



Report 2021



STUDENTS' LAB:

GOETHE GOES ENVIRONMENT







## Imprint

### Editors

Prof. Dr. Dr. h.c. Henner Hollert

Marc Wollenweber

Markus Schmitz

### Contact

E-Mail: [studentslab@bio.uni-frankfurt.de](mailto:studentslab@bio.uni-frankfurt.de)

Telefon: +49 (0)69 798 42160

Fax: +49 (0)69 798 42161

### Evolutionary Ecology & Environmental Toxicology (E<sup>3</sup>T)

Goethe University Frankfurt

Max-von-Laue-Str. 13

60438 Frankfurt/Main

Germany

## Preface of Prof. Dr. Dr. h.c. Henner Hollert

### German:

Die Ökotoxikologie leistet durch die Verknüpfung chemischer und biologischer Risikobewertung einen essentiellen Beitrag zur (inter-)nationalen Umweltforschung und -schutz. Jedoch haben bereits 2003 das Umweltbundesamt (UBA) und der Verband der Chemischen Industrie (VCI) auf den eklatanten Mangel an gut ausgebildeten Nachwuchswissenschaftlern aufmerksam gemacht. Wichtige Stellen in Behörden und Industrie können nach wie vor nicht optimal besetzt werden. Als eine kurz- bis mittelfristige Lösung wurde von den beiden maßgeblichen Fachgesellschaften, der Gesellschaft Deutscher Chemiker e.V. (GDCh) Fachgruppe Umweltchemie und Ökotoxikologie und der Society of Environmental Toxicology and Chemistry (SETAC) Europe – German Language Branch e.V., ein bundesweiter Postgradualstudiengang zum Fachökotoxikologen entwickelt. Langfristig sehen jedoch das UBA und der VCI die dringende Notwendigkeit, das wissenschaftliche Fach der Ökotoxikologie an den Universitäten zu fördern und entsprechende Inhalte bzw. einen entsprechenden Studiengang an deutschen Universitäten anzubieten. Ganz anders als es die aktuelle hohe Nachfrage an gut ausgebildeten Ökotoxikologinnen und Ökotoxikologen vermuten lässt, ist die aktuelle Situation an deutschen Hochschulen in diesem Fachbereich völlig widersprüchlich. Trotz des hohen Ausbildungsrückstandes und der Tatsache, dass der Ökotoxikologie auch gesellschaftlich eine stetig wachsende Bedeutung zukommt, werden immer mehr Lehrstühle in der Bundesrepublik geschlossen. Entgegen des großen Bedarfs an innovativer Forschung und Lehre an den Universitäten, verlieren Umweltchemie und Ökotoxikologie seit vielen Jahren an Unterstützung, Ressourcen und Anerkennung. Es ist jedoch von entscheidender Bedeutung, dass die Umweltchemie und Ökotoxikologie in der akademischen Wissenschaft verwurzelt sind, und folglich mit Ausrüstung, Ressourcen und Entwicklungsperspektiven zukunftsweisend ausgestattet werden. Auch in der Nationalen Akademie der Wissenschaften Leopoldina wurde kürzlich diese negative Entwicklung kritisiert und die Dringlichkeit fördernder Maßnahmen aufgezeigt. Nicht zuletzt verdeutlichen leider gerade die sich immer stärker häufenden Negativschlagzeilen über den allgemeinen ökologischen Zustand der Natur in Deutschland und weltweit (wie z. B. Biodiversitätskrise, extreme Wetterlagen, Waldsterben, belastete Grundwasser), dass eine gute ökotoxikologische Ausbildung in Zukunft von wirtschaftlicher, sozialer und ökologischer Bedeutung, und somit auch auf lange Sicht ein Berufsfeld mit nachhaltiger Zukunft sein wird. Um diesem Fachkräftemangel in der Ökotoxikologie entgegenzuwirken wurde von mir 2009 an der RWTH Aachen das Konzept des Studierendenlabors „Faszination Umwelt“ entwickelt und umgesetzt. Ziel dieses Konzeptlabors war es, Studierende an der RWTH Aachen eine exzellente Ausbildungsumgebung zu bieten und ihnen bereits früh den Einstieg in die universitäre Forschung zu ermöglichen. Das Studierendenlabor „Faszination Umwelt“ war für mich an der RWTH Aachen gerade im Hinblick auf die Entwicklung von Konzepten zur Forschungs-orientierten Lehre bedeutend. Studentinnen und Studenten können hier bereits sehr früh an die wissenschaftliche Forschung herangeführt und von erstklassigen Bedingungen für die akademische Ausbildung profitieren. In diesem Kontext wurde das Studierendenlabor "Faszination Umwelt" an der RWTH Aachen mit Spenden und Gerätedauerleihgaben mit einer Summe von über 1,5 Mio. € ausgestattet und ermöglichte im Laufe der letzten Jahre über 100 Abschlussarbeiten sowie unzählige Praktika für Studentinnen und Studenten sowie Schülerinnen und Schülern.

Aufgrund der einzigartigen Erfolgsgeschichte des Studierendenlabors "Faszination Umwelt" habe ich in meinem Forschungs- und Lehrkonzept an der Goethe-Universität Frankfurt am Main diese Idee aufgegriffen und möchte an der Goethe-Universität nun das Students' Lab: Goethe Goes Environment aufbauen. So können evolutionsbiologisches und ökotoxikologisches Praxiswissen aus erster Hand gelehrt und gelernt werden. Die hier vermittelten Inhalte umfassen dabei nicht nur wie mit modernen bioanalytischen und analytischen Verfahren retrospektive Umweltforschung betrieben werden kann, sondern auch, dass im Sinne einer Green Toxicology neue umweltfreundlichere Chemikalien und Produkte entwickelt werden können. Des Weiteren wird das Students' Lab: Goethe Goes Environment auch einen wichtigen Beitrag bei der Ausbildung von Studierenden und forschungsorientierter Lehre im Kontext der Exzellenzclusterinitiative „RobustNature“ leisten, die eine der sieben geförderten Clusterinitiativen der Goethe-Universität darstellt. In der Clusterinitiative RobustNature „Robustheit und Resilienz von Natur-Gesellschaftssystemen im sich entwickelnden Anthropozän“ soll mit einem Multiskalenansatz Prozessverständnis zum Themenkomplex Robustheit und Resilienz aus fachlich-disziplinärer, interdisziplinärer und transdisziplinärer Sicht erarbeitet werden. RobustNature befasst sich mit dem Rückgang der Biodiversität, Auswirkungen des Klimawandels und der Chemischen Belastung. In Stellungnahmen der Leopoldina und den Berichten der Internationalen Panels IPCC, IPBES und IPCP/NORMAN werden die Interaktionen und Interdependenzen dieser drei Bereiche als große Herausforderungen für die internationale Forschung in den nächsten Jahren angesehen. RobustNature will hier konkret auf die Themenfelder Wasser, Organismische Interaktionen und Systemische Risiken fokussieren, um wissenschaftliche Transformationsforschung von Natur-Gesellschaftssystemen an den Beispielen Biodiversität und Wasser zu entwickeln – vom Wissen zum Handeln. Wie kürzlich in der Frankfurter Rundschau beschrieben, soll so ein Beitrag geleistet werden, damit „Menschen um nicht nur die kommenden 50, sondern die kommenden 500 000 Jahre auf der Erde leben können“.

Wir freuen uns Ihnen hiermit den Jahresbericht des Students' Lab: Goethe Goes Environment vorstellen zu können und würden uns sehr freuen, wenn wir Sie in Zukunft auch persönlich im Studierendenlabor begrüßen können.



**Prof. Dr. Dr. h.c. Henner Hollert**



**English:**

By linking chemical and biological risk assessment, ecotoxicology makes an essential contribution to (inter)national environmental research and protection. However, as early as 2003, the Federal Environment Agency (UBA) and the German Chemical Industry Association (VCI) drew attention to the glaring shortage of well-trained young scientists. Important positions in government agencies and industry still cannot be optimally filled. As a short- to medium-term solution, the two relevant professional societies, the German Chemical Society eV (GDCh) Fachgruppe Environmental Chemistry & Ecotoxicology and the Society of Environmental Toxicology and Chemistry (SETAC) Europe - German Language Branch eV, have developed a nationwide postgraduate course to become a specialist ecotoxicologist. In the long term, however, the UBA and the VCI see an urgent need to promote the scientific subject of ecotoxicology at universities and to offer corresponding content or a corresponding course of study at German universities. Quite contrary to what the current high demand for well-trained ecotoxicologists would suggest, the current situation at German universities in this field is completely contradictory. Despite the high backlog in training and the fact that ecotoxicology is also becoming increasingly important in society, more and more chairs are being closed in the Federal Republic. Contrary to the great need for innovative research and teaching at universities, environmental chemistry and ecotoxicology have been losing support, resources and recognition for many years. However, it is crucial that environmental chemistry and ecotoxicology are rooted in academic science, and consequently provided with equipment, resources, and development prospects for the future. Also, in the National Academy of Sciences Leopoldina this negative development was recently criticized and the urgency of promoting measures was pointed out. Last but not least, the increasingly frequent negative headlines about the general ecological state of nature in Germany and worldwide (such as biodiversity crisis, extreme weather conditions, forest dieback, polluted groundwater) unfortunately make it clear that good ecotoxicological training will be of economic, social and ecological importance in the future, and thus also a professional field with a sustainable future in the long term. To counteract this shortage of specialists in ecotoxicology, I developed and implemented the concept of the student laboratory "Fascination Environment" at RWTH Aachen University in 2009. The aim of this concept laboratory was to offer students at RWTH Aachen University an excellent training environment and to enable them to enter university research at an early stage. The student laboratory "Fascination of the Environment" was significant for me at RWTH Aachen University, especially with regard to the development of concepts for research-oriented teaching. Students can be introduced to scientific research here at a very early stage and benefit from first-class conditions for academic training. In this context, the student laboratory "Fascination of the Environment" at RWTH Aachen University has been endowed with donations and equipment loans totaling more than € 1.5 million and has enabled more than 100 final theses and countless internships for students and schoolchildren over the past years.

Due to the unique success story of the student lab "Fascination of the Environment", I have taken up this idea in my research and teaching concept at Goethe University Frankfurt am Main and would now like to establish the Students' Lab: Goethe Goes Environment at Goethe University. In this way, practical knowledge in evolutionary biology and environmental toxicology can be taught and learned first-hand. The contents taught here not only include how retrospective environmental research can be carried out using modern bioanalytical and analytical methods, but also how new, more environmentally friendly chemicals and products can be developed in the sense of green toxicology. Furthermore, the Students' Lab: Goethe Goes Environment will also make an important contribution to the education of students and research-oriented teaching in the context of the Cluster of Excellence

initiative “RobustNature”, which is one of the seven cluster initiatives funded by Goethe University. In the cluster initiative RobustNature “Robustness and Resilience of Nature-Society Systems in the Evolving Anthropocene”, a multiscale approach will be used to develop process understanding on the topic complex of robustness and resilience from a disciplinary, interdisciplinary and transdisciplinary perspective. RobustNature addresses the decline of biodiversity, impacts of climate change and chemical stress. In statements of the Leopoldina and the reports of the International Panels IPCC, IPBES and IPCC/NORMAN, the interactions and interdependencies of these three areas are seen as major challenges for international research in the coming years. RobustNature wants to focus specifically on the topics of water, organismic interactions and systemic risks in order to develop knowledge-based transformation research of nature-society systems using the examples of biodiversity and water - from knowledge to action. As recently described in the “Frankfurter Rundschau”, the aim is to contribute so that “humans can live on earth for not only the next 50, but the next 500,000 years”.

We are pleased to present the annual report of the Students' Lab: Goethe Goes Environment and would be very happy to welcome you personally to the Students' Lab in the future.



**Prof. Dr. Dr. h.c. Henner Hollert**



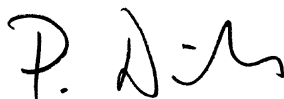
## Preface of Prof. Dr. Paul Dierkes (Academic Dean, Faculty 15)

### German:

Das Students' Lab: Goethe Goes Environment ist ein Labor, das durch Berufungsmittel von Prof. Hollert, dem Institut Ökologie, Evolution und Diversität, dem Bundesministerium für Bildung und Forschung, dem Fonds der Chemischen Industrie (FCI) und zahlreiche Industriekooperationen in den letzten beiden Jahren in der Abteilung Evolutionsökologie und Umwelttoxikologie aufgebaut wurde. Die Konzeption ist es, in unserem Fachbereich ein großartiges Labor für die Lehre und Forschung im Bereich der Umweltforschung zu etablieren. Als Studiendekan des Fachbereiches 15 Biowissenschaften der Goethe-Universität freue ich mich besonders über ein solch exzellentes Studierendenlabor. Das Students' Lab: Goethe Goes Environment bietet unseren Studierenden herausragende Möglichkeiten, sehr praxisnah im Bereich der chemikalienbasierten Umweltforschung und von multiplen Stressoren zu forschen und forschungsorientierte Lehre in das Curriculum noch besser zu integrieren. Auch wissensbasierte Transformationsforschung von Natur-Gesellschaftssystemen an den Beispielen Biodiversität und Wasser soll so im Kontext der RobustNature Exzellenzinitiative und des Profilbereiches „Sustainability & Biodiversity“ der Goethe-Universität umgesetzt werden. Das Students' Lab: Goethe Goes Environment ergänzt das Konzept des Fachbereichs 15 der Goethe-Universität, in dem auch das Goethe BioLab angesiedelt ist, welches sich primär an Schülerinnen und Schüler wendet. Durch die ergänzende Ausrichtung der beiden Lehr- und Forschungslabore und eine exzellente instrumentelle Ausstattung können sowohl Studierende als auch Schülerinnen und Schüler schon früh ein Verständnis und Bewusstsein für naturwissenschaftliche und umweltrelevante Fragestellungen vermittelt bekommen. Das Students' Lab: Goethe Goes Environment hilft uns die Situation in der experimentellen Ausstattung unserer Praktikumsräume zu verbessern und stellt somit ein wichtiges Element dar, unsere Forschung und Ausbildung auf ein neues Level zu bringen. Durch die schon jetzt umfangreiche Ausstattung des Students' Lab: Goethe Goes Environment kann dieses ab sofort von den Studierenden der Biologie, der Masterstudiengänge Umweltwissenschaften sowie Ökologie und Evolution, für Forschungspraktika und für den Postgradualkurs Ökotoxikologie der SETAC/GDCh und auch die Exzellenzclusterinitiative RobustNature genutzt werden. Das neue Labor wird auch bei der Weiterentwicklung des Masterstudiengangs Evolution & Ökologie hin zu einem internationalen Masterstudiengang „Biodiversity and Ecosystem Health“ eine herausragende Bedeutung haben.

Ich wünsche dem Studierendenlabor alles Gute und viel Erfolg!

Mit freundlichen Grüßen,



**Prof. Dr. Paul Dierkes**

Studiendekans des FB15 der Goethe-Universität Frankfurt





Gesamtprobe

BAR11-2022-01 - Gesamtprobe  
BAR34-11-2022-01  
Gesamtpr

181  
BAR

963

BAR10-2022-02 Gesamtprobe  
Assay

11-2022

07

**English:**

The Students' Lab: Goethe Goes Environment is a laboratory that has been established in the Department Evolutionary Ecology & Environmental Toxicology (E<sup>3</sup>T) through appointment funds from Prof. Hollert, the Institute of Ecology, Evolution and Diversity, the Federal Ministry of Education and Research, the Chemical Industry Fund (FCI), and numerous industry collaborations over the past two years. The concept is to establish a great laboratory for teaching and research in environmental science in our department. As the academic dean of faculty 15 (Biosciences) at the Goethe University Frankfurt, I am particularly pleased to have such an excellent student lab. The Students' Lab: Goethe Goes Environment offers our students outstanding opportunities to conduct very hands-on research in the field of chemical-based environmental research and multiple stressors, and to better integrate research-oriented teaching into the curriculum. Knowledge-based transformation research of nature-society systems, using the examples of biodiversity and water, will also be implemented in the context of the RobustNature Excellence Initiative and the profile area "Sustainability & Biodiversity" of the Goethe University Frankfurt. The Students' Lab: Goethe Goes Environment complements the concept of faculty 15, which is also home to the Goethe BioLab, which is primarily aimed at school students. The complementary orientation of the two teaching and research labs and excellent instrumental equipment enable both students and pupils to gain an understanding and awareness of scientific and environmentally relevant issues at an early age. The Students' Lab: Goethe Goes Environment helps us to improve the situation in the experimental equipment of our internship rooms and thus represents an important element in bringing our research and education to a new level. Due to the already extensive equipment of the Students' Lab: Goethe Goes Environment, it can be used from now on by students of Biology, the Master's programs Environmental Sciences as well as Ecology and Evolution, for research practicals and for the postgraduate course Ecotoxicology of SETAC/GDCh and also the Cluster of Excellence initiative RobustNature. The new laboratory will also be of outstanding importance in the further development of the Master's program Evolution & Ecology towards an international Master's program "Biodiversity and Ecosystem Health".

