Integrated Effect Database for Toxicological Observations (INTOB), a comprehensive data management tool that standardizes collection of metadata and phenotypic observations using a controlled vocabulary. By incorporating data from more than 600 experiments into the database and subsequent comprehensive data analyses, we demonstrate its utility in improving the comparability and interoperability of toxicity data. Our results show that the ZFET can detect toxicity spanning seven orders of magnitude at the scale of effect concentrations. We also highlight the potential of read-across analyses based on morphological fingerprints and their connection to chemical modes of action, provide information on control variability of the ZFET, and highlight the importance of time for mechanistic understanding in chemical exposure-effect assessments. We provide the full FAIR dataset as well as the analysis workflow and demonstrate how professional data management, as enabled with INTOB, marks a significant advancement by offering a comprehensive framework for the systematic use of zebrafish embryo toxicity data, thus paving the way for more reliable, data-driven chemical risk assessment.

10) Maternal Exposure to Low-Dose PFOS and GenX Induced Weight Gain and Impaired Insulin Sensitivity in female Offspring

Bingyu Wang, Tobias Polte, Marita Wagner, Ana Claudia Zenclussen

Environmental Immunology

Per- and polyfluorinated alkyl substances (PFAS) are endocrine disruptors that are widely used in consumer products and have been linked to the development of several diseases. A particular focus of recent epidemiologic and experimental studies has been the influence of environmental exposures during pregnancy and the early postnatal period. This developmental window is particularly vulnerable to the effects of environmental factors due to rapid growth and high cell division rates. However, the long-term transgenerational effects of exposure to very low levels of long-chain PFOS and the short-chain substitute GenX, which is considered safer by the industry, in terms of metabolic changes are still unclear. In this study, the effects of maternal exposure to PFOS and GenX on weight development and metabolic alterations in the offspring were investigated in an in vivo disease model. Therefore, mice were orally exposed to subtoxic concentrations of PFOS and GenX during pregnancy and lactation, with the concentrations used corresponding to the acceptable daily intake (ADI) and no-observed-adverse-effect level (NOAEL) established by regulatory authorities. Preliminary results showed that maternal exposure to PFOS/ADI resulted in significantly increased body weight and fat mass in the female offspring. In contrast, the higher NOAEL concentration had no effect on the female offspring, as both concentrations had no effect on the male offspring. Maternal exposure to GenX resulted in increased body weight in female offspring at both concentrations, although again no effects were observed in male offspring. The increased weight in the female offspring of PFOS/ADI and GenX/ADI-exposed dams was associated with impaired glucose and insulin tolerance. In addition, levels were elevated in these animals. In conclusion, the results obtained so far indicate that exposure during pregnancy/lactation, even at very low PFAS concentrations, leads to weight gain and metabolic alterations in the offspring, with the effects being observed in a sex-specific manner. Ongoing studies to further investigate the impact of PFAS on weight/body size, as well as studies of PFAS effects on gene expression, DNA methylation, or the gut microbiome in the offspring, along with in vitro adipocyte differentiation experiments should clarify the underlying mechanism of PFAS effects on adipogenesis and metabolic function.

11) Investigating Placental Barrier Integrity Under Exposure to a Real-Life PFAS Mixture

Verena M. Mailänder, Sergio Gómez-Olarte, Qiuguo Fu, Violeta Stojanovska, Nicole Meyer, Ana C. Zenclussen

Environmental Immunology

Background: Per- and polyfluoroalkyl substances (PFAS) are persistent pollutants with long half-lives. Several studies have reported that PFAS can cross the placental barrier, impacting placental function and fetal development. Although in utero exposure diminishes fetal growth, it remains unclear whether PFAS affect fetal metabolism directly or via disruption of the placental barrier. In particular, most assessments have focused on single entities rather than PFAS mixtures. This study, therefore, investigates the impact of a PFAS mixture on placental barrier formation and integrity.
Method: An in vitro placental barrier model was established on a collagen-coated Transwell (3 µm pore size) by coculturing the BeWo b30 trophoblast cell line (apical) with primary HUVEC endothelial cells (basolateral). Before seeding on the Transwell, cells in co- or monoculture (barrier formation control) were maintained separately in complete DMEM and endothelial cell medium with 5% FBS. The composition of a real-life PFAS mixture was chosen based on a screening of 56 PFAS measured in 1st trimester human placentas, resulting in a mixture of PFNA, PFOS, PFBA, PFOA, PFHxS, and PFDA in