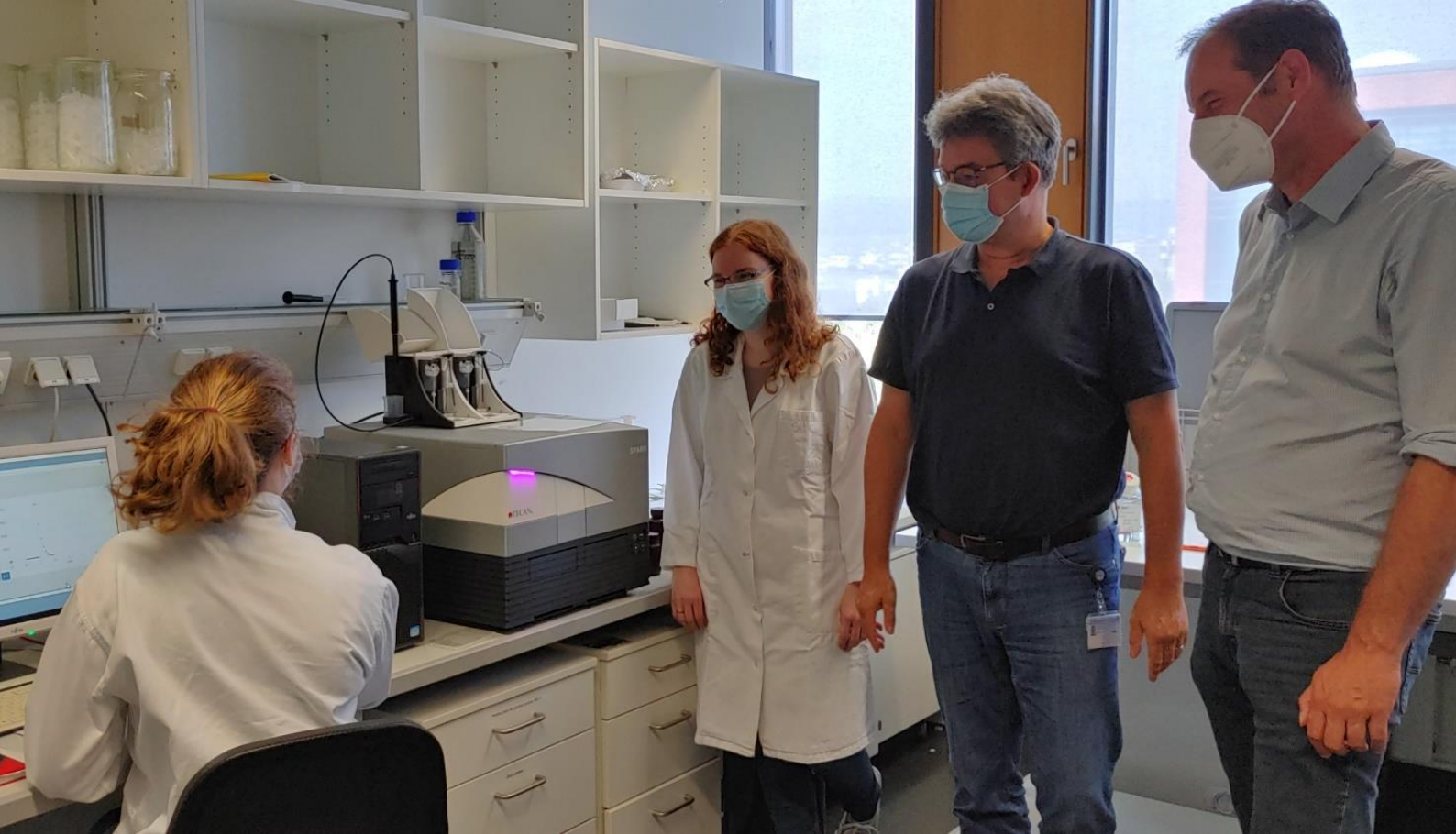
I wish the student lab all the best and much success!

Yours sincerely,



**Prof. Dr. Paul Dierkes**

Academic dean of faculty 15 of the Goethe University Frankfurt



For more information, please visit the Students' Lab: Goethe Goes Environment website using the following QR code.



*Students' Lab: Goethe Goes Environment website*

## Contents

Imprint.....	i
Preface of Prof. Dr. Dr. h.c. Henner Hollert.....	ii
Preface of Prof. Dr. Paul Dierkes (Academic Dean, Faculty 15) .....	vi
Contents .....	x
Students' Lab Partners .....	1
Scientific Cooperations.....	2
Student Impressions.....	4
International Team Members .....	5
Dr. Xingchen Zhao .....	5
Yuan Meng, B.Sc. ....	5
Teaching .....	7
Practical Course plus Film Course .....	7
Press Releases .....	8
Flood water: Toxins from the riverbed .....	8
Goethe University successful in industry open call for replacement of animal components .....	10
Sustainable bioeconomy: development of environmentally friendly bio-shampoos and plant protection agent technologies .....	12
Equipment & Devices .....	14
Investigating pesticide exposure on honeybees using ViewPoint's ToxmateLab .....	15
New (Old) Clean Bench IBS Integra Biosciences.....	16
Projects.....	18
RoadTox: Ecotoxicological assessment of tire abrasion in stormwater runoff from heavily frequented roads (2021–2025) .....	19
SensoryTox - Impairment of sensory organ development in petroleum-exposed fish early life stages .....	22
Ecotoxicological investigation and chemical characterization of the organic fraction in urban particulate matter and its influence on aquatic systems.....	24

---

Der Einfluss multipler chemischer Stressoren auf freilebende Fischpopulationen – eine Fallstudie zur kombinierten Anwendung von Biomarkern sowie Transkriptom- und Genomanalysen .....	26
NC3R – CRACK it Challenge 36: Animal-free in vitro .....	28
KONTRISOL – Konzentrate aus der Trinkwasseraufbereitung – Lösungsansätze für die technischen, rechtlichen und wirtschaftlichen Hemmnisse beim Einsatz von NF/RO-Prozessen in der Trinkwasseraufbereitung (2019–2023) .....	31
SENTINELL-II – Freshwater pollution and the links to the distribution of Schistosoma host snails in Western Kenya (2020–2023) .....	34
TeToxBeScheit – Technical and toxicological evaluation of emission reduction technologies used in small-scale wood log furnaces (2019–2022) .....	19
DreamResourceConti (2019-2022) .....	31
EfectroH2O: Effect-based Monitoring demonstrates Efficiency of Electrically-driven Water treatment Processes to Remove Salts and Micropollutants from Process Water (2020–2023) .....	35
DFG Cluster of Excellence - Fuel Science Centre (2019–2025) .....	36
Further development of the Ames fluctuation and Ames $\mu$ RAMOS assay for optimized and more reproducible mutagenicity detection (2020-2021) .....	40
PEPcat – Energieeffiziente erweiterte Oxidation zur Elimination organischer Substanzen aus Abwasser mittels plasmonisch verstärkter Photokatalyse (2019–2022) .....	42
GreenToxiConomy - Green Toxicology for a Green Bioeconomy (2019–2021) .....	44
Resurrected Daphnia as a model organism to investigate micro-evolutionary adaptations of natural populations to multiple stressors in the environment (2019–2022) .....	45
SIGN-2 – Sino-German water supply Network (2018–2021) .....	46
Effects of pesticides on aquatic and soil non-target organisms on different levels of biological organization (2019-2021) .....	48
Depth related analysis of sediment and pore-water in microcosms (2018–2021) .....	49
Publications .....	50



TEMP  
**37.0** °C  
Set 37.0 °C

100



TIMER 

-- h : -- m

End

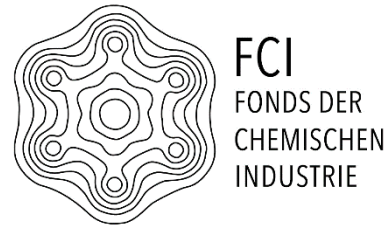


FUP

0.0

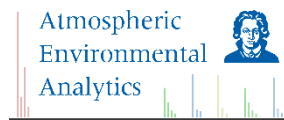
## Students' Lab Partners

The Students' Lab: Goethe Goes Environment offers students of Goethe University an excellent research facility through state-of-the-art equipment. This foundation was only made possible through intensive partnerships between academia and industry. This includes sponsoring and high discounts as well as loans. We would like to take this opportunity to express our sincere thanks to our partners.



## Scientific Cooperations

As part of the Students' Lab: Goethe Goes Environment, we promote a close network of academic and industrial cooperations in teaching and research.



BioDetection Systems







Sensortechnik & mikrobielle Anlagensicherheit



## Student Impressions

*“In the NC3R Animal-free in vitro project I had a great opportunity to gain valuable experience in the field of inventive research and human derived cell culture.”*

Julien Colas (Master’s student)

*“Thanks to the state-of-the-art technology available to me in the department, I can easily analyze multiple sublethal endpoints with the model organism Danio rerio. Here, the high-quality cameras from Leica on the microscopes and the ZebraBox, a behavioral assessment technology from ViewPoint, provide amazing high-resolution results.”*

Salina Seibold (Master’s student)

*“The Students’ Lab enables me to work with the CELENA X, a boxed microscope to image and analyze my AR CALUX® cells I am currently working with. This opens the opportunity for me to work very targeted on reducing the animal compounds from in vitro test methods.”*

Florian Jünger (Bachelor’s student)



## International Team Members

With respect to our broad international cooperations, we regularly invite international students and researchers to our lab. We are proud to welcome two new team members from China, Xincheng and Yuan.

### Dr. Xingchen Zhao

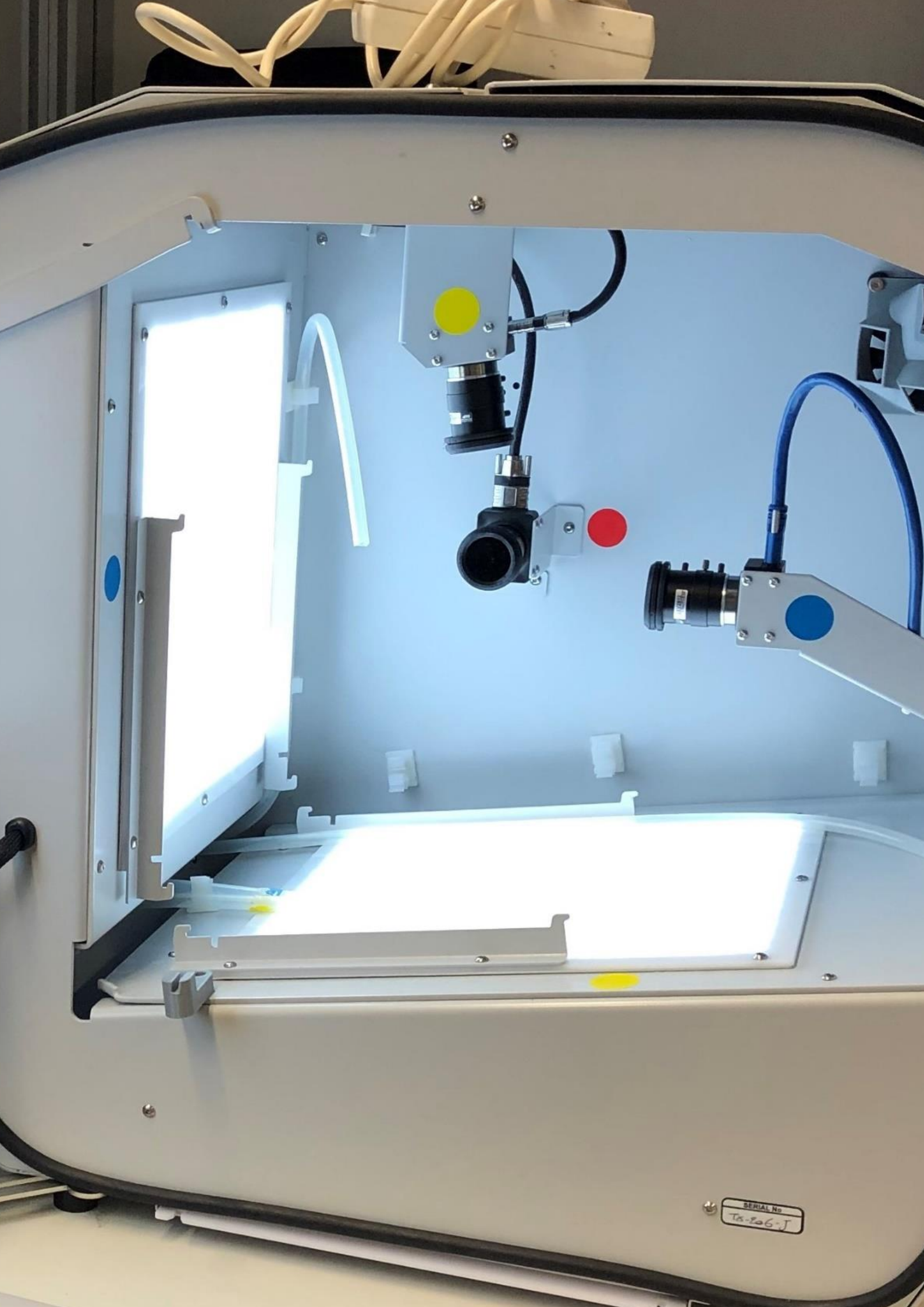
Xingchen is a postdoctoral researcher from China. He obtained his Ph.D. degree in 2017 from the Chinese Academy of Science in Beijing. He moved to Hong Kong to pursue his postdoctoral research career. In 2021, Xingchen obtained the highly regarded Humboldt fellowship and continues his research at the Department Evolutionary Ecology & Environmental Toxicology (E<sup>3</sup>T). His primary research interests include developing models for the risk assessment of emerging pollutants in aquatic and terrestrial ecosystems and identifying relevant mechanisms. Current research concerns the impact of microplastics and persistent organic pollutants on trophic cascade strength and aquatic biodiversity, with algae as a particular focus.



### Yuan Meng, B.Sc.

Yuan is a PhD student from Tongji University in China, majoring in environmental science. She has been working as an exchange student in the “Forschungszentrum Jülich” and at E<sup>3</sup>T since 2021. Yuan’s research interests focus on the identification and toxicity of organic pollutants in the environment, with special respect to the pulmonary bioaccessibility and toxicity based on oxidative stress and inflammatory reaction of organophosphate ester (OPE). Currently, Yuan is working on the metabolism of typical OPEs simulated by electrochemistry and S9 fraction as well as the cytotoxic potential before and after metabolism. Here at E<sup>3</sup>T, she will perform a broad array of different *in vitro* assays, such as the Neutral Red Retention assay, various CALUX<sup>®</sup> systems, micro-EROD and the micronucleus test.





SERIAL No  
T8-206J

## Teaching

One major aspect of the Students' Lab Goethe Goes Environment is the hand-to-hand teaching that facilitates strong didactics and provides an excellent environment for Bachelor's, Master's and PhD candidates.