a ratio of 80:7:5:4:2:2. The in vitro placental barrier was exposed to this PFAS mixture at 100 µM or to the solvent control (0.1% DMSO). The treatment was added daily to the apical (maternal) side during media refreshment. Barrier formation was monitored daily via transepithelial electrical resistance (TEER) and Na-fluorescein translocation measurements. Placental integrity was assessed with FITC-Dextran (40 kDa) translocation over 1–21 hrs.
Results: Compared to the acellular control, TEER values increased and Na-fluorescein values decreased over time in all culture conditions, validating the in vitro placental barrier formation. BeWo cells in mono- and coculture showed the highest TEER values (~500 and ~400 Ω/cm^2), while those of HUVEC in monoculture were similar to the acellular control (~100 Ω/cm^2). FITC-Dextran translocation decreased with culture time, with the coculture having the lowest permeability. PFAS mixture (100 µM) treatment did not alter any placental barrier endpoints in the co- or monocultures when compared to the DMSO control (p-value > 0.05).
Conclusion: Our findings suggest that a mixture of six PFAS at 100 µM in the ratio found in human placenta does not induce measurable effects on the formation and integrity of an in vitro placental barrier model.

12) RetiNAM: Identification of Sensitive Biomarkers for the disruption of the retinoic acid signaling pathway

Vanessa Saalmann, Éva Fetter, Susanne Walter-Rohde, Jürgen Arning, Stefan Scholz

Ecotoxicology

Already during the 1970's, imposex development led to a marine mollusk population decline. Today, exposure to organotins in anti-fouling paints on ships is considered as a cause. Laboratory exposure to tributyltin (TBT) confirmed its potency to cause imposex in mollusks. The disruption of the retinoic acid (RA) pathway by binding the retinoid X receptor (RXR) has been later identified as the underlying mechanism. The RA pathway is a highly conserved endocrine signaling pathway with a central role to control essential physiological and cellular processes including (neuro)development and cellular metabolism. Regulatory interest in the retinoid system is growing and the OECD has initiated efforts to review and harmonize regulations, focusing on key organ systems, while highlighting the need for validated assays.
Focusing on environmental organisms, a literature research was conducted to collect evidence for the relevance of RA signaling disruption. Disruption of the RA pathway by environmentally relevant chemicals was reported for sites all globally across several species. Observed effects beyond imposex included malformations, altered retinoid tissue levels and effects on population-relevant traits leading to population decline in various phyla. Furthermore, molecular biomarkers for the disruption of the RA signaling were identified.
Current identification of endocrine disrupting (ED) properties for active substance testing via standardized test methods only considers EATS (estrogen, androgen, thyroidal, steroidogenic) modalities. Validated test methods for the identification of endocrine disruptors of other pathways like the RA pathway are lacking. With reduction of animal testing inevitable, new approach methods are required for testing disruption of other endocrine signaling pathways.
As the disruption of the RA pathway can affect organisms up to population-relevant levels, a better understanding of the relationships between molecular key events and apical adverse effects is crucial. The research objective of RetiNAM is to establish sensitive endpoints and biomarkers for disruption of the RA pathway by RXR or retinoic acid receptor (RAR) ligand binding. The literature research provides the basis to develop NAM's for the zebrafish embryo model, by combining morphology, behavioral fingerprinting and transcriptomics in order to identify ED's interfering with the RA pathway.

13) The zebrafish Visual and Acoustic Motor Response (VAMR) assay adds value to the Developmental Neurotoxicity In Vitro Battery (DNT-IVB)

Julia Spath, Nadia Herold, Stefan Scholz, Tamara Tal

Ecotoxicology

Exposure to chemicals can pose a significant threat to the developing nervous system, yet few chemicals are evaluated for their potential to cause developmental neurotoxicity (DNT) using OECD rodent-based test guidelines. A promising alternative to rodent studies is the New Approach Method (NAM)-centered DNT in vitro test battery (DNT-IVB). A recent study identified nine false negative compounds that were developmentally neurotoxic in guideline rodent studies but negative in the DNT-IVB. We hypothesized that multi-behavioral phenotyping in metabolically competent early-life stage zebrafish can detect DNT effects and add value to the DNT-IVB. To assess the neurotoxic potential of false negative DNT-IVB chemicals, 5-day post fertilization (dpf) zebrafish were exposed to 4.4-80 µM chlorpyrifos, maneb, PFOA, ketamine, 5,5-Diphenylhydantoin, triethlytin bromide, nicotine, or BDE-99 or 0.4% DMSO 60 min before behavioral analysis using the Visual and Acoustic Motor Response (VAMR) NAM. This behavior test measures motor activity following a range of visual and acoustic stimuli and consists of 26 behavioral endpoints including visual and acoustic startle responses, non-associative habituation learning, memory retention, and movement during inter-stimulus intervals. Collectively, pronounced neuroactivity signatures were identified for six out of eight false negative reference chemicals (5,5-Diphenylhydantoin, chlorpyrifos, ketamine, maneb, nicotine, and triethyltin bromide but not PFOA or BDE-99). Inclusion of VAMR NAM as part of the DNT-IVB resulted in an increase in battery sensitivity from 84% to 94%. In addition to the identification of false negative chemicals, we also hypothesized that the VAMR NAM can detect neuroactivity effects at lower concentrations, relative to the DNT-IVB. To explore this, 5 dpf zebrafish were exposed to 0.00004-80 µM of the true positive DNT-IVB chemicals haloperidol, hexachlorophene, tebuconazole, or trichlorfon. For all true positive chemicals, effects were visible with an increased potency of 3.1 to 4.8 orders of magnitude, as compared to the DNT-IVB. Taken together, the VAMR NAM adds value to the DNT-IVB by increasing test battery sensitivity and enhancing potency detection. More broadly, this work shows that multi-behavioral phenotyping in early life stage zebrafish yields rapid, useful information on the ability of chemicals to disrupt nervous system development and function.