### Practical Course plus Film Course

A thing we are particularly proud of is that despite all ongoing Corona-restrictions we again were able to offer a full-scale in-person practical course for our Master's program in early summer. A balanced mixture of field work with *in situ* water quality assessment, invertebrate taxonomy and ecology and our departments bioanalytical profile including introductions to fish embryo toxicity assessment, behavioral analyses, *in vitro* toxicity testing and evolutionary ecology and toxicology enabled the students to dive deep into our ecotoxicological everyday live. An extensive virtual introduction to environmental chemistry and analytics was given by our associates from the Helmholtz Centre for Environmental research (UFZ) and with lectures given by our recently assigned Professor Dr. Werner Brack, head of the Department of Effect-Directed Analysis. To complete the program, Wolfgang Kübel from Berlin-based Rixdorf Film Studios offered again a comprehensive side-course in developing media competencies for young scientists in front and behind the camera. We firmly believe that few things in the world of academic teaching are as important as hands-on and personal practical experiences. With an average evaluation of 5.8 out of 6 possible points given by our students we are incredibly happy that our department's mutual effort put into our courses pay off and excite and motivate our students for environmental sciences!



## Press Releases

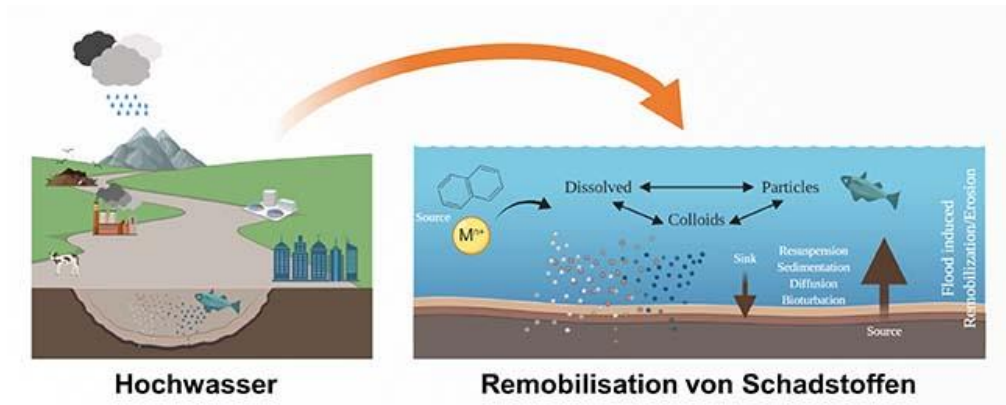
### Flood water: Toxins from the riverbed

Press release of the Goethe University Frankfurt

**A long-term hazard from flood water is often underestimated: The raging rivers swirl up pollutants out of their sediments that stem from environmental pollution decades or centuries ago. Such harmful substances can not only cause ecological damage in the river. They can also deposit themselves on flooded areas and affect crops, grazing livestock and humans. This has been pointed out by an international research team in a review of scientific studies on flood events throughout the world. The paper has been published in the Journal of Hazardous Materials and was produced under the leadership of Goethe University.**

Sediments are regarded as a river's long-term memory. They mainly comprise particles that are eroded from the ground, ending up at some point in river deltas or the sea. However, sediments can also remain stable for a relatively long time – and bind pollutants which, for example, have entered the rivers through mining or industrial wastewater. As a consequence, many old river sediments contain pollutants as “chemical time bombs”, such as heavy metals or dioxins and dioxin-like compounds that are not easily degradable. During flood events in the more industrial regions of Europe, North America and Asia, old sediments can be churned up as a result of the high speeds at which the water is flowing. In the process, the pollutants bound in them are regularly released in one go and contaminate flooded areas. An interdisciplinary team of researchers from Goethe University, RWTH Aachen University and the University of Saskatchewan in Canada, along with other partners, has compiled a review of previous scientific studies on this topic. In it, the researchers, headed by junior research group leader Dr Sarah Crawford in Frankfurt and Canadian researcher Professor Markus Brinkmann, show, for example, which pollutant loads were measured after various flood events, which test systems were developed for different pollutants and how different sediments behave when water flows at high speeds. It describes the risks for drinking water production, the influence of temperature on pollutant intake by fish and methods for assessing the economic costs associated with the remobilization of pollutants.





The remobilization of pollutants from sediments during severe flooding is a so far underestimated consequence of extreme events. Graphics: Crawford, S. et al. (2021) J. Haz. Mat.

Despite the many years of research on this subject, Henner Hollert, professor of environmental toxicology at Goethe University and senior author of the publication in hand, is greatly concerned: “I have the impression that the problem of pollutants from old sediments is greatly underestimated in Germany and also in Europe as a whole. One reason for this could also be that to date there have been practically no studies at all on the economic consequences of this problem, as we’ve been able to show. However, contaminated sediments are a ticking time bomb that can explode each time there’s a flood. What we need now is good river management across the board that not only looks at immediate hazards for humans, animals and infrastructure but also at the long-term consequences resulting from pollutants in the riverbeds. It’s imperative, for example, that we examine flooded areas used agriculturally for river-specific pollutants so that these do not end up on our plates in the form of meat and dairy products.”

In an interdisciplinary approach, researchers from Goethe University Frankfurt, in collaboration with RWTH Aachen University, the University of Saskatchewan in Canada, the Helmholtz Centre for Environmental Research in Leipzig, the Institute for Social-Ecological Research (ISOE), the Senckenberg Institute, the LOEWE Centre for Translational Biodiversity Genomics and many other partners, are also studying the recent extreme flood events in Rhineland-Palatinate and North Rhine-Westphalia in terms of hydraulic engineering and the biological, ecotoxicological, ecological, geoscientific but also the social-ecological and economic consequences. These studies are embedded in the new research cluster RobustNature at Goethe University, which is examining the robustness and resilience of nature-society systems in the changing Anthropocene and aims to contribute to knowledge-based transformation research using the examples of biodiversity and water – that is, from knowledge to action.



## Goethe University successful in industry open call for replacement of animal components

Press release of the der Goethe University Frankfurt

**While many studies take place in a petri glass in toxicology research, for some processes there is still a need for animal components such as serum or liver cell tissue. A team of researchers headed by Goethe University now seeks to develop a new cell culture technique to replace the use of animal components. Their project won the “CRACK IT” innovation challenge by NC3Rs, a British organization that works to reduce reliance on animal models in research. The challenge is sponsored by AstraZeneca and Unilever.**

Studies using cell cultures are necessary in toxicology research because they make it possible to test whether new substances exhibit undesirable effects. In these studies, the serum of unborn calves (Fetal Calf Serum, FCS) is often used as animal component in the cell cultures. Other *in vitro* toxicity tests also frequently use components of animal origins. The livers of laboratory rats, for example, are used to create an enzyme cocktail that helps investigate whether liver enzymes transform the substance being tested into toxic products.

Pharma producers and companies in the cosmetic industry want to find substitutes for both components, serum and liver tissue. The reasons are not only ethical nature. Tissue and serums that are taken directly from animals also introduce inaccuracies, as their composition varies depending on origin. In addition, not all components, including those of fetal calf serum, are known. That jeopardizes the reproducibility of the results. In the “CRACK IT 36: Animal-free in vitro” challenge, products of animal origin are therefore to be replaced by precisely defined and reproducible alternatives.





## **No more animal components in cell culture nutrient solutions**

Prof. Henner Hollert und Dr. Andreas Schiwy from the Department for Evolutionary Ecology and Environmental Toxicology at Goethe University and the LOEWE Centre TBG, together with the environmental toxicologist Prof. Beate Escher from the Helmholtz Centre for Environmental Research in Leipzig (UFZ) and the companies Biodetection Systems in Amsterdam and Scinora in Heidelberg seek to find alternatives to these animal components.

In a first step, chemically defined nutrient solutions for cell cultures will be developed – without animal components. These nutrient solutions are already common in drug manufacturing, not least for safety reasons, as they eliminate the risk that diseases such as BSE (bovine spongiform encephalopathy) are transmitted through the calf serum. Up to now, there have been only very few such systems for toxicological testing, because the amounts required are low in comparison with pharmaceutical production. To develop them, the metabolic processes of the cells must be known in detail.

## **Dispensing with laboratory rats**

In a second step, the researchers want to replace the enzyme cocktail from laboratory rats by having liver cell lines metabolize the substances to be tested instead. The liver cell lines are to be grown under chemically defined culture conditions. Subsequently, the metabolic products will be extracted and their effect tested in the adapted toxicological cell cultures that were developed in the first step.

Hollert and his team will first test the process on the model substance benzo[a]pyrene, a substance also found in cigarette smoke. Benzo[a]pyrene is transformed into toxic substances in the human liver, which causes damage to cell DNA and impairs hormonal balance. Funding during the first phase amounts to 100,000 pounds, or about 114,000 euros. Following a successful evaluation, the researchers can apply in the same year for a second phase of the challenge, in which the equivalent of about 685,000 euros over another three years may be awarded.

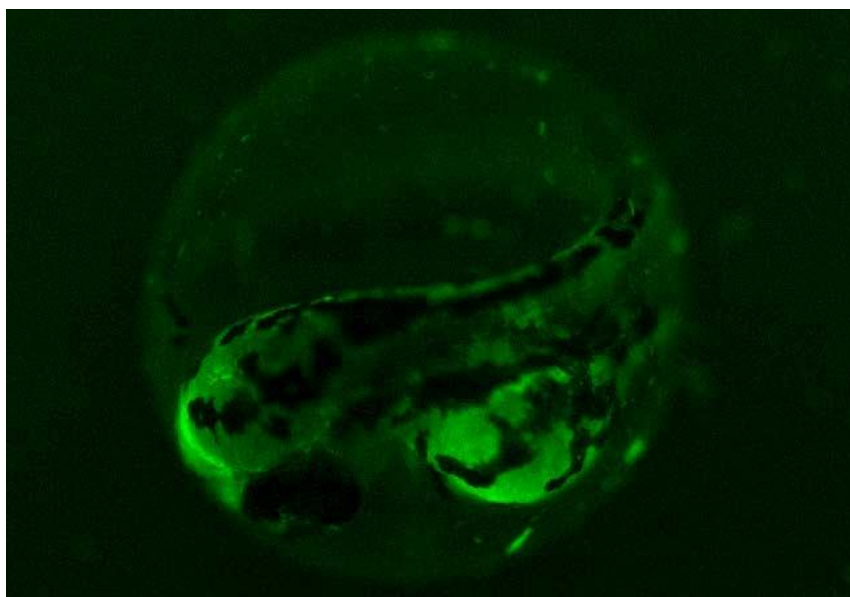


*Press release of the Goethe University Frankfurt*

## Sustainable bioeconomy: development of environmentally friendly bio-shampoos and plant protection agent technologies

Press release of the der Goethe University Frankfurt

**With the early assessment of sustainable, newly developed chemicals and products it is possible to assess a potential risk of toxic substances being released at a later point in product cascades. This has been revealed in a proof-of-concept study jointly coordinated by Goethe University Frankfurt and RWTH Aachen University. In the course of the study the toxicity of sustainable biosurfactants, potentially applied in, e.g., bio-shampoos, and of a new technology for the economical deployment of plant protection agents were analyzed using a combination of computer modelling and laboratory experiments. The study is the first step towards a safe bioeconomy from an eco-toxicological stance, and which uses sustainable resources and processes to reduce environmental burdens significantly.**



The natural resources of the planet are running short, yet at the same time they are the basis for our prosperity and development. A dilemma which the EU intends to overcome with the aid of its revised bioeconomy strategy. Rather than relying on fossil-based materials, the economy is to be based on renewable materials. These include plants, wood, microorganisms and algae. At some point in time everything is to be found in closed loops, yet the implementation of a circular bioeconomy requires a shift in the manufacture of chemicals. These also have to be produced from bio-materials rather than crude oil. Based on these requirements the American chemists Paul Anastas and John C. Warner formulated their twelve principles of green chemistry in 1998. One of their principles has very much been neglected to date, however: the reduction of the environmental toxicity of newly developed substances.

It is precisely here that the interdisciplinary project “GreenToxiConomy”, which is part of the scientific alliance Bioeconomy Science Center (BioSC), comes into play. The objective was to examine bio-based substances and innovative technologies with a view to their toxic impact on the environment at an early stage in product development and to incorporate the resulting findings into product design. Project partners from Aachen, Jülich and Düsseldorf provided two of their bio-based product candidates for the analyses: microgel containers for crop protection agents and biosurfactants.

The wash-active biosurfactants for use in shampoos and detergents at BioSC are based on the synthesis abilities of the *Pseudomonas putida* bacterium and the *Ustilago maydis* fungus, respectively, rather than on crude oil. The microgel technology allows for the controlled delivery of crop protection agents because the containers ensure that the active ingredients still adhere to the plants in the event of rain.

Dr. Sarah Johann, the lead author for the study and the head of a working group in the department of evolutionary ecology and environmental toxicology at the Institute for Ecology, Evolution and Diversity at Goethe University Frankfurt, explains: “For the analysis of novel substances and technologies we have selected a broad range of concentration to be able to adequately estimate potential hazards for humans and the environment. We wanted to examine whether the bio-based surfactants were more environmentally friendly than conventional chemical surfactants. In addition, we investigated whether the microgel containers per se induce any toxicity.”

To ensure the ecotoxicological evaluation was as precise as possible, the project team combined two elements in the determination of the toxicity: computer-aided prognoses (in silico) and experiments in the laboratory (in vitro and in vivo). The computer models work with the toxicity data of known chemicals, whose structure they compared with the structure of the new bio-based substances to forecast the toxicity. The experiments were conducted on aquatic and terrestrial organisms that represent specific organism groups, among them earthworms, springtails, water fleas and zebrafish embryos at a very early stage.

The result: both biosurfactants and microgels are highly promising candidates for use in a future bioeconomy whose products must be sustainably manufactured while not causing any environmental damage or harm to humans both during and after their utilization. “We can only make statements within certain limits, however, as the transfer of laboratory results to the reality in the open field or in other applications is complicated,” says Johann. More research is necessary for a holistic assessment of the risk potential, which is why follow-up projects are planned.

Prof. Henner Hollert, head of the evolutionary ecology and environmental toxicology department at Goethe University Frankfurt, underlines the significance of the close interdisciplinary collaboration on “GreenToxiConomy”. In the project biotechnologists and engineers jointly designed a new product, and this was evaluated during the development stages by eco-toxicologists from Goethe University together with a team at RWTH Aachen headed by Prof. Dr. Martina Roß-Nickoll. “This continuous process is the major strength of the project.” Although it is only a first step towards a bioeconomy that is safe in eco-toxicological terms, for Hollert it is already clear that eco-toxicology and green toxicology will play a key role in the plans being drawn up by the EU. “Whenever it is a question of future bio-based product development and product design, we have to clarify the consequences for humans and the environment at an early stage. In this respect our approach can provide valuable results.”



## Equipment & Devices

With its state-of-the-art equipment, the Students' Lab: Goethe Goes Environment offers an excellent research and educational atmosphere. In the following we would like to present our latest acquisitions and insight.



## Investigating pesticide exposure on honeybees using ViewPoint's ToxmateLab

### Multi-Species Behavior Monitoring



The evaluation of pesticides is based on various toxicological and efficacy-specific tests that are carried out by regulatory authorities before registration. However, new knowledge is constantly being gained about the toxicity of pesticides in honeybees and other model organisms, which are not yet covered by the conventional toxicological tests recommended in the regulatory testing guidelines. Recent studies aim to additionally investigate sublethal effects. Different behavioral and reproductive performances of honeybees are utilized for the risk assessment of pesticides, with different criteria for the test systems, such as the possibility of standardization, the simplicity of monitoring and the ecological relevance. Automated video tracking systems like the ToxmateLab of ViewPoint Behavior Technology are promising tools for the investigation of sublethal impacts of pesticides on the behavior of various organisms. The ToxmateLab is able to detect small behavioral changes on a predefined timescale, e.g., the moved distances of individuals inside the chambers, the movement speed, and inactivity intervals, which would not be noticeable by human observation. In addition, up to 48 individuals can be tracked simultaneously, which allows for higher throughput and better comparability of the data than single observation of each individual in turn by one or more experimenters. Previously, the ToxmateLab was mainly used to test aquatic organisms like *Gammarus*, but we also investigated the possibility of testing other organisms, such as honeybees.

Our first experiments with honeybees in the ToxmateLab indicated the suitability of the tracking system. One part of our study for evaluating sublethal effects of amitraz on honeybees was the walking behavior. Amitraz is used as a veterinary treatment for managed beehives (livestock) for pest control of the ectoparasitic *Varroa* mite. Initial results indicate that exposure to amitraz reduced the walking behavior compared to the negative control. We are currently repeating these experiments to validate the robustness of the data obtained.

### New (Old) Clean Bench IBS Integra Biosciences

Not every single machine and equipment we got in the Student's Lab is necessarily brand new: With rather a little bit of a nostalgic value we were excited when our clean bench from IBS Integra Biosciences moved from our former lab in Aachen to our Student's Lab at the Goethe University Frankfurt. Already back then many, many bioassays have been performed in this biosafety cabinet, including yeast estrogenic and androgenic screens (YES/YAS), Ames assays and many more requiring sterile conditions. Here in Frankfurt, our "new old bench" perfectly adds to our everyday *in vitro* bioanalytics and greatly enhances our capacities for high-throughput biotesting and enabling students to work on their research thesis. Current applications in our *in vitro* testing include amongst others the micro-EROD, different CALUX® test systems, and research on animal-free, chemically defined sera and S9 supplements.

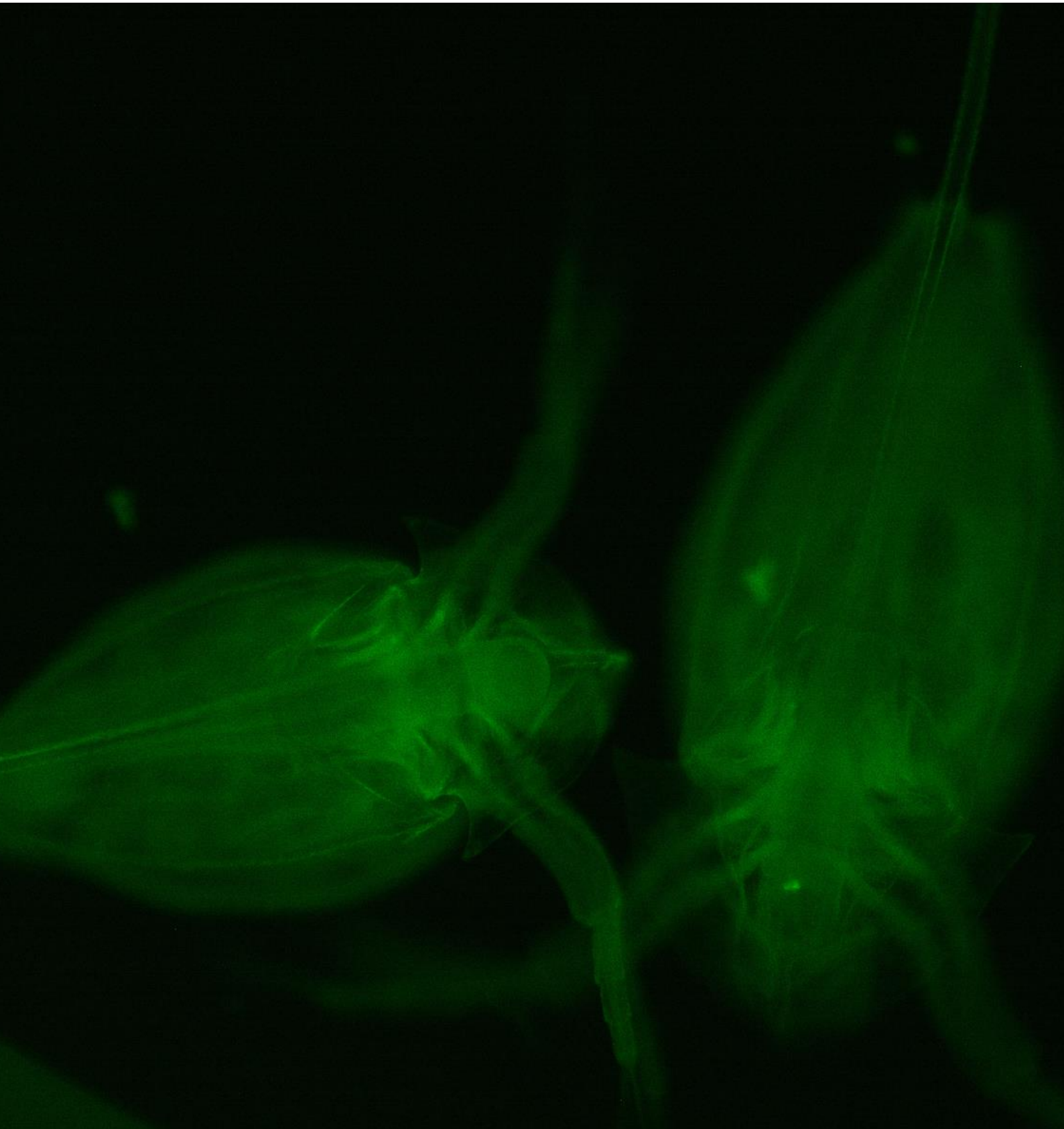


A photograph of a white wall corner. A purple LED light strip is mounted along the top edge of the wall, casting a soft purple glow. The Tecan logo, consisting of a red circle with a white dot inside, followed by the word "TECAN." in a serif font, is printed on the wall. The lighting is dim, with the purple light being the primary source of illumination.

TECAN.

## Projects

As part of the Students' Lab: Goethe Goes Environment, many national and international projects and studies are being carried out. Please find them on the following pages.





## TeToxBescheit – Technical and toxicological evaluation of emission reduction technologies used in small-scale wood log furnaces (2019–2022)

Funding:



Cooperation partners:



The increasing use of renewable resources for bioenergy leads to the question of potential implications to human health and the environment. It is well known that emissions from wood smoke contain a complex mixture of compounds, which exert adverse effects on human health and ecosystems. Detailed information on wood smoke emissions are provided, but fail to evaluate whether emission reduction technologies supplied with small-scale wood log furnaces are sufficient to efficiently remove pollutants from exhaust gases. We aim to address this question within the TeToxBescheit project. TeToxBescheit is a joint project between the Unit of Technologies of Fuels (TEER, RWTH Aachen University), the Institute for Infection Prevention and Hospital Epidemiology (University Medical Center Freiburg), the Institute for Occupational, Social and Environmental Medicine (University Hospital Aachen) and the Department of Evolutionary Ecology and Environmental Toxicology (E<sup>3</sup>T, Goethe University Frankfurt am Main) and is funded by Fachagentur Nachwachsende Rohstoffe e. V. (FNR). The main objectives are to (1) evaluate the technical efficiency of selected emission reduction technologies used in small-scale wood log furnaces and to (2) evaluate pre- and post-reduction emissions (gaseous and particulate phase) regarding relevant (eco)toxicological effects. The subproject at E<sup>3</sup>T aims to evaluate emissions from small-scale wood log furnaces regarding ecotoxicological endpoints to provide information on potential impacts on the environment, with strong emphasis on aquatic ecosystems. Samples prepared from exhaust gases will be analyzed using a bioassay battery, which was successfully applied in the joint EU project SOLUTIONS. The bioassay battery accounts for the different trophic levels fish (*Danio rerio*, fish embryo toxicity, DIN EN ISO 15088, OECD 236), zooplankton (*Daphnia magna*, inhibition of mobility, DIN EN ISO 6341) and phytoplankton (*Raphidocelis subcapitata*, growth inhibition, DIN EN ISO 8692), as well as for various mode-of-action specific effects like endocrine (ER/AR CALUX<sup>®</sup> assay, ISO 19040-3) and dioxin-like potentials ( $\mu$ EROD assay, EU 709/2014). Our goal is to provide expert opinions on the efficiency of emission reduction technologies. Additionally, the data collected may be used by regulators to provide opinions on safer operation of small-scale wood log furnaces.

## RoadTox: Ecotoxicological assessment of tire abrasion in stormwater runoff from heavily frequented roads (2021–2025)

Funding:

Ministerium für Klimaschutz, Umwelt,  
Landwirtschaft, Natur- und Verbraucherschutz  
des Landes Nordrhein-Westfalen



Cooperation partners:

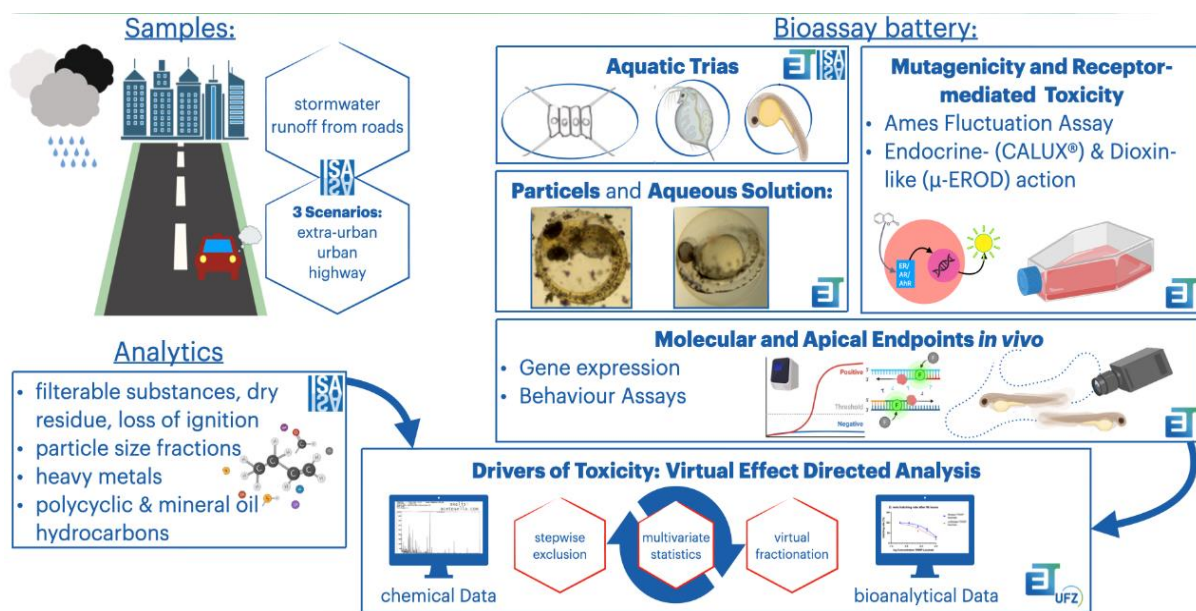


Tire and road wear particles (TRWP) have long been off the scope of aquatic environmental risk assessment. Although, over the past few years, awareness and knowledge about TRWP as part of microplastic sources have significantly increased. Recently, modelling approaches indicated an annual emission of up to 97,000 tons of TRWP per year on German roads alone, of which up to 20,000 tons may enter aquatic environment. Yet, while wastewater treatment plants were extensively studied as a sink for microplastic inputs in the past, the fate and ecotoxicological effects of tire abrasion outside these systems are insufficiently understood. Furthermore, there is a lack of data about how tire abrasion interacts with other pollutants from road runoff.

The aforementioned knowledge gaps pose particular challenges in assessing the risk for aquatic environments emerging from TRWP affected stormwaters. **(1)** Due to the high variability of the structural and chemical makeup of TRWP, no standardized sample preparation protocols for (bio-) chemical assessment are available; **(2)** only few and partly contradictory effect data investigating environmental samples or concentrations frequently measured in the environment have been reported complicating *a priori* decisions on endpoints of interest to investigate. Appreciating these challenges, comprehensively investigating the ecotoxicity of TRWP demands for a scientific bottom-up approach generating a broad knowledge base covering both chemical and biological effect information for a variety of environmental model scenarios.

In such an interdisciplinary approach, the project RoadTox—jointly managed by the institute of environmental engineering (ISA) RWTH Aachen, Germany and the department for evolutionary ecology and environmental toxicology (E<sup>3</sup>T), Goethe University Frankfurt, Germany—aims for a quantitative ecotoxicological risk assessment in stormwater runoff sampled from urban and extra-urban roads as well as federal highways. For this purpose, a comprehensive multi-endpoint bioassay

battery paired with hydrological, physical, and chemical characterization of the received runoff and its particulate matter will be performed following recent recommendations to assess complex exposure scenarios in aquatic environments. At the ISA, the runoff samples will be analyzed for various standard parameters, including particle size distribution and chemically analyzed for heavy metals (ICP-OES) and polyaromatic (PAHs) and mineral oil hydrocarbons (MKWs) (pyr-GC-MS), as well as microplastic share. Additionally, acute toxicity will be assessed in algae (*Desmodesmus subspicatus*) (E DIN 38412-59) and *Daphnia magna* (DIN EN ISO 6341). Addressed toxicological endpoints measured at E<sup>3</sup>T include dioxin-like activity (micro-EROD assay), endocrine disruption (estrogen/androgen receptor (ant-)agonism in CALUX<sup>®</sup> assay system), mutagenicity (Ames fluctuation assay), and sublethal, transcriptional and behavioral effects in *Danio rerio* embryos (DIN EN ISO 15088, extended). The received ecotoxicological information will be interactively analyzed and combined with literature data to enable a meaningful risk assessment of the input of tire abrasion into the environment. **Fehler! Verweisquelle konnte nicht gefunden werden.**



**RoadTox project outline:** ISA: Institute for Environmental Engineering, RWTH Aachen University; E<sup>3</sup>T: Dpt. for Evolutionary Ecology & Environmental Toxicology, Goethe University, Frankfurt; UFZ: Helmholtz Centre for Environmental Research.

The chosen focus of the proposed project will not be on the individual tire and road wear particles but on the overall ecotoxicological impact of the road wastewater sample. Based on the quantitative results of the input pathways and the ecotoxicological risk assessment, interdisciplinary recommendations for actions aiming to minimize tire abrasion inputs will be developed.

At the current state, several complete sample sets of stormwater runoff from the federal highway (Bundesautobahn) A4 have been acquired and are assessed in the described multi-endpoint bioassay battery. In 2021, one Bachelor's thesis and one Master's thesis have been included in the project at E<sup>3</sup>T's Students' Lab supervised by one of our Ph.D. candidates, Markus Schmitz. Current project advancements include the further exploitation of the fish embryo toxicity assay with novel non-standard endpoints investigating the road runoff toxicity in different matrices received by different runoff fractionation and extraction methods.