14) Developing a high-content imaging workflow to investigate the impact of endocrine disruptors on intestinal macrophages in zebrafish larvae

E.K. Nicolay, R. Massei, D. Trofimova, R. Haase, F. Isensee, A.C. Zenclussen , T. Tal

Ecotoxicology

Environmental chemicals are ubiquitous in water and food, making the gut a primary site of exposure and potential risk for toxic effects. We therefore hypothesize that intestinal exposure to chemicals can provoke gut inflammation. Macrophages are innate immune cells crucial for maintaining intestinal homeostasis and can potentially serve as a key readout of the immunomodulatory effects of chemical exposure. To elucidate the underlying cellular mechanisms, robust and multi-modal high-content assays are necessary to detect the potential effects of environmental chemicals on intestinal health. However, existing methods often lack high-content capacity, limiting their ability to efficiently screen a wide range of chemicals. To address these limitations, we developed a novel approach utilizing a 96-well

setup to acquire 3D confocal images of transgenic zebrafish larvae expressing a fluorophore under a macrophage-specific promoter (mpeg1:mCherry). We developed an open-source workflow for 3D image processing, which is currently operational in beta-version. This semi-automatic workflow requires user input to identify the intestine in transmitted light images, and these regions are then transferred to corresponding fluorescence channels to enable downstream quantitative analysis within the gut. In a refined workflow, a 3D nnU-Net (No New U-Net) was trained for semantic segmentation of the fish body and gut using the transmitted light channel, based on partially annotated volumes with manual labels provided on selected slices. Preliminary results demonstrate that the workflow can detect changes in cell number following exposure to reference chemicals such as dextran sodium sulfate and trinitrobenzene sulfonic acid. As part of the ENDOMIX consortium, the gut inflammation assay will be used to test mixtures of Endocrine Disrupting Chemicals (EDCs) that co-occur in human populations in Europe. Prolonged intestinal exposure may disrupt the gut-brain axis. Future work will develop a deep learning approach to explore enteric neuron–macrophage interactions, providing detailed insights into their spatial and morphological responses to chemical exposure. Overall, our imaging tool represents a sophisticated and robust strategy for computerized batch processing of high-content images, which can be broadly applied in the fields of developmental biology and toxicology.

15) Composition, bioaccumulation, and acute behavioral effects of tire wear particle leachate on zebrafish reveal new insights into suspects and underlying mechanisms of action

David Leuthold, Bettina Seiwert, Daniel Zahn, Nicole Schweiger, Renee Owen, Tamara Tal

Ecotoxicology

Tire wear particles, a major source of synthetic polymer pollution from road transport, release complex chemical mixtures into aquatic environments via roadway runoff, posing risks to ecological and human health. However, data on their acute neurotoxicity remain limited, especially for complex mixtures.

To address this, we combined targeted analytical chemistry with a zebrafish-based New Approach Method (NAM) using a battery of visual and acoustic behavioral assays. Cryo-milled tire tread (CMTT; 0.3–31.2 g/L) from the U.S. Tire Manufacturers Association was leached for 72 h under controlled conditions (20 °C, 14:10 h light:dark, 600 rpm). Zebrafish larvae were acutely exposed for 1 hour at 5 days post-fertilization, and behavior was assessed via light–dark transitions, acoustic startle responses, and learning/memory paradigms.

Following acute exposure, larvae exhibited dark-phase-specific hyperactivity, also present during acoustic stimulation. This effect persisted for several hours and was partially reversible after washout. A similar phenotype was induced by leachates from field-collected tunnel dust, supporting environmental relevance.

Twenty-five organic compounds, including para-phenylenediamines (PPDs, e.g., 6PPD), benzothiazoles, and guanidines, were quantified in CMTT leachates and exposed larvae. However, reconstituted mixtures of these compounds and a quinone mix including 6PPD-quinone failed to reproduce the phenotype, excluding these as sole drivers. Filtration and solid-phase extraction did not alter leachate bioactivity, suggesting polar compounds as likely effectors.