## SensoryTox - Impairment of sensory organ development in petroleum-exposed fish early life stages

Funding: Cluster of Excellence RobustNature



Cooperation partners:



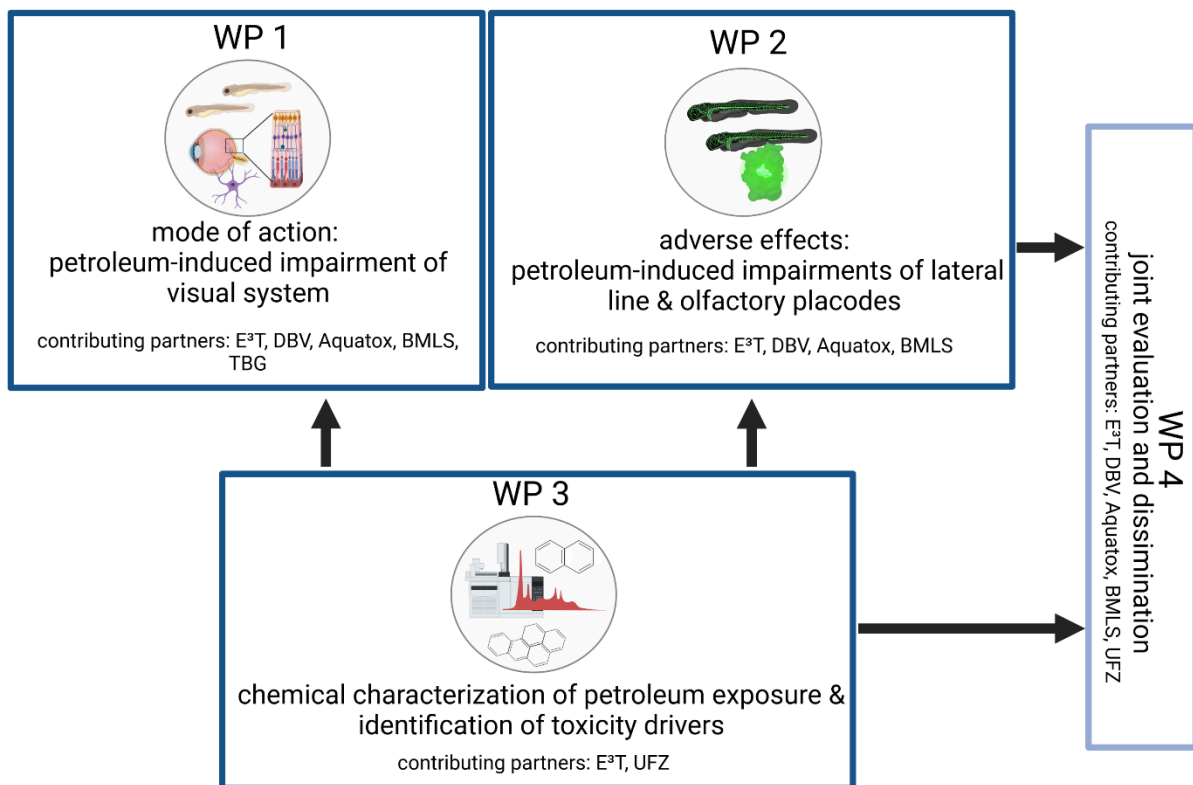
RUPRECHT-KARLS-  
UNIVERSITÄT  
HEIDELBERG



Institut für  
sozial-ökologische  
Forschung



The risk for the contamination of the environment with crude or refined oil is of high relevance not only for marine but also for freshwater ecosystems, considering incidents related to tanker collisions, offshore oil production platforms, shipping traffic or accidents in huge industry sectors or private households. The recent extreme flood event in Germany (summer 2021) is a particular example for unexpected petroleum contamination in freshwater ecosystems through thousands of tons of fuel oil released from household heating or vehicle tanks. Several previous laboratory and long-term monitoring field studies demonstrated that spilled oil can have severe consequences for the exposed biota with especially embryonic and larval stages of developing fish being highly sensitive. The toxicity of petroleum products has mainly been attributed to hydrocarbons (e.g., polycyclic aromatic compounds) causing acute as well as chronic adverse effects such as genotoxicity or carcinogenicity. Though morphological alterations and especially cardiotoxicity as modes of action are well described, the underlying diversity of molecular mechanisms and additional mechanism of toxicity such as the increasingly described impairment of the visual system are not well understood.



The overall goal of the proposed project is to investigate and provide detailed information on the oil-induced toxicity during fish embryo development with the focus on the impairment of the visual system and other sensory organs. In particular, the present project aims to:

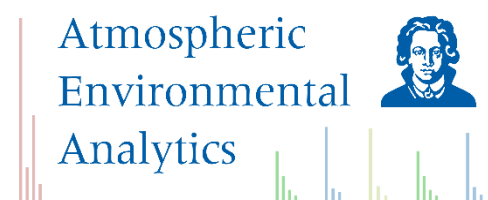
- improve the understanding of the underlying modes of action associated with the impairment on the visual system in oil-exposed fish embryos and larvae
- investigate the impact of oil exposure on other sensory organs like the lateral line and olfactory epithelium
- evaluate the role of chemical dispersion as an oil spill response measure in fish embryo toxicity

## Ecotoxicological investigation and chemical characterization of the organic fraction in urban particulate matter and its influence on aquatic systems

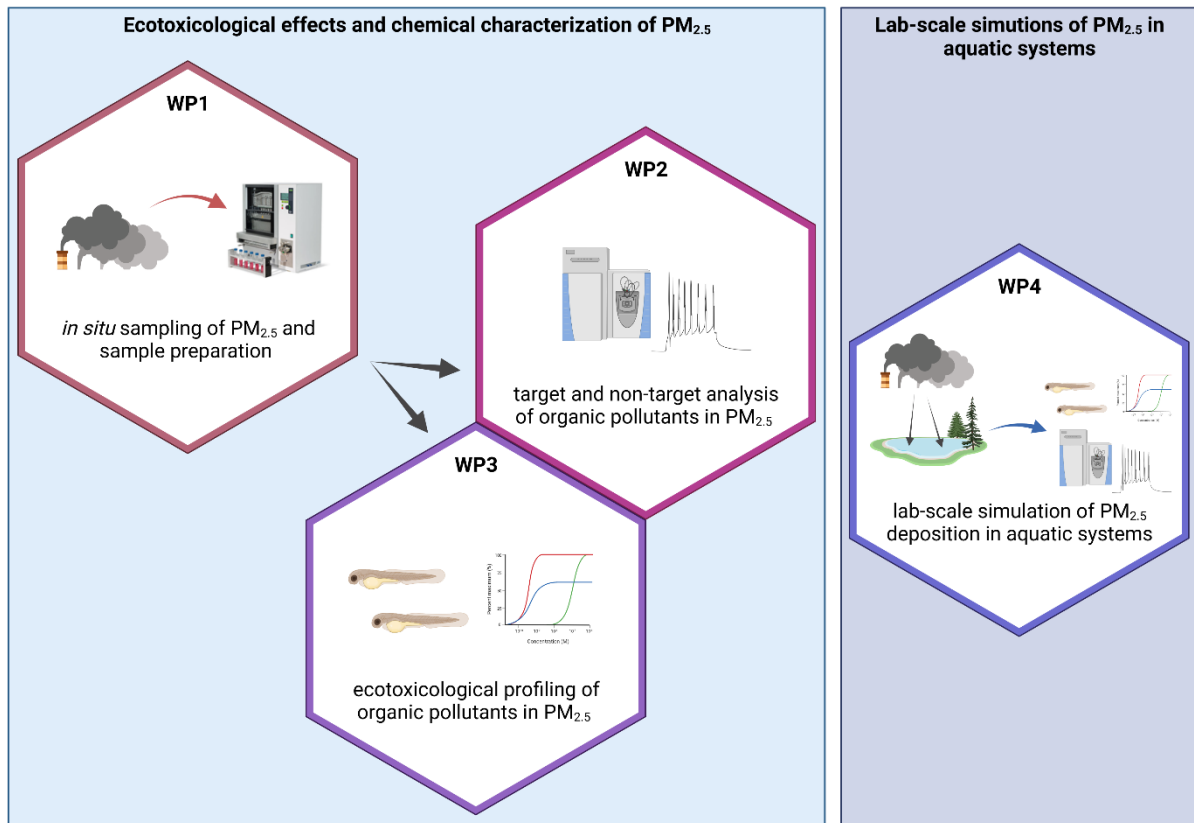
Funding: Cluster of Excellence RobustNature



Cooperation partners:



Air pollution is overall a major global problem and a serious threat for humans and the environment. In particular, PM<sub>2.5</sub>, one important fraction of airborne particulate matter (PM) has caught increasing attention in the scientific community. Due to its small size PM<sub>2.5</sub> has a wide array of exposure pathways. PM<sub>2.5</sub> consists not only of primary organic aerosols (PAOs; e.g., particles formed during incomplete combustion of organic matter) but largely of secondary organic aerosols (SAOs), organic compounds formed through oxidation of gas-phase organic compounds and condensation of pre-existing airborne particles. Although considerable resources have been spent on air pollution research and PM<sub>2.5</sub> in particular one major factor has been neglected: Its fate in aquatic systems and impact of aquatic biota. Airborne particles can enter aquatic systems by various means and components of PM<sub>2.5</sub> have shown to cause serious adverse effects in aquatic biota. However, despite decades of air pollution research there is a significant knowledge gap regarding fate, behavior and exposure of airborne PM<sub>2.5</sub> in aquatic systems. Here, we propose a proof-of-concept study that seeks to elucidate the relationship between air and water pollution on the example of urban PM after entering aquatic systems.



**Project outline:** Urban particulate matter will undergo various analyses, including chemical and ecotoxicological investigations. One major part of the project will be the lab-scale simulation of particulate matter deposition.



Der Einfluss multipler chemischer Stressoren auf freilebende Fischpopulationen – eine Fallstudie zur kombinierten Anwendung von Biomarkern sowie Transkriptom- und Genomanalysen

Funding: Cluster of Excellence RobustNature



Cooperation partners:



**UNIVERSITY OF  
GOTHENBURG**

**SENCKENBERG**  
world of biodiversity



Nowadays, water pollution patterns are characterized by a steadily growing number of pollutants that are present in complex mixtures. The ecological consequences of this environmental pollution





## NC3R – CRACK it Challenge 36: Animal-free in vitro

Funding:



# Innovation Platform

Exploiting 3Rs technologies

Cooperation partners:



**BioDetection Systems**



To improve in vitro toxicology animal derived products, need to be substituted as they introduce variability and reduce the reproducibility. Especially, the cell culture component serum is of concern as it is harvested from fetal blood of calves and its composition is not defined and various production problems can impact its quality. It has been suggested that serum quality alone can be the cause of a reproducibility crisis. Nevertheless, important OECD test guidelines (TG) are still based on media containing serum. As requested in the CRACK IT Challenge 36 animal-derived components need to be substituted with a defined and reproducible alternative. A solution has been developed in context of the biopharmaceutical production with mammalian cell lines, which applies chemically defined media (CDM) since 1980s. In these media every component is defined, and its composition is reproducible. However, this development has been conducted only for a few cell lines like CHO cells and no OECD relevant cell lines. These media have been requested by the European Centre for the Validation of Alternative Methods (ECVAM) for the development of new cell-based methods and the OECD guidance document on "Good In vitro Method Practices (GIVIMP)" additionally states the importance of CDM. In the scientific community this problem has been addressed and a database for serum-free media has been established. One partner of our consortium, Scinora, develops and commercializes CDM for established cell lines (VERO, CHO, HROC) as well for the development of new cell lines (CHOsulu-ER). The GU has also experience with this technology in context of V79 cells and liver cell lines. For phase 1 of the challenge, we propose to adapt and to cultivate the relevant human cell lines for the OECD TGs 487 (A549) and 455 (ER CALUX®, ER-GeneBLazer and CHOsulu-ER) in CDM.

In the context of effect-based methods the mammalian metabolism is an important element in in vitro toxicology that needs to be improved to achieve a realistic hazard assessment. This is of particular importance for compounds like Benzo[a]pyren (B[a]P) that show toxic effects after metabolic activation. In in vitro methods metabolic activation is simulated via animal-derived components like rat liver S9 or human organ donor derived S9. Again, these products, similar like serum, do not comply with 3R, are not defined and show high batch to batch variations. Here, we propose to substitute

animal-derived products with a biotechnologically produced cocktail of metabolic enzymes called ewoS9. The biotechnological approach follows two principles: (1) The cultivation of cells in CDM, and (2) scaling of the process by cultivating the cells in suspension. The ewoS9 has been optimized for a high cytochrome P450 production through induction with selected P450 inducers. The activity of the cytochromes is monitored via biochemical assays. A quality criterion is the Cytochrome P450 CYP1A activity determined via the EROD assay. Following, the cells are harvested, processed and, like animal derived S9, the supernatant after centrifugation is obtained as the final product ewoS9R. This product has been evaluated within in vitro methods like the micronucleus assay with V79 and KCB H2B-eGFP cells. Furthermore, ewoS9 has been evaluated with the Ames fluctuation test according to ISO 1135011. Finally, we have investigated the metabolism of B[a]P after incubation with ewoS9R. The results showed the kinetics of B[a]P metabolism is comparable to rat derived S9. The advantage of ewoS9 as biotechnological produced cocktail of metabolic enzymes is the reduced variability.





## DreamResourceConti (2019-2022)

Funding: BMBF (033RC002)



Cooperation partners:



The objective of the DreamResourceConti Project is to increase resource efficiency, broadening of the resource base as well as to improve the environmental friendliness of fatty alcohol ethoxylates, poloxamers and hard foam polyols. The results of the predecessor project “Dream Resource” lay the basis for this project where the usage of CO<sub>2</sub> as a main building block was assessed. DreamResourceConti will investigate the usage of ethylene oxide and CO<sub>2</sub> as a precursor for high-volume applications. Within this project, the biodegradability and ecotoxicity of newly synthesized polymeric surfactants will be examined using a Green Toxicology approach. A compound-specific bioassay battery will be used to investigate and compare the newly developed products relative to conventional reference products. Namely, biodegradability, toxicity tests across a range of trophic levels, toxicity tests at the sediment water interphase, and possible endocrine activity with cell-based assays will be investigated. Specifically, ready biodegradability (OECD 301F), algae inhibition test (OECD 201), acute immobilization with *Daphnia magna* (OECD 202), reproduction test with *Daphnia magna* (OECD 211), the Fish Embryo Toxicity (FET) test with *Danio rerio* (OECD 236), ER $\alpha$  CALUX<sup>®</sup> assay, Ames assay, Nf $\kappa$ B-based assays, and the Nrf2 CALUX<sup>®</sup> assay are being performed with the newly synthesized surfactants and the reference surfactants. Using this bioassay battery, a high number of compounds will be assessed and a short feedback loop established to forward the information gained through the ecotoxicological assessment to improve the developmental process and produce green products.

KONTRISOL – Konzentrate aus der Trinkwasseraufbereitung – Lösungsansätze für die technischen, rechtlichen und wirtschaftlichen Hemmnisse beim Einsatz von NF/RO-Prozessen in der Trinkwasseraufbereitung (2019–2023)

# KONTRISOL

Funding:

BMBF (02WAV1530)

FONA (W 201807-A)



Cooperation partners:



IWW ZENTRUM WASSER



LAGOTEC GMBH

Sensortechnik & mikrobielle Anlagensicherheit



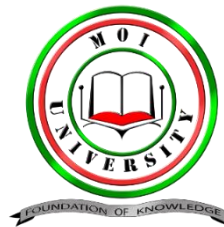
In der Trinkwasseraufbereitung werden zunehmend Membrantrennverfahren Nanofiltration und Umkehrosmose (NF und UO) eingesetzt, um z.B. die Konzentration von Härtebildnern, anorganischen Wasserinhaltsstoffen, natürlichen organischen Stoffen oder anthropogenen Spurenstoffen zu reduzieren. NF und RO trennen dabei gelöste Stoffe weitgehend ab und sind somit Alternativverfahren wie Aktivkohlefiltration oder Ionentausch deutlich überlegen, wenn es um die Entfernung polarer organischer Stoffe oder um kombinierte Verfahrensziele (bspw. Härtereduktion und Spurenstoffentfernung) geht. Bei NF/RO-Prozessen entstehen unterschiedliche Konzentratmengen mit einer entsprechend höheren Konzentration der abgetrennten Stoffe. Für einen störungsfreien Betrieb der NF/RO Trennstufe werden zudem fast immer sogenannte Scaling-Inhibitoren (Antiscalants, überwiegend Phosphonate und Carboxylate) in geringen Konzentrationen zugesetzt, die i.d.R. ebenfalls im Konzentrat verbleiben und dann über Direkt- oder Indirekteinleitung in Gewässer gelangen. In den letzten Jahren wird die Einleitung von Konzentraten in ein Gewässer allerdings durch die zuständigen Genehmigungsbehörden zunehmend kritisch betrachtet. Insbesondere dann, wenn die Konzentrate naturfremde anthropogene Spurenstoffe inklusive der zugesetzten Aufbereitungsstoffe (u. a. Polyphosphonate und -Carboxylate) oder Nährsalze in hohen Konzentrationen enthalten. Die Auswirkungen der Inhaltsstoffe von Konzentraten inkl. Antiscalants im Zusammenhang mit der Direkteinleitung insbesondere in kleine Gewässer sind nur unzureichend auf ihre Wirkungen bewertet. Daher soll die öko- und humantoxikologische Bewertung von Konzentraten bzw. behandelten Wässern sowie von bekannten Antiscalants und Mischungen aus organischen Spurenstoffen und Antiscalants in Oxidationsverfahren mit Hilfe einer Biotestbatterie untersucht werden. Dazu wird in dem Vorhaben die akute Toxizität *in vivo* gegenüber Algen, Daphnien und frühen Stadien von Zebraquärlingen (*Danio rerio*) untersucht. Als Proxies für das langfristige Schädigungspotential wird zudem *in vitro* die endokrine Wirksamkeit über den ER, AR und GR CALUX sowie den H295r Assay getestet und das mutagene bzw. genotoxische Potential (Reactive Chemicals) mithilfe des Ames-Fluktuations-Tests sowie des Mikrokerntests bestimmt. Darüber hinaus kann oxidativer Stress als wichtiger Endpunkt eines spezifischen Modes of Action getestet werden. Der Verhaltenstest mit frühen Lebensstadien von *Danio rerio* kann dabei Aufschluss über eine mögliche Beeinträchtigung des Nervensystems geben.