In silico target prediction showed that PPDs share a predicted interaction with kcnh3, a Kv12.2 voltage-gated potassium channel. The Kv12.2 inhibitor ASP2905 partially phenocopied CMTT-induced behavior and shared target overlap with 6PPD, suggesting a possible shared mechanism.

This study identifies a novel neurobehavioral phenotype in zebrafish after acute exposure to CMTT and tunnel dust and highlights Kv12.2 as a previously unrecognized target of PPDs, with implications for risk assessment of tire-derived pollutants.

16) Establishing a high-content analysis of complex mixtures with the zebrafish embryo model

Emmanuel Ogwu Chukwu, Stefan Scholz and Wibke Busch

Ecotoxicology

Chemicals are integral to modern society, but their widespread use has led to environmental contamination, posing risks to human and ecological health. Traditional risk assessments focus on individual chemicals, overlooking the combined effects of complex mixtures found in the environment. This research aims to enhance environmental risk assessment by analyzing complex chemical mixtures using zebrafish embryos (Danio rerio). By integrating chemical monitoring with effect-based approaches, the study seeks to better understand the toxicological impacts of chemical pollutants. The research follows a structured approach: the first phase involves high-content assessment of artificial model mixtures, followed by evaluating prototype mixtures informed by environmental data in the second phase, and applying findings to real environmental samples in the third phase. A key aspect of the study is the selection of chemicals based on their Toxic Ratio (TR), calculated as the ratio of baseline toxicity to observed toxicity (LC50). Compounds with TR values exceeding tenfold were prioritized for analysis. The study also categorizes these chemicals based on their mechanisms of action, including anticancer agents, antibiotics, pesticides, enzyme inhibitors, and environmental toxicants. By leveraging zebrafish embryo models and high-content screening, this research provides insights into the cumulative and interactive effects of chemical mixtures. The findings aim to improve regulatory frameworks for environmental protection and contribute to a more comprehensive understanding of chemical mixture toxicity.

17) Neuroactive Behavioral Fingerprinting of Crude Oil-Derived Water Accommodated Fractions in Larval Zebrafish Using a New Approach Method

Nadia Herold, Lisbet Sørensen, Mari Egeness Creese, Jasmine Nahrgang, Nicole Schweiger, Stefan Scholz, Tamara Tal

Ecotoxicology

Petroleum spills release complex chemical mixtures into aquatic environments, posing neurodevelopmental risks to wildlife. Water Accommodated Fractions (WAF), which contain bioavailable constituents, are of concern, yet the neuroactive properties of their chemical classes remain unclear. We applied a behavior-based new approach method (NAM) to identify neuroactive effects of a crude oil-derived WAF and its fractions in larval zebrafish.

Zebrafish larvae (5 dpf) were exposed to WAF concentrations (9.8–100 %, 1 g/L WAF loading) and assessed using a 26-endpoint Visual and Acoustic Motor Response (VAMR) assay. WAF exposure induced concentration-dependent effects: lower concentrations caused hypoactivity and reduced startle responses, while higher doses (≥ 56.6 %) elicited light-phase hyperactivity, seizure-like motor patterns, and impaired habituation learning.

To identify chemical drivers, WAF was fractionated into PAHs, resins, naphthalenes, monoaromatics, and saturates. All disrupted behavior; PAH and resin fractions best mirrored total WAF profiles. GCxGC-MS identified phenanthrene as the dominant PAH (50 %). Resin fractions were chemically diverse, with no compound exceeding 5 % abundance.

Top three compounds from each fraction were tested. Phenanthrene reproduced habituation deficits, while 2-methylanthracene and ethyl 4-ethoxybenzoate contributed to late dark-phase hypoactivity. Resin compounds, including 1H-phenalen-1-one, 7-methyl-1-indanone, and dihydrocarvone, had varied effects, suggesting the resin fraction's profile emerges from cumulative low-abundance constituents.

Behavioral fingerprints were aligned with a library of 63 neuroactive reference compounds. Resin fractions clustered with GABAA receptor antagonists, indicating disrupted inhibitory signaling. High-dose WAF clustered with neurodevelopmental disruptors targeting mTOR, PPARδ, RXR, and BMP pathways.

This study demonstrates how chemically diverse crude oil mixtures exert neurotoxicity via distinct pathways. Our findings support behavioral fingerprinting as a scalable, mechanism-informative strategy for mixture toxicity analysis and chemical hazard assessment.