## SENTINELL-II – Freshwater pollution and the links to the distribution of *Schistosoma* host snails in Western Kenya (2020–2023)

Funding:



Cooperation partners:



Schistosomiasis is the second most pressing tropical disease after malaria, with 218 million people infected worldwide. It is caused by parasitic flatworms of the genus *Schistosoma* sp. which parasitize humans as their definitive host and freshwater snails of the family planorbidae as their intermediate hosts. Hot spots of schistosomiasis infections in Sub-Saharan Africa are typically characterized by extensive agriculture and heavy rainfalls. In such conditions, there is a high risk of surface run-off that washes pesticides from agricultural fields into adjacent freshwaters. This agrochemical pollution affects the macro-invertebrate community structure such that this favors highly tolerant host snails of *Schistosoma*. Currently, the global control strategies generally rely on morbidity control through human treatment with drugs that kill adult worms harbored in human hosts, but it cannot prevent the re-infection. Accordingly, re-infection from freshwater biotopes needs to be restricted. This is where the SENTINEL project comes in. In the project SENTINEL-I, we have already identified those environmental factors through field monitoring as well as laboratory experiment that increase the occurrence of the host snails – namely pesticide pollution and eutrophication. Now, in the follow-up project SENTINEL-II, we aim to understand the environmental factors that are relevant for describing the infection rate of snails. For this, we will investigate the spatiotemporal population dynamics and recolonization of host snails, and their schistosomes throughout the year. Additionally, we aim to investigate spatiotemporal variation in pesticide pollution and its association with macroinvertebrate community structure, abundance of host snails and the temporal dynamics of schistosomes in the host snails. Finally, we also aim to investigate the effect of additional environmental stressors on the dynamics of parasite burden in host snails. We will address all of the research questions through field studies as well as laboratory investigations. We anticipate that the combined results of SENTINEL-I and SENTINEL-II will draw a complete picture of the ecotoxicological processes and interrelations driving the occurrence and infection rate of the parasites and their host based on the prevailing environmental conditions.

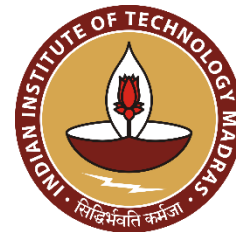


## EfectroH2O: Effect-based Monitoring demonstrates Efficiency of Electrically-driven Water treatment Processes to Remove Salts and Micropollutants from Process Water (2020–2023)

Funding: BMBF (01DQ20010A)



Cooperation partners:



This project aims to improve process water treatment in the textile industry to reduce harmful toxicological effects in the environment. In the process, process streams are to be recycled and resources recovered. The technology used for desalination and dye removal is capacitive deionization (CDI), which can be used especially for highly concentrated brines. In addition, advanced oxidation processes are used to remove micropollutants. The novel treatment technologies are being tested in a pilot plant in the textile industry in India after development in the laboratory. To verify water quality and treatment efficiency, effect-based methods (EBM) specifically adapted to textile wastewater are used to complement the chemical target analyses. The advantage of toxicological screening by EBM is that it allows a holistic statement on toxicological effects of complex mixtures typical for process waters, including unknown oxidation by-products and synergistic effects. For this purpose, a variation of bioassays will be adapted to the specific requirements of textile wastewater and transferred from Germany to India as a test battery. The project includes the Sustainable Development Goal 6 of the United Nations Environment Programme to ensure the availability and sustainable management of water and sanitation for “all”, as recycling of process water can reduce water consumption in water scarce regions like India.

## DFG Cluster of Excellence - Fuel Science Centre (2019–2025)



Funding: DFG Excellence Initiative



Cooperation partners:

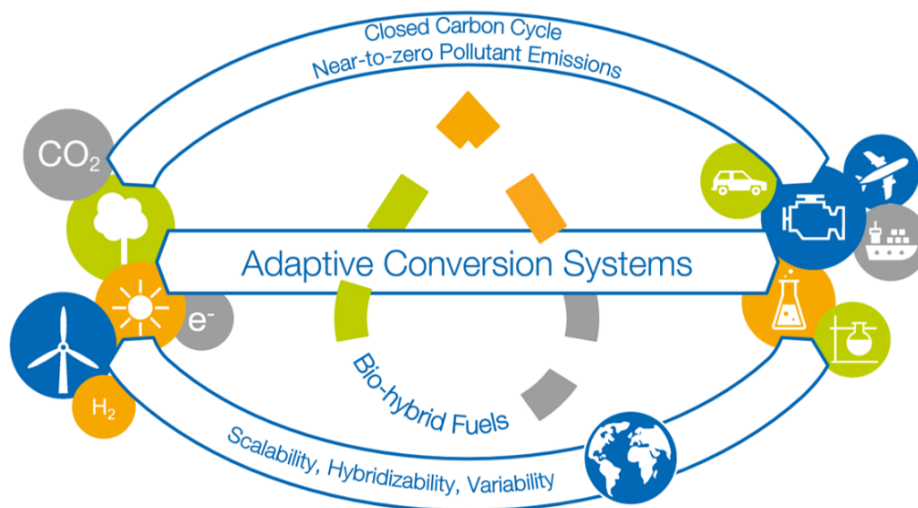


The increasing availability of non-fossil energy technologies opens unprecedented possibilities to re-design the interface of energy and material value chains towards a sustainable future. The fundamental research in the Cluster of Excellence “The Fuel Science Center – Adaptive Conversion Systems for Renewable Energy and Carbon Sources” (FSC) aims to integrate renewable electricity with the joint utilization of bio-based carbon feedstocks and CO<sub>2</sub> to provide high-density liquid energy carriers (“bio-hybrid fuels”), which enable innovative engine concepts for highly efficient and clean combustion. FSC will generate fundamental knowledge as well as novel scientific methodologies to

replace today's fossil fuel-based static scenario by adaptive production and propulsion systems that are based on renewable energy and carbon resources under dynamic system boundaries.

Current research on renewable fuels is focused on fuel replacements for present-day engine technology that are either biofuels from non-food biomass or e-fuels from CO<sub>2</sub> capture and utilization. FSC goes far beyond this approach by defining the scientific basis for the development of bio-hybrid fuels through integrated design of production and propulsion systems. The targeted technologies are adaptive to anticipate the increasing diversification of energy supply and carbon feedstock availability for a mobility sector in transformation. The (electro-)catalytic production of fuels as well as chemicals is envisaged as an important enabler for flexible and economic value chains. Molecularly controlled combustion systems are targeted to maximize efficiency and minimize emissions during the recovery of the chemically stored renewable energy. Methodological approaches will be developed to assess and ultimately predict the environmental impact, economic viability, and societal relevance of the technical developments.

FSC strengthens disciplinary competences in natural sciences, engineering sciences, and social sciences and converges them in a dynamic team science approach. Forward-integration occurs from fundamental science to the complex systems of fuel production, mobility, and transportation. Simultaneously, system-level information is propagated back by inverse methodologies to enable an integrated molecular and machine design.



FSC capitalizes on achievements of the Cluster of Excellence “Tailor-Made Fuels from Biomass (TMFB)” to act as a structuring element at RWTH Aachen University and its partner institutions. Together with the Forschungszentrum Jülich and the two Max Planck Institutes at the Campus Mülheim, a world-class research environment will be established, which is embedded in a network of strategic partnerships with globally leading research institutions and companies. Joint appointment models for junior research groups, tenure track and lighthouse professorships will create attractive career paths within the German academic landscape. The Cluster of Excellence “The Fuel Science Center – Adaptive Conversion Systems for Renewable Energy and Carbon Sources” (FSC) is an unique and interdisciplinary research cluster aiming towards the integration of renewable electricity with the joint utilization of bio-based carbon feedstocks and CO<sub>2</sub> to provide high-density liquid energy carriers (“bio-hybrid fuels”). These fuels enable innovative engine concepts for highly efficient and clean combustion for a sustainable future. Of the total number of 63 projects that are part of this cluster, two projects will be conducted in the Department of Ecosystem Analysis at RWTH Aachen University in close cooperation

with E3T at Goethe University in Frankfurt. In this context, a Junior Research Group (Dr. Miaomiao Du, “Toxicity assessment and prediction”) has been established and funded by the FSC.

### **Subprojects at RWTH Aachen University (Institute of Environmental Research) in close Cooperation with E<sup>3</sup>T at Goethe University in Frankfurt**

#### **The effects of bio-hybrid fuel exhaust on human health**

Biofuels are considered to be potential alternatives for fossil fuels due to their promising benefits, therefore the research activities on biofuels are increasing rapidly during last few years. Recently, concerns have increased regarding the potential adverse impacts of biofuels on the environment and human health. However, limited publications are available regarding the effects of biofuels or their emissions on the environment and human health. One main reason for developing renewable bio-hybrid fuels (derived from biomass and CO<sub>2</sub>) in the CoE “FSC” is to reduce the harmful effects of fossil fuel on both the environment and human health. The long-term vision is to find a sustainable approach for the production and usage of bio-hybrid fuels as well as to develop bio-hybrid fuels with no adverse effects to human health. The exhaust emissions from biofuels have been extensively characterized under field and laboratory conditions, but there are a limited number of studies regarding the effects of biofuel exhaust on human health. The goal of this project is to establish a rapid, *in vitro* and relatively inexpensive method for the early identification and characterization of adverse effects of biofuel exhaust on human health.

FSC aims to develop new and innovative technical solutions to valorize renewable energy and alternative carbon feedstocks into liquid energy carriers for CO<sub>2</sub>-neutral and near-to-zero emission propulsion systems. Our subproject will generate fundamental knowledge as well as reliable test strategies for assessing the potential effects of bio-hybrid fuel exhaust on human health. Additionally, this project will provide invaluable supporting information needed to achieve near-to-zero human health impacts from emission, and will aid in the incorporation of human health impacts into Life Cycle Assessments (LCA) for bio-hybrid fuels.

#### **Green Toxicology for the Prediction of Fuel Mixtures**

Compared to the ecotoxicological investigations of single biofuel candidates in the TMFB, this project will target the ecotoxicological investigation of biohybrid fuel mixtures and subsequent prediction of mixture effects of varying fuel mixtures. In first proof-of-concept experiments, insight on mixture effects for binary biofuel mixtures could be generated, revealing the high relevance of mixture toxicity for bio-hybrid fuels. However, the investigation of biohybrid fuel mixtures and the prediction of varying composition of biohybrid fuels differ from the approaches for investigation of environmental samples. In particular the fast and prospective prediction of varying (fuel) mixtures during the fuel development requires a novel approach and represents a challenge.

Ecotoxicological information obtained either by experiments or by predictions will then be integrated in a predictive design method for a sustainability assessment based on Life-Cycle Assessment. This combination of experimental and predictive data, as well as an LCA-based prediction method could lead to a so called “Comprehensive Environmental Assessment”, which enables an early and comprehensive evaluation of potential environmental impacts of bio-hybrid fuels.



## Further development of the Ames fluctuation and Ames $\mu$ RAMOS assay for optimized and more reproducible mutagenicity detection (2020-2021)

Funding:



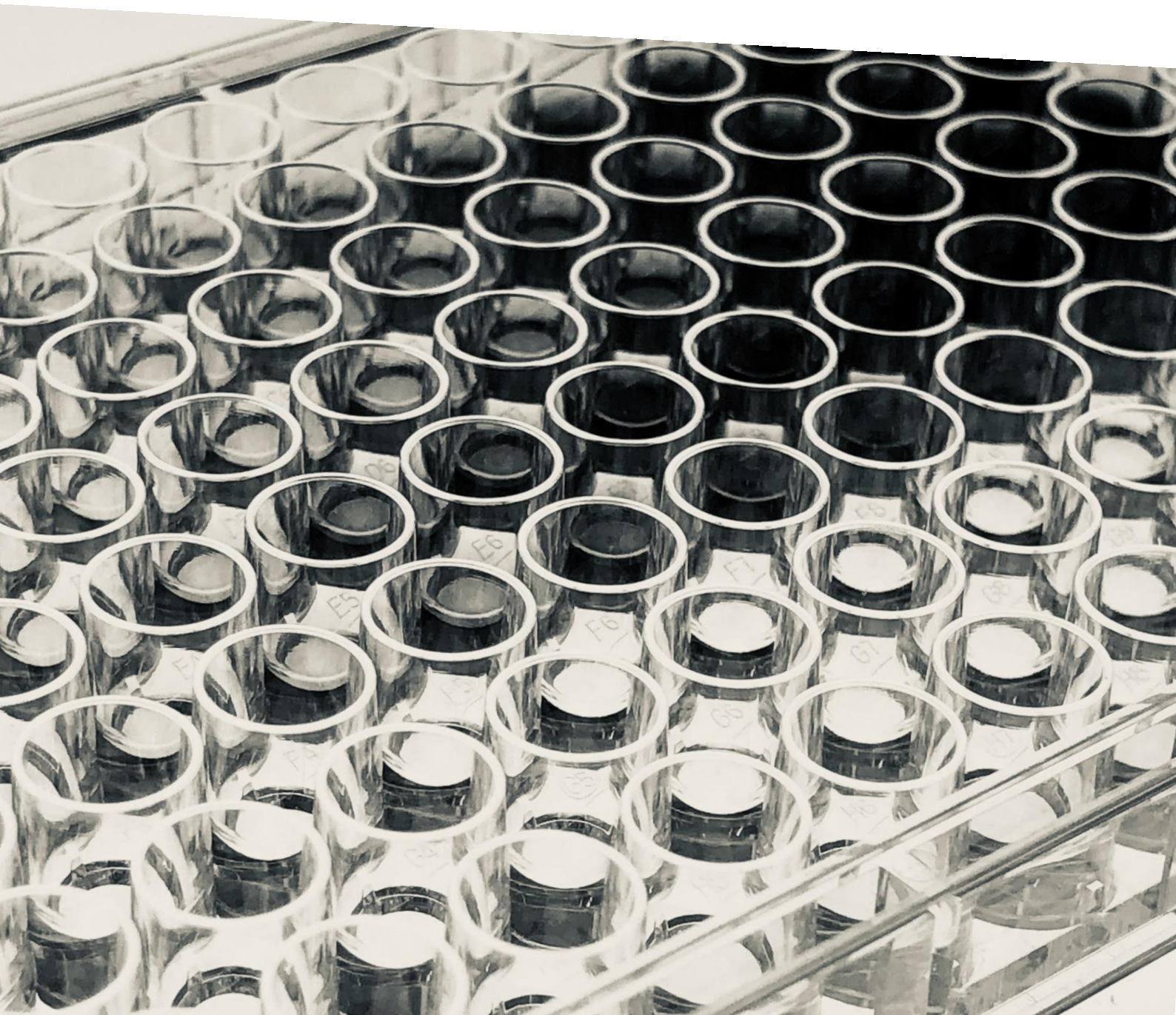
Cooperation partners:



The Ames test developed by Ames and Maron in 1971 is one of the most important bacterial tests with regard to mutagenicity. The Ames test is based on the back mutation of histidine auxotrophic bacteria (*Salmonella typhimurium*) towards histidine prototrophy. The more bacteria that reverse mutate to histidine prototrophy, the more bacteria will grow in a histidine deficient medium. Growth is detected by the detection of visible bacterial colonies on agar plates (Ames agar plate test) or using color indicators in liquid culture in microtiter plates (Ames fluctuation test). Both test methods are standardized by the OECD and ISO, respectively. Within the framework of a DBU project, a new Ames test system was developed in microtiter plates using the  $\mu$ RAMOS technology developed at the Chair of Biochemical Engineering (Ames-RAMOS project, AZ 32654). The RAMOS technology allows defined, well-mixed, non-O<sub>2</sub>-limited culture conditions and a very precise measurement of the oxygen consumption of the test microorganisms (oxygen transfer rate, OTR) of submerged microbial cultures. Based on the OTR, significant conclusions can be drawn about the metabolic activity and growth of a culture. This has already resulted in an optimized, more resource-efficient and, above all, more informative Ames test in 48-well microtiter plate format (Ames- $\mu$ RAMOS test). Mutagenicity is determined by the time frame of OTR increase due to growth of back-mutated bacteria. However, the previous project also revealed glaring deficiencies in the original Ames fluctuation assay that were equally applicable to the newly developed Ames  $\mu$ RAMOS assay. In part, these deficiencies have already been corrected in the newly developed Ames  $\mu$ RAMOS test. However, further investigations and optimizations are also required here, just as the already standardized Ames fluctuation test should be optimized with regard to these deficiencies. In order to exploit all the advantages of the Ames  $\mu$ RAMOS test, it must first be transferred from the 48-well prototype format to the 96-well  $\mu$ RAMOS system to be commercialized by Kuhner Shaker GmbH. Only in this way can the Ames  $\mu$ RAMOS test be applied on a broader basis in other laboratories. This will allow increased throughput and resource savings. In addition, the increased reproducibility already achieved in the Ames  $\mu$ RAMOS assay (Ames-RAMOS project) should also be transferred to the Ames fluctuation assay through rational preculture management without histidine carryover into the main culture. By investigating and eliminating the

batch differences of the bacterial test strains (*Salmonella typhimurium* TA 98 and TA 100) already uncovered in the Ames-RAMOS project, both the Ames- $\mu$ RAMOS test and the already standardized Ames-fluctuation test are to be made even more reproducible. The batches of test strains are to be more closely examined and standardized on the one hand by RAMOS technology (Chair of Bioprocess Engineering, RWTH) and on the other hand by molecular biological identification by means of sequencing (Teaching and Research Area Ecosystem Analysis RWTH or in future by the appointment of Prof. Hollert to the Goethe University Department of Evolutionary Ecology and Environmental Toxicology in Frankfurt via a service at a sequencing facility). New quality control systems for the test strains will be developed.

Both the optimized Ames  $\mu$ RAMOS test and the optimized Ames fluctuation test will subsequently be evaluated with regard to improved reproducibility (GU and SME Hydrotox). The results of the internal validation of the optimized Ames- $\mu$ RAMOS test at the Chair of Biochemical Engineering will be incorporated into the further commercialization of this test system. Both the optimized Ames  $\mu$ RAMOS test and the process optimization of the Ames fluctuation test are to be implemented in the existing guidelines (ISO and OECD) in the future.



PEPcat – Energieeffiziente erweiterte Oxidation zur Elimination organischer Substanzen aus Abwasser mittels plasmonisch verstärkter Photokatalyse (2019–2022)



Funding:

BMBF (02WCL1519A-E)



Bundesministerium  
für Bildung  
und Forschung

Cooperation partners:



中国科学院  
CHINESE ACADEMY OF SCIENCES



**HOLINGER**  
the art of engineering





In times of climate change extreme weather events such as long periods of drought are becoming more frequent. Therefore, water reuse gets more important. Water reuse describes the use of purified municipal or industrial wastewater that has been treated for further use and can then be applied in various areas. The potential of a possible reuse depends on the concentration of pollutants and nutrients, the volume flow, the technically and economically reasonable treatment processes and the legal framework.

In the joint project PEPcat a novel process for oxidative water purification is being developed. The aim is to increase the elimination of so-called organic trace substances, such as pharmaceutical residues, which are insufficiently retained in the normal treatment process. The project aims to develop a coating technology to enable the post-treatment of wastewater treatment plant effluents using sunlight. Thus, enabling a sustainable process in which no energy or chemicals are used. In this demonstration project plasmonically enhanced photocatalysis with sunlight will be applied for a further treatment of waste water. Experiments with waste water will be carried out at the wastewater treatment plants Aachen-Soers and Beijing Dongba. To analyse the efficiency of the wastewater technologies (UV/TiO<sub>2</sub> and Solar-TiO<sub>2</sub>) a battery of effect-based methods will be applied to examine the treated wastewater for its ecotoxicological potential. In addition to the three standard test organisms (algae, daphnia, fish), mechanism specific investigations (e.g., endocrine and mutagenic effects) will be performed.



## GreenToxiConomy - Green Toxicology for a Green Bioeconomy (2019–2021)

Funding: SEED FUND 2.0, BioSc - Bioeconomy Science Centre and MKW NRW

Cooperation partners:



The project GreenToxiConomy addresses the implementation of a GreenToxicology strategy in developmental processes of novel, and sustainable compounds that are elaborated within the context of the BioEconomy Science Center (BioSC). A GreenToxicology concept aims to evaluate the safety and environmental impacts of new products already during early steps of product development and optimization, thus enabling to focus on less toxic products without delay or financial loss. The focus of bioeconomy is to replace compounds of modern life with environmentally friendly counterparts that are based on renewable resources. Within this it is of high importance to investigate the ecotoxicological potential for such “green” chemicals, as environmental impacts cannot be excluded in principle. This project aims to develop a substance-specific and exposure-based proof-of-concept strategy for the two product categories biosurfactants (rhamnolipids, mannosylerythritol lipids, ustilagic acids) and microgel containers as well as anchoring peptides for the latter. Biosurfactants have a broad range of potential applications including in detergents, remediation or pharmacy. Microgel containers are planned as novel pesticide release systems for plant protection significantly reducing the application amount. Against this background, both substance classes have a high potential to end up in the aquatic and terrestrial environment.

The planned project approach includes as first step to develop and establish ecotoxicological test batteries for the individual product categories considering physical-chemical properties as well as the fields of application. For this literature data and in silico prediction tools will be applied to obtain initial information on toxic potencies of the substances and prioritize biological endpoints of further testing. Afterwards bioassays will be adapted to the specific compounds’ requirements before the biosurfactants and microgels will be finally investigated to evaluate their ecotoxicological potential.

## Resurrected *Daphnia* as a model organism to investigate micro-evolutionary adaptations of natural populations to multiple stressors in the environment (2019–2022)

Funding: Start-Up Postdoctoral funds; GU GRADE FOCUS Junior Researcher funds



This research examines the effects of environmental contaminants in combination with changes in temperature in natural populations of lake systems. Long-term exposure to environmental stressors can lead to genetic adaptations in exposed populations of aquatic organisms, such as *Daphnia* (Crustacea: Cladocera), which are a keystone species in lake systems (food webs). Eggs produced by *Daphnia* sp., can become dormant as a result of unfavorable environmental conditions, and can settle and be archived in sediments. Dormant eggs can be dated and hatched to produce clonal lineages (i.e., same genotypes) of historical populations (i.e., resurrection ecology). The emerging research fields of evolutionary toxicology and resurrection ecology offer powerful tools that have not previously been used to investigate changes in sensitivities and adaptive trajectories of populations exposed to multiple environmental stressors. We will examine how genotypes of clonal lineages of *Daphnia* sp. from single populations, separated through generations of evolution, differ in their response to exposure of environmental stressors. Since increased temperatures are expected to occur according to climate change scenarios, exposure of historical and recent clones of *Daphnia* to both contaminants and increasing temperatures will provide insight into the sensitivity and fitness of a keystone sp. (figure). Additional insight will be gained regarding the micro-evolutionary adaptations of genes in response to multiple stressors, to better understand future evolutionary changes of *Daphnia* in response to changing environments. Toxicological assessments and genomic data obtained from exposure of *Daphnia* populations will provide unprecedented opportunities to gain insight into long-term and potentially future evolutionary responses of a keystone sp. in the face of changing environments, providing feedback for risk assessment and future management of lake systems (i.e., collapse of grazers/impacts on upper trophic levels).

## SIGN-2 – Sino-German water supply Network (2018–2021)

Funding: BMBF (02WCL1471A-M)



Cooperation partners:



Sauberes Trinkwasser ist eine der wichtigsten Fragen der Menschheit, die sowohl ökologische als auch soziale und wirtschaftliche Fragen beinhaltet. Die chinesischen Behörden sind sich der zunehmenden Schwierigkeiten bewusst, ihre Bevölkerung, Landwirtschaft und Industrie mit sauberem Trinkwasser zu versorgen. Zur Bewältigung dieser Herausforderungen sind deutsche Erfahrungen und Technologien in China bereits heute wegen ihrer Problemlösungskapazität und ihrer technischen Zuverlässigkeit hoch anerkannt. Der Transfer deutscher Technologie nach China und gemeinsame Forschungsprojekte chinesischer und deutscher Partner aus Wissenschaft und Wirtschaft zur Suche nach neuen Lösungen für die nach wie vor bestehenden Probleme in den Bereichen Ressourcenschutz, Trinkwasseraufbereitung sowie Trinkwasserverteilung sind ständig gefragt. Die Trinkwasserqualität selbst und der sichere Transport des Trinkwassers zum Endverbraucher sind daher wichtige, aber nicht die einzigen Aspekte des beabsichtigten FuE-Vorhabens SIGN-2. Ressourcenschutz, der eine geringere Verschmutzung der Rohwasserquellen in der Zukunft impliziert, sollte zugleich auch die für die Gewinnung von sicherem Trinkwasser erforderliche Aufbereitungsintensität verringern. Kurz gesagt, die Aufgaben von SIGN-2 umfassen

- Detaillierte Betrachtung des Tai-See (Taihu) als heutzutage stark beeinträchtigte Rohwasserressource in Bezug auf die Dynamik des Schadstoffaustausches während der Wasser-Sediment-Mischprozesse, die die Rohwasserqualität erheblich beeinflussen
- Trinkwasseraufbereitung mit innovativen dicht beschichteten Membranen und optimierten (in Bezug auf Wasserqualität und Energieeffizienz) Aufbereitungsketten
- Trinkwasserverteilung mit neuer integraler Anlagenverwaltung durch Software-Tools, die Spül-, Leckerkennungs- und Ventilwartungsdaten integrieren
- Verbreitung der Erkenntnisse und Ausbildung von Personen in den beiden Schwerpunktregionen des chinesischen Mega-Wasserprogramms im Rahmen des 13. chinesischen 5-Jahres-Plans: die Regionen Taihu und Peking
- Eintritt deutscher Unternehmen in den chinesischen Markt durch Pilotdemonstrations- und Markteinführungsaktivitäten

In Deutschland werden bei dem geplanten FuE-Vorhaben sowohl Partner aus der Industrie (überwiegend KMU) als auch aus Forschungsinstituten zusammenarbeiten, um sowohl 1) eine Weiterentwicklung gegenüber dem aktuellen Stand der Technik als auch 2) die praktische Anwendbarkeit der entwickelten Lösungen zu erreichen. Kooperationspartner in China sind die führenden Forschungsinstitute sowie die zuständigen Behörden und Akteure, die die nachhaltige Umsetzung der Projektergebnisse in China sicherstellen. Die Ziele dieses Teilprojekts von SIGN2 sind: 1) die Entwicklung und Validierung der auf In-vitro Effekt basierten Methoden für die Implementierung eines biotechnologischen Metabolisierungssystems durch den Vergleich mit dem aus Tieren gewonnenen S9-Rattenleberhomogenat, und 2) die Untersuchung des umfassenden ökotoxikologischen Risikos in der Taihu-Region anhand mehrerer Proben, einschließlich Rohwasser, Sedimenten, Oberflächenwasser und Trinkwasser nach der Behandlung in einer Batterie toxikologischer und ökotoxikologischer Testsysteme mit dem neuen biotechnologischen Metabolisierungssystem und konventionellem S9. Weitere Informationen zum SIGN2-Projekt können unter dem folgenden QR-Code aufgerufen werden.

## Effects of pesticides on aquatic and soil non-target organisms on different levels of biological organization (2019-2021)

Funding:



Cooperation partners:



In recent years the usage of pesticides has been a highly debated topic. Biodiversity declines have been observed worldwide and researchers have linked these negative effects to the widespread usage of pesticides. As pesticides are directly applied in the environment in target areas but often end up in different environmental compartments, they pose a threat not only to soil but also to aquatic ecosystems. Their effects do not only include biodiversity declines but could also indirectly impact ecosystem services. As the complex effects of pesticides on non-target organisms are often not clear, it is important to investigate their potential impact on the environment more thoroughly.

Therefore, the aim of the present project is the improvement of the assessment of pesticide effects on both soil and aquatic organisms, to be able to understand their modes of action in more detail and to extend the available data for pesticides that are already in use in Germany. As a more detailed assessment however often calls for a higher amount of test organisms, this project aims to minimize the usage of laboratory animals. A comprehensive test-battery will be used and a workflow established to assess multiple sub-lethal endpoints. Namely, fluorescence-based methods for oxidative stress measurement and MXR activity detection as well as behavioral changes and biomarker measurements with a minimal number of test organisms will be used to assess the effects of commonly used pesticides (thiacloprid, esfenvalerate, prosulfocarb and dimethenamid-p).

## Depth related analysis of sediment and pore-water in microcosms (2018–2021)

Funding:



Sediments are habitats for numerous organisms like bacteria, plant and invertebrates. Therefore, the anthropogenic contamination of aquatic systems is of great interest in the framework of the EU registration process for plant protection products (PPP). Within the aquatic compartment the environmental risk assessment therefore covers not only the water body but as well the sediment.

This implementation of the aquatic environmental risk assessment is addressed by three groups in the department of Environmental Safety at Bayer (Experimental Environmental Exposure, Aquatic Organism, Exposure Modelling).

The ecotoxicological potential of PPPs on sediment dwelling organisms is examined in sediment test systems according to OECD test guideline (TG) 218 (Sediment-Water Chironomid Toxicity Using Spiked Sediment) and OECD TG 219 (Sediment-Water Chironomid Toxicity Using Spiked Water). The environmental fate and behavior of PPPs in aquatic sediment systems is investigated in studies according to OECD TG 308 (Aerobic and Anaerobic Transformation in Aquatic Sediment Systems). Calculations of the redistribution and the transport of the test item in the mentioned water-sediment microcosms can be performed using the mechanistic TOXSWA model (toxic substances in surface waters). However, up to now there is a lack of experimental data to confirm the model predictions.

In this project, we generated experimental data by using a newly developed sampling methodology enabling the depth-related analysis of sediment and pore-water. In a previous study we tested this novel methodology and compared the experimental with the modelled data. The obtained results were promising.

The mechanistic model considers the diffusion of PPPs via pore water as relevant transport process. Since the diffusion via pore-water is depended on sorption properties of the model compounds, three model compounds with low, medium and high sorption affinity were selected. Further, we are using two natural sediments providing a low and high organic carbon (used in OECD TG 308 tests) and an artificial sediment (acc. to OECD TG 218/219) to follow the spatio-temporal dynamics of the three model compounds in the different experimental approaches.