Research Theme: Environmental Chemistry

1) Degradation of per- and polyfluoroalkyl substances in fungi

Ann-Cathrin Krause, Emily Lichtenwald, Dietmar Schlosser, Lukas Y. Wick, Thorsten Reemtsma, Daniel Zahn

Environmental Analytical Chemistry

Per- and polyfluoroalkyl substances (PFAS) are very persistent anthropogenic chemicals of increasing concern due to their links to human health risks and associated costs. PFAS form an extensive class that exceeds seven million registered compounds (1). Yet, only a few PFAS are well-characterized, regularly monitored, and regulated. While few PFAS have been shown to transform into the persistent perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs), the primary biodegradation and transformation products of other classes remains poorly understood. Particularly, the transformation of PFAS by fungi has received little attention.
In this work, three fungi that differ in their enzymatic systems are investigated in their effectiveness to degrade and transform nine PFAS. The selected fungal species include the ascomycete Phoma sp. (UHH 5-1-03) and the basidiomycete Gloeophyllum trabeum (brown-rot fungus), previously shown to degrade 7:2 fluorotelomer alcohol (FTOH) (2), and Pleurotus ostreatus (white-rot fungus), which produces laccase, an enzyme implicated in PFAS degradation (3).
The study expands beyond the commonly studied PFAS perfluorooctanoic acid, perfluorooctanesulfonic acid and FTOHs by including a perfluoroalkane sulfonamide, a perfluoroalkane sulfonamidoacetic acid, a fluorotelomer carboxylic acid, a hydrogenated PFCA, a polyfluorinated aromatic compound, a perfluoroalkyl phosphinic acid and two perfluorinated ethers. For some of the investigated PFAS, a concentration decline was observed with distinct differences between the examined fungal species, which may aid in uncovering underlying degradation mechanisms. Furthermore, conclusions about an order of transformation are made and the formation of transformation products, such as PFCAs, is studied.

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2) Microplastics in Elbe River Sediments Analyzed by Thermal Extraction-Desorption Gas Chromatography-Mass Spectrometry (TED-GCMS)

Siqi Wu, Alexander Böhme, Mara Römerscheid, Martin Simoneit, Andreas Schäffer, Martin Krauss, Norbert Kamjunke, Anna Matoušů, Tina Sanders, Ingeborg Bussmann, Björn Raupers, Holger Brix, Annika Jahnke

Exposure Science

Microplastic pollution is a globally emerging environmental concern, with sediments serving as major sinks for certain polymers in aquatic systems. Traditional environmental analysis of microplastics typically involves spectroscopic methods, such as Fourier Transform Infrared and Raman spectroscopy. While effective for identifying microplastic morphology, these methods are time-consuming and may overlook quantitative (mass) data. In contrast, methods like Thermal Extraction-Desorption Gas Chromatography-Mass Spectrometry (TED-GCMS) can provide valuable mass-based measurements of microplastics. In this study, we applied this thermal method to investigate microplastic pollution in sediments from the Elbe River. Twenty-seven sediment samples were collected along the river, covering the Czech Elbe, the free-flowing inland Elbe, the tidal Elbe, and the North Sea. After density separation and digestion, sediment samples were analyzed. Microplastic patterns covering seven common polymers, i.e. polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyamide (PA), polylactic acid (PLA), and polymethyl methacrylate (PMMA), will be presented.

3) Removal of polyamide microplastics from artificial water samples using electrosorption and electrooxidation

Navid Saeidi, Alexander Böhme, Susanne Stenz, Jane Neshovski, Annika Jahnke, Anett Georgi, Falk Harnisch

Exposure Science

A class of emerging pollutants detected in wastewater treatment plants (WWTPs) and other water bodies are microplastics (MPs, particle size < 5 mm). They can decrease the cleaning efficiency of WWTPs, pollute water resources, or act as vectors in spreading other toxic pollutants. The removal of MPs from wastewater, however, is a resource-intensive process, and, depending on the treatment method, may lead to breakdown of the plastic into smaller particles. Within the transfun-funded project ELECTROREM4PLASTICS we show on the example of polyamide MPs their removal from artificial water samples (composed of deionized water supplemented with 100 mM sodium sulfate to adjust the ionic strength and approximate conditions found in natural aquatic environments) using electrochemistry-based approach. This method offers a promising alternative to conventional treatments by targeting a more controlled and potentially less invasive removal process, reducing the risk of further fragmentation. Polyamide was chosen for this case study because of its high use in the textile industry (≈ 8 million tons in 2024) and its easy handling in the lab. First, we used highly porous carbon electrodes to enrich the MPs in order to allow, secondly, for an efficient follow-up oxidation through OH radicals generated at boron doped diamond electrode. The concentration of the polyamide MPs (size ≥ 1 µm) in the water samples were quantified using a pyrolysis balance coupled to a gaschromatograph with a mass spectrometric detector (TED-GC-MS). The results of this case study showed that polyamide MPs can be efficiently enriched at the porous carbon electrode and subsequently degraded through electrooxidation by up to 85% of the particle fraction ≥ 1 µm, both at mild, energy-saving conditions (0.5 Wh per mg of degraded polyamide MPs).