The presented project will contribute to a more realistic sediment risk assessment, as it enables the examination of a depth integrated more realistic exposure concentration. These data allow a better estimation of the real exposure concentrations for benthic organisms living predominantly on and on the upper sediment layer.

## Publications

The projects carried out in the Students' Lab: Goethe Goes Environment have also resulted in a large number of international scientific publications in 2021:

1. M., Altenburger, R., Anlanger, C., Baborowski, M., Bärlund, I., Beckers, L.-M., Borchardt, D., Brack, W., Brase, L., Busch, W., Chatzinotas, A., Deutschmann, B., Eligehausen, J., Frank, K., Graeber, D., Griebler, C., Hagemann, J., Herzsprung, P., Hollert, H., Inostroza, P.A., Jäger, C.G., Kallies, R., Kamjunke, N., Karrasch, B., Kaschuba, S., Kaus, A., Klauer, B., Knöller, K., Koschorreck, M., Krauss, M., Kunz, J.V., Kurz, M.J., Liess, M., Mages, M., Müller, C., Muschket, M., Musolff, A., Norf, H., Pöhlein, F., Reiber, L., Risse-Buhl, U., Schramm, K.-W., Schmitt-Jansen, M., Schmitz, M., Strachauer, U., von Tümpling, W., Weber, N., Wild, R., Wolf, C., Brauns, M., 2021. Disentangling multiple chemical and non-chemical stressors in a lotic ecosystem using a longitudinal approach. *Science of The Total Environment* 769, 144324. <https://doi.org/10.1016/j.scitotenv.2020.144324>
2. Brendt, J., Crawford, S.E., Velki, M., Xiao, H.X., Thalmann, B., Hollert, H., Schiwy, A., 2021. Is a liver comparable to a liver? A comparison of different rat-derived S9-fractions with a biotechnological animal-free alternative in the Ames fluctuation assay. *Science of the Total Environment* 759. <https://doi.org/10.1016/j.scitotenv.2020.143522>
3. Brendt, J., Lackmann, C., Heger, S., Velki, M., Crawford, S.E., Xiao, H.X., Thalmann, B., Schiwy, A., Hollert, H., 2021. Using a high-throughput method in the micronucleus assay to compare animal-free with rat-derived S9. *Science of the Total Environment* 751. <https://doi.org/10.1016/j.scitotenv.2020.142269>
4. Brinkmann, M., Ouellet, J.D., Zennegg, M., Buchinger, S., Reifferscheid, G., Hollert, H., 2021. Combined sediment desorption and bioconcentration model to predict levels of dioxin-like chemicals in fish. *Science of the Total Environment* 758. <https://doi.org/10.1016/j.scitotenv.2020.143891>
5. Dogruer, G., Kramer, N.I., Schaap, I.L., Hollert, H., Gaus, C., van de Merwe, J.P., 2021. An integrative approach to define chemical exposure threshold limits for endangered sea turtles. *Journal of Hazardous Materials* 420. <https://doi.org/10.1016/j.jhazmat.2021.126512>
6. Dorn, A., Hammel, K., Dalkmann, P., Faber, D., Hellpointner, E., Lamshoef, M., Telscher, M., Bruns, E., Seidel, E., Hollert, H., 2021. What is the actual exposure of organic compounds on Chironomus riparius? - A novel methodology enabling the depth-related analysis in sediment microcosms. *Chemosphere* 279, 130424. <https://doi.org/10.1016/j.chemosphere.2021.130424>
7. Esteban-Sánchez, A., Johann, S., Bilbao, D., Prieto, A., Hollert, H., Seiler, T.-B., Orbea, A., 2021. Multilevel responses of adult zebrafish to crude and chemically dispersed oil exposure. *Environ Sci Eur* 33, 106. <https://doi.org/10.1186/s12302-021-00545-4>
8. Ford, A.T., Agerstrand, M., Brooks, B.W., Allen, J., Bertram, M.G., Brodin, T., Dang, Z.C., Duquesne, S., Sahm, R., Hoffmann, F., Hollert, H., Jacob, S., Kluver, N., Lazorchak, J.M., Ledesma, M., Melvin, S.D., Mohr, S., Padilla, S., Pyle, G.G., Scholz, S., Saaristo, M., Smit, E., Steevens, J.A., van den Berg, S., Kloas, W., Wong, B.B.M., Ziegler, M., Maack, G., 2021. The Role of Behavioral Ecotoxicology in Environmental Protection. *Environmental Science & Technology* 55, 5620–5628. <https://doi.org/10.1021/acs.est.0c06493>
9. Forsten, E.C., Kauffmann, K., Schiwy, A., Hollert, H., Buchs, J., 2021. Batch-to-batch variation of Ames test results for *S. typhimurium* TA 98 and TA 100 correlates with growth kinetics. *Toxicology Letters* 350, S97–S97.
10. Ganatra, A.A., Kandie, F.J., Fillinger, U., McOdimba, F., Torto, B., Brack, W., Liess, M., Hollert, H., Becker, J.M., 2021. Calibration of the SPEAR(pesticides) bioindicator for cost-effective pesticide



- monitoring in East African streams. *Environmental Sciences Europe* 33. <https://doi.org/10.1186/s12302-021-00497-9>
11. Gundlach, M., Augustin, M., Smith, K.E.C., Kampfer, D., Paulzen, M., Hollert, H., 2021. Effects of the antidepressant mirtazapine on the swimming behaviour and gene expression rate of *Danio rerio* embryos-Is the sedating effect seen in humans also evident for fish? *Science of the Total Environment* 792. <https://doi.org/10.1016/j.scitotenv.2021.148368>
  12. Gunthardt, B.F., Hollender, J., Scheringer, M., Hungerbühler, K., Nanusha, M.Y., Brack, W., Bucheli, T.D., 2021. Aquatic occurrence of phytotoxins in small streams triggered by biogeography, vegetation growth stage, and precipitation. *Science of the Total Environment* 798. <https://doi.org/10.1016/j.scitotenv.2021.149128>
  13. Heger, S., Brendt, J., Hollert, H., Ross-Nickoll, M., Du, M.M., 2021. Green toxicological investigation for biofuel candidates. *Science of the Total Environment* 764. <https://doi.org/10.1016/j.scitotenv.2020.142902>
  14. Johann, S., Gossen, M., Mueller, L., Selja, V., Gustavson, K., Fritt-Rasmussen, J., Wegeberg, S., Ciesielski, T.M., Jenssen, B.M., Hollert, H., Seiler, T.B., 2021. Comparative toxicity assessment of in situ burn residues to initial and dispersed heavy fuel oil using zebrafish embryos as test organisms. *Environmental Science and Pollution Research* 28, 16198–16213. <https://doi.org/10.1007/s11356-020-11729-5>
  15. Johann, S., Weichert, F.G., Schröer, L., Stratemann, L., Kämpfer, C., Seiler, T.-B., Heger, S., Töpel, A., Sassmann, T., Pich, A., Jakob, F., Schwaneberg, U., Stoffels, P., Philipp, M., Terfrüchte, M., Loeschcke, A., Schipper, K., Feldbrügge, M., Ihling, N., Büchs, J., Bator, I., Tiso, T., Blank, L.M., Roß-Nickoll, M., Hollert, H., 2021. A plea for the integration of Green Toxicology in sustainable bioeconomy strategies – Biosurfactants and microgel-based pesticide release systems as examples. *Journal of Hazardous Materials* 127800. <https://doi.org/10.1016/j.jhazmat.2021.127800>
  16. Lackmann, C., Brendt, J., Seiler, T.-B., Hermann, A., Metz, A., Schäfer, P.M., Herres-Pawlis, S., Hollert, H., 2021. The Green toxicology approach: Insight towards the eco-toxicologically safe development of benign catalysts. *Journal of Hazardous Materials* 416, 125889. <https://doi.org/10.1016/j.jhazmat.2021.125889>
  17. Lackmann, C., Velki, M., Bjedov, D., Ecimovic, S., Seiler, T.B., Hollert, H., 2021. Commercial preparations of pesticides exert higher toxicity and cause changes at subcellular level in earthworm *Eisenia andrei*. *Environmental Sciences Europe* 33. <https://doi.org/10.1186/s12302-021-00455-5>
  18. Leusch, F.D.L., Hollert, H., Holmes, G., 2021. Editorial - Virtual special issue (VSI) green turtles as silent sentinels of pollution in the Great Barrier Reef - Rivers to Reef to Turtles project. *Science of the Total Environment* 757. <https://doi.org/10.1016/j.scitotenv.2020.144188>
  19. Liess, M., Liebmann, L., Vormeier, P., Weisner, O., Altenburger, R., Borchardt, D., Brack, W., Chatzinotas, A., Escher, B., Foit, K., Gunold, R., Henz, S., Hitzfeld, K.L., Schmitt-Jansen, M., Kamjunke, N., Kaske, O., Knillmann, S., Krauss, M., Kuster, E., Link, M., Luck, M., Moder, M., Müller, A., Paschke, A., Schafer, R.B., Schneeweiss, A., Schreiner, V.C., Schulze, T., Schuurmann, G., von Tumpling, W., Weitere, M., Wogram, J., Reemtsma, T., 2021. Pesticides are the dominant stressors for vulnerable insects in lowland streams. *Water Research* 201. <https://doi.org/10.1016/j.watres.2021.117262>
  20. Machate, O., Dellen, J., Schulze, T., Wentzky, V.C., Krauss, M., Brack, W., 2021. Evidence for antifouling biocides as one of the limiting factors for the recovery of macrophyte communities in lakes of Schleswig-Holstein. *Environmental Sciences Europe* 33. <https://doi.org/10.1186/s12302-021-00500-3>
  21. Mangold-Doring, A., Grimard, C., Green, D., Petersen, S., Nichols, J.W., Hogan, N., Weber, L., Hollert, H., Hecker, M., Brinkmann, M., 2021. A Novel Multispecies Toxicokinetic Modeling

- Approach in Support of Chemical Risk Assessment. *Environmental Science & Technology* 55, 9109–9118. <https://doi.org/10.1021/acs.est.1c02055>
22. Meyer-Alert, H., Wiseman, S., Tang, S., Hecker, M., Hollert, H., 2021. Identification of molecular toxicity pathways across early life-stages of zebrafish exposed to PCB126 using a whole transcriptomics approach. *Ecotoxicology and Environmental Safety* 208. <https://doi.org/10.1016/j.ecoenv.2020.111716>
  23. Müller, A.-K., Markert, N., Leser, K., Kämpfer, D., Schiwy, S., Riegraf, C., Buchinger, S., Gan, L., Abdallah, A.T., Denecke, B., Segner, H., Brinkmann, M., Crawford, S.E., Hollert, H., 2021. Bioavailability and impacts of estrogenic compounds from suspended sediment on rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology* 231, 105719. <https://doi.org/10.1016/j.aquatox.2020.105719>
  24. Muschket, M., Brack, W., Inostroza, P.A., Beckers, L.M., Schulze, T., Krauss, M., 2021. Sources and Fate of the Antiandrogenic Fluorescent Dye 4-Methyl-7-Diethylaminocoumarin in Small River Systems. *Environmental Toxicology and Chemistry* 40, 3078–3091. <https://doi.org/10.1002/etc.5181>
  25. Nanusha, M.Y., Krauss, M., Sorensen, B.G., Schulze, T., Strobel, B.W., Brack, W., 2021. Occurrence of plant secondary metabolite fingerprints in river waters from Eastern Jutland, Denmark. *Environmental Sciences Europe* 33. <https://doi.org/10.1186/s12302-021-00464-4>
  26. Reiber, L., Knillmann, S., Kaske, O., Atencio, L.C., Bittner, L., Albrecht, J.E., Gotz, A., Fahl, A.K., Beckers, L.M., Krauss, M., Henkelmann, B., Schramm, K.W., Inostroza, P.A., Schinkel, L., Brauns, M., Weitere, M., Brack, W., Liess, M., 2021. Long-term effects of a catastrophic insecticide spill on stream invertebrates. *Science of the Total Environment* 768. <https://doi.org/10.1016/j.scitotenv.2020.144456>
  27. Reinwald, H., Konig, A., Ayobahan, S.U., Alvincz, J., Sipos, L., Gockener, B., Bohle, G., Shomroni, O., Hollert, H., Salinas, G., Schafers, C., Eilebrecht, E., Eilebrecht, S., 2021. Toxicogenomic fin(ger)prints for thyroid disruption AOP refinement and biomarker identification in zebrafish embryos. *Science of the Total Environment* 760. <https://doi.org/10.1016/j.scitotenv.2020.143914>
  28. Riegraf, C., Reifferscheid, G., Moscovici, L., Shakibai, D., Hollert, H., Belkin, S., Buchinger, S., 2021. Coupling high-performance thin-layer chromatography with a battery of cell-based assays reveals bioactive components in wastewater and landfill leachates. *Ecotoxicology and Environmental Safety* 214. <https://doi.org/10.1016/j.ecoenv.2021.112092>
  29. Shao, Y., Zhu, L.Y., Chen, Z.L., Thalmann, B., Zhou, S.B., Hollert, H., Seiler, T.B., 2021. Evidence of increased estrogenicity upon metabolism of Bisphenol F-Elucidation of the key metabolites. *Science of the Total Environment* 787. <https://doi.org/10.1016/j.scitotenv.2021.147669>
  30. Svava, V., Krauss, M., Michalski, S.G., Altenburger, R., Brack, W., Luckenbach, T., 2021. Chemical Pollution Levels in a River Explain Site-Specific Sensitivities to Micropollutants within a Genetically Homogeneous Population of Freshwater Amphipods. *Environmental Science & Technology* 55, 6087–6096. <https://doi.org/10.1021/acs.est.0c07839>
  31. Weitere, M., Altenburger, R., Anlanger, C., Baborowski, M., Barlund, I., Beckers, L.M., Borchardt, D., Brack, W., Brase, L., Busch, W., Chatzinotas, A., Deutschmann, B., Eligehausen, J., Frank, K., Graeber, D., Griebler, C., Hagemann, J., Herzsprung, P., Hollert, H., Inostroza, P.A., Jager, C.G., Kallies, R., Kamjunke, N., Karrasch, B., Kaschuba, S., Kaus, A., Klauer, B., Knoller, K., Koschorreck, M., Krauss, M., Kunz, J.V., Kurz, M.J., Liess, M., Mages, M., Muller, C., Muschket, M., Musolff, A., Norf, H., Pohlein, F., Reiber, L., Risse-Buhl, U., Schramm, K.W., Schmitt-Jansen, M., Schmitz, M., Strachauer, U., von Tumpling, W., Weber, N., Wild, R., Wolf, C., Brauns, M., 2021. Disentangling multiple chemical and non-chemical stressors in a lotic ecosystem using a longitudinal approach. *Science of the Total Environment* 769. <https://doi.org/10.1016/j.scitotenv.2020.144324>

32. Weltmeyer, A., Dogruer, G., Hollert, H., Ouellet, J.D., Townsend, K., Covaci, A., Weijs, L., 2021. Distribution and toxicity of persistent organic pollutants and methoxylated polybrominated diphenylethers in different tissues of the green turtle *Chelonia mydas*. *Environmental Pollution* 277. <https://doi.org/10.1016/j.envpol.2021.116795>
33. Westhaus, S., Weber, F.A., Schiwy, S., Linnemann, V., Brinkmann, M., Widera, M., Greve, C., Janke, A., Hollert, H., Wintgens, T., Ciesek, S., 2021. Detection of SARS-CoV-2 in raw and treated wastewater in Germany - Suitability for COVID-19 surveillance and potential transmission risks. *Science of the Total Environment* 751. <https://doi.org/10.1016/j.scitotenv.2020.141750>
34. Zhou, S.B., Peng, S.C., Brack, W., Doering, J.A., Seiler, T.B., Hollert, H., 2021. Bioanalytical equivalents and relative potencies for predicting the biological effects of mixtures. *Science of the Total Environment* 763. <https://doi.org/10.1016/j.scitotenv.2020.143030>