4) nanoINHALE: Plastic-associated chemicals in terrestrial biota, including simulated leaching

Alexander Böhme, Annika Jahnke, Kristin Schubert, Ursula Siebert und Elisa Rojo-Nieto

Exposure Science

Between 1950 and 2015, a total of 6300 Mt of plastic waste was generated, with 60% discarded and directly entering various parts of the environment [1]. Due to weathering, this plastic is fragmented into smaller particles, such as microplastics (<5 mm, MP) and nanoplastics (<1 µm, NP). These particles can be found in the air in both urban and rural regions. In addition to known exposure routes like skin contact and ingestion, inhalation is another potential pathway for micro- and nanoplastic (MNP) uptake. Due to their small size, NPs can penetrate deep into biological systems and cause damage by crossing cell membrane barriers [2]. Beyond the risks posed by their size, MNPs usually contain additives, such as plasticizers, UV stabilizers, and flame retardants, that enhance their properties like flexibility, durability, or heat-stability. These compounds may leach out of the polymer matrix, as they are often not covalently bound. Once leached out, they can harm biota and persist in the environment [3]. Regarding exposure to plastic-associated chemicals, only a few studies on terrestrial wildlife are available focusing on ingestion and dermal contact only, while MNP-based respiratory uptake as well as corresponding additive leaching is still a knowledge gap. [4]
This sub-project of the nanoINHALE-PhD cohort focuses, amongst others, on the significance of uptake via breathable MNPs in the overall burden of plastic-associated chemicals in air-breathing mammals. The goal is to assess whether airborne MNPs play a critical role in the exposure of air-breathing animals to plastic-associated chemicals. To this end, lung and lipid tissues from racoons sampled from differently polluted environments (urban versus remote/rural areas) will be studied. A wide range of plastic-associated chemicals (including plasticizers, UV-stabilizers, and flame retardants) will be analyzed in those tissues by GC-MS/MS and LC-MS/MS. Furthermore, using artificial lung fluid, the leaching kinetics of plastic-associated chemicals from different polymers and particle size will be investigated, considering that size can influence the penetration depth in different areas of the lungs and residence time. In addition, the lung proteome of a subset of samples will be studied, to explore potential links between airborne exposure and the effects of plastic-associated chemicals. Finally, we will employ chemoassays to profile the reactivity of plastic-associated chemicals toward lung proteins to assess their potential to cause respiratory diseases such as asthma.

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5) Removal of Persistent, Mobile, Organic Compounds (PMOC) using Activated Sludge-Based Hydrochars in a Decentralized Municipal Wastewater System

Sampriti Chaudhuri, Lisa Röver, Maria Balda, Anett Georgi, Martin Krauss, Ariette Schierz

Technical Biogeochemistry

Existing decentralized wastewater treatment systems already meet legal standards for water quality parameters (EU 2020/741). However, recent studies have demonstrated the uptake of persistent, mobile, organic compounds (PMOC) such as pharmaceuticals, tire additives and industrial chemicals by irrigated crops, raising concerns about human exposure through food consumption. The revised EU Urban Wastewater Treatment Directive (UWWTD 2024) outlines the need to include a quaternary treatment step that addresses micropollutants for safe water reuse in urban areas.
Hydrothermal carbonization (HTC) is a promising technology for the treatment of wet biological waste - it can recover nutrients and produce hydrochar, a carbon-rich solid residue with multiple applications. The aim of this study is to develop a sustainable sorbent from HTC of sewage sludge for the removal of PMOC from municipal wastewater. Sewage sludge, either alone or in combination with manure and seasonal biomass, is subjected to temperatures of 180-280°C and pressures of 10-65 bar to produce hydrochar. The resulting hydrochar is activated by steam in a N2 atmosphere at temperatures between 800 and 900°C for 1-4 hours to increase its carbonization and micro- and mesoporosity, which is beneficial for PMOC sorption. Preliminary results from sorption studies using 8 different activated sludge-based hydrochars show efficient removal of model PMOC perfluorobutanoic acid, perfluorooctanoic acid and sulfamethoxazole (logarithmic sorption coefficients Kd ranging between 3.3 to 5.4 for PMOC concentrations of 40 to 150 µg L-1). The results also indicate that a de-functionalized, micro- and mesoporous sorbent performs best in terms of sorption of these compounds.
The best-performing activated hydrochar will be tested at an existing pilot site where wastewater from 8 population equivalents is treated by an aerated constructed wetland (CW). Water from the CW will pass through a slow trickling sand filter to reduce the bacterial load, followed by the reactor packed with the tailored activated hydrochar to remove residual PMOC. The final water quality from the decentralized treatment system is expected to be suitable for horticulture thereby improving water resilience in urban areas.

6) Sustainable treatment technologies to remove the 'forever chemicals' PFAS from water

Anett Georgi, Ariette Schierz, Navid Saeidi, Sarah Sühnholz, Robert Köhler, Maria Balda, Katrin Mackenzie

Technical Biogeochemistry

PFAS (Per- and polyfluorinated alkyl substances) are man-made chemicals with extreme stability. They have been shown to cause adverse health effects at trace levels (ppb) when ingested through food and water. There is an urgent need to cut PFAS emissions from hot spots. Environmental technologies need to become more sustainable as we strive for carbon neutrality. Current PFAS removal technologies fail to be affordable and/or have a high carbon footprint. We will present our approach: innovation in adsorption water treatment with 2 examples. Overall, our work shows that we can make environmental technologies more sustainable, even for the challenging PFAS.

7) What is the impact of waste water treatment plants on the Danube River? – An integrated assessment in the framework of the Joint Danube Survey (JDS)-5

Charlotte Henkel, Martin Krauss, Werner Brack

Exposure Science

Increasing chemical pollution is a threat to sustainable water resources worldwide. Waste water treatment plants (WWTPs) are a main source contributing to this chemical burden as the complexity of chemical pollution mixtures entering waste water treatment plants pose challenges to common treatment technologies. As a consequence, effluents containing an unknown number of various contaminants might impact surface water bodies, with unknown effects for aquatic organisms and, ultimately, for humans. Elucidating the chemical complexity of contaminants in waste water (both influents and effluents) is required to understand the contribution of waste water treatment plants to chemical pollution of the aquatic environments and to determine the associated risks.

The Danube River is the second largest river in Europe and an important water resource for many countries. However, the fact that many large cities and waste water treatment plants are located in the (catchment) area of the Danube River, makes it susceptible to the entry of a large number of domestic and industrial pollutants. The Joint Danube Surveys (JDS)-3, JDS-4, and many other studies have assessed different types of stressors in waste water individually, but a comprehensive characterization and harmonized evaluation of mixtures of all types of chemicals emitted via WWTP effluents to the Danube catchment are missing. This study aims at closing this knowledge gap by integrating the analysis of a broad range of organic and inorganic contaminants including heavy metals, rare earth elements, hydrophilic and hydrophobic organic micropollutants, micro- and nanoplastics as through chemical analysis, but also bioassays and antibiotic resistance genes.

In the framework of the Joint Danube Survey 5, waste water influent, effluent, sludge and combined sewer overflow samples will be taken in ten waste water treatment plants in the Danube catchment. In this study, the samples will be analyzed by international laboratories using complementary tools, combining chemical and bioanalytical methods with the overarching goal being a comprehensive integrated assessment.

8) Chemical Profiling of Hydrophobic Organic Compounds in Terrestrial Mammals at High Trophic Levels Using Silicone Chemometers

Caglar Akay, Elisa Rojo-Nieto, Claudia A. Szentiks, Ursula Siebert and Annika Jahnke

Exposure Science

Passive equilibrium sampling using silicone chemometers is an effective tool for sampling and assessing hydrophobic organic compounds (HOCs) in biota. Silicone chemometers have been applied to evaluate HOCs in lipid tissues of various marine mammal species; however, their use for the bioaccumulation assessment of pollutants in terrestrial mammals remains largely underexplored. Here, we address this gap by characterizing the chemical exposome of four terrestrial and two semi-terrrestial mammal species, representing different feeding habits and habitats.

We used silicone chemometers to extract and analyze approximately 100 legacy and emerging HOCs from 83 lipid-rich blubber samples collected from nutria (n=10), otter (n=10), fox (n=3), raccoon dog (n=20), raccoon (n=10), and wolf (n=30). Targeted chemical analysis was performed using gas chromatography coupled with high-resolution mass spectrometry (GC-HRMS Orbitrap Q-Exactive), covering polybrominated diphenyl ethers (PDBEs), polychlorinated biphenyls (PCBs), chlorinated hydrocarbons (CHCs), and organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), pyrethroids, musks and other industrial compounds. Here we focus on PBDEs, PCBs, OCPs, and CHCs, as data evaluation for the other HOCs is ongoing.

We identified 19 out of 37 target compounds in at least one of the 83 blubber samples, while 18 were absent from all samples. PCBs and OCPs were the most frequently detected HOCs across all species. Furthermore, PCB153, PCB170 and PCB180 were present at elevated concentrations

compared to other PCBs. The metabolite 4,4'-Dichlorodiphenyldichloroethylene (DDE) was determined in all otter samples (up to 119 pg/mgPDMS), half of the raccoons (up to 36 pg/mgPDMS), and 12 wolves (up to 22 pg/mgPDMS) but was absent in nutria. The presence of DDE indicates historical contamination with 4,4'-Dichlorodiphenyltrichloroethane (DDT), with DDE being detected five times more frequently than its precursor. Higher concentrations and detection frequencies of HOCs in otters than in other species investigated in our study suggest distinct exposure through their diet and habitat.
Overall, our findings highlight the persistence and bioaccumulation of legacy pollutants, such as PCBs and DDE, in high trophic level terrestrial mammals, despite regulatory bans on these HOCs. Future work will expand the range of HOCs and explore patterns across terrestrial mammals, marine mammals and humans in our HGF project EXPOSO-METER.

9) Redox-differentiation enhances the microbial transformation of sulfamethoxazole in lab-scale bank filtration columns

Jimmy Köpke, Caglar Akay, Simon Klaes, Irantzu Vergara-Luis, Chang Ding, Lorenz Adrian, Aki S. Ruhl

Molecular Environmental Biotechnology

We used sulfamethoxazole (SMX), which is a detected antibiotic residual in surface water, groundwater and even drinking water. We used SMX as model substance and hypothesized, that the fate of SMX during bank filtration is dependent on the present redox-conditions. We fed tap water spiked with 0 to 10,000 µg/L SMX to long-term operated lab-scale sediment columns in parallel first under oxic conditions (0 to 159 days), later under oxic-anoxic conditions (160 to 600 days). The lower concentration ranges were closer to detected residual concentrations, the higher ranges allowed better fate assessment of SMX transformation products (TPs). During oxic-anoxic operation, the first part of the column was oxic-anoxic (0 – 8 cm) and deeper purely anoxic (8 – 24 cm). The highest SMX transformation occurred within the oxic-anoxic part but still considerably within the purely anoxic parts. SMX TPs with different attacking sites and transformation mechanisms were identified. The abundance of major SMX TPs increased along the column depth, but mainly within the oxic-anoxic column parts. Additionally, amplicon sequencing demonstrated the prevalence of potentially transforming communities such as Meiothermus. Shotgun proteomics and resistance genes determination will also be conducted soon. The so far results demonstrated that redox-differentiated columns improved the overall transformation of SMX, whereby many SMX TPs are being formed and released via column effluent. Residual SMX and formed TPs could have strong implications for the safety of treated water. To study the exact contribution of oxic and anoxic conditions to SMX transformation pathways, alternating redox-
sequences during bank filtration have to be investigated.

10) Smart Manure: Developing novel bioelectochemical systems for the degradation of antibiotics and recovery of heavy metals from manure

Florian Fischer, Qiuguo Fu, Navid Saeidi, Benjamin Korth

Environmental Analytical Chemistry

Livestock manure represents a beneficial resource for fertilization of agricultural land and is used for bioenergy production.[1,2] For instance, 120 million tons of cow and pig liquid manure are annually produced in Germany. However, current on farm manure management results in an accumulation of antibiotics and heavy metals in manure.[1] This poses a risk to human health and the environment through the contamination of soils and aquifers and promotes the emergence of antibiotic-resistant microorganism.[2] Thus, novel manure management strategies are required to remove these contaminants while preserving the fertilizing properties of manure. Therefore, the project Smart Manure aims to develop a reactor system that electrochemically utilizes greenhouse gases emitted during manure storage, removes antibiotics, recovers heavy metals and aims to evaluate the impact of the decontaminated manure on the soil microbiome and cash crops. Within Smart Manure, we develop a bioelectrochemical system for the removal of antibiotics and recovery of heavy metals via electrosorption and electroactive microorganisms.

Conventional adsorption technologies are either water intensive, require chemical agents or have a high energy consumption to achieve adsorbent regeneration.[3] In contrast, electrosorption on activated carbon felt has the potential to increase adsorption capacity and rate through electrostatic attraction without these drawbacks.[3] Contaminant desorption is facilitated by reversing the electrode charge allowing a low-cost and on-site adsorbent regeneration.[3] In the first reactor system heavy metals and antibiotics are adsorbed at the anode and cathode, according to their charge. After desorption, electroactive microorganisms degrade antibiotics and facilitate the deposition of heavy metals. Electroactive microorganisms are cultivated in a mixed microbial culture from contaminated manure in a one-chamber bioelectrochemical system. Subsequently, different antibiotics and mixtures thereof (Amoxicillin, Enrofloxacin, Sulfadiazine, Tetracycline and Trimethoprim) are added to explore the robustness and degradation capabilities of the system.

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