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PROJECT PRESENTATION

DanTox—a novel joint research project using zebrafish (*Danio rerio*) to identify specific toxicity and molecular modes of action of sediment-bound pollutants

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Abstract
Introduction The European Water Framework Directive aims to achieve a good ecological and chemical status in surface water of European rivers by the year 2015. Since sediments and particulate matter act as secondary sources for pollutants, applied sediment toxicology is perceived to play a major role for obtaining new knowledge that can contribute to successful attainment of the goal. However, the existing bioassays for sediment toxicity analyses do not provide sufficient data concerning bioavailability of environmental pollutants. In this regard, there is an urgent need to combine sediment contact assays with gene expression analysis to investigate mechanism-specific sediment toxicity.
Purpose The aim of the novel joint research project is to develop a eukaryotic test system, which can be used to investigate the ecotoxicological effects of contaminated sediments on gene expression level (DNA-array and RT-PCR). Current ecotoxicological research customarily involves a battery of bioassays to cover different toxicological endpoints (e.g., teratogenicity, genotoxicity, mutagenicity, Ah-receptor-mediated toxicity, neurotoxicity). In contrast, methods that detect alterations in gene expression offer deeper insight by elucidating how chemical exposure and/or environmental challenge affect multiple metabolic pathways leading to these particular kinds of toxic response. Gene expression profiles reflect the way cells and organisms adapt or respond to a changing environment.
Conclusion The present project aspires to increase the fundamental molecular and physiological knowledge concerning the mode of action of environmental toxicants in zebrafish (*Danio rerio*). By working with partners from the academic and research institutions as well as from industry and waterway regulations, the success of this basic research-driven joint project in terms of development and implementation of novel sediment toxicity methods will be realized.

Keywords *Danio rerio* · Genomics · Sediments · Toxicity

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1 Introduction and background

The European Water Framework Directive (EWFD) aims to achieve a good ecological and chemical status in surface water of European rivers by the year 2015. In order to fulfill this legal obligation, acquisition of new knowledge from basic research definitely plays a huge role. In particular, since sediments and particulate matters are well known for being sinks and secondary sources for pollutants, applied sediment toxicology is of major relevance in achieving the objectives set by the EWFD (Förstner 2008; Wölz et al. 2008). Article 2 of the EWF daughter directive (EC 2006) requires from the member states that the concentration of selected priority pollutants (Parts A and B of Annex I) should not increase in sediments and biota.

Previous research projects have already demonstrated that the list of priority pollutants needs to be reviewed and updated (for review see Hecker and Hollert 2009). Furthermore, the climate change induces extreme weather conditions which could remobilize and expose sediment-associated contamination (EWFD—Guidance document no. 24, Wölz et al. 2008). According to the German Strategy for Adaptation to Climate Change, the implementation of the EWFD and the Directive 2007/60/EC (Assessment and Management of Flood Risks) will necessitate the conduct of different research activities to be supported by the Federal Cabinet under consideration of different methodical aspects.

Initially, sediment quality was not integrated in the EWFD. Meanwhile, many studies on the effects of contaminated sediments on different aquatic ecosystems (e.g., Blaise et al. 2004; Chen and White 2004; Kammann et al. 2004; Verweij et al. 2004) clearly demonstrate that sediment contamination as a global phenomenon is still underestimated. Detailed studies on sediment and soil extracts from the catchment areas of several German rivers showed a clear mutagenic, genotoxic, endocrine, and dioxin-like hazard potential (e.g., Keiter et al. 2009a, b). Within ecotoxicology and sediment toxicology, a shift of focus from problems caused by acute toxic concentrations of pollutants to those caused by chronic exposure to a mixture of low concentrations of pollutants has been observed (Hollert et al. 2002). A conclusion of this observation was that specific efforts from water research, stress ecology, national legislations for water management, and the EWFD should be integrated in order to evaluate these new aspects in sediment toxicology. In particular, the detection of specific toxic effects will be of major concern within the prospective research concepts of environmental science (Chen and White 2004). Specific effect mechanisms include among others the teratogenicity, neurotoxicity, Ah-receptor mediated and associated toxicity, genotoxicity, mutagenicity, and carcinogenicity. Recently, just with few exceptions, the detection of these specific, probably irreversible adverse effects is implemented neither in conventional guidelines nor in national and international legislations.

A detailed characterization of the hazard potential of sediments is possible with different exposure scenarios using several biotest systems. The investigations of sediment extracts is a useful tool in order to simulate long-term exposure scenarios; however, following the weight-of-evidence concept the definite verification of the bioavailable fraction in sediments is only achievable with bioassays testing native sediments (e.g., Kosmehl et al. 2008; Chapman et al. 2002). Since several professional associations and international networks (i.e., Society of Environmental Toxicology and Chemistry (SETAC), European network SedNet and the European SETAC/SedNet Advisory group) claim the implementation of specific effects in test strategies and legislations, there is a need for further research in order to develop and optimize contact assays for the detection of specific effects in sediments. Previous investigations have already adopted the fish egg assay for the measurement of teratogenic and embryotoxic effects in native sediments (Hollert et al. 2003). Other studies showed that native sediments can also be used for the detection of DNA-fragmentation in single cells of *Danio rerio* (e.g., Kosmehl et al. 2008). Thus, it is possible to measure the bioavailable hazard potential of native sediments.

Nevertheless, there is still a dire need for basic research concerning the molecular mechanisms and the physiological adaptation of embryo in response to changing environmental conditions and the compensation of contamination during exposure. Since the abundance and species composition are required in the EWFD as major criteria to achieve a good ecological status, it follows that there is an urgent need for research about molecular mechanisms and physiological response. In addition, for a successful implementation of the EWFD, it is important to develop sensitive mechanism-specific methods that can define prospective statements about possible pollution sources impairing fish health. The combination of effect-oriented analyses and mechanism-specific as well as molecular test systems also provides a very promising approach for a more effective toxicity identification and toxicity reduction evaluation (Hecker and Hollert 2009).

2 Aims

Public authorities for water management require the implementation of specific hazard effects criteria in legislations; therefore, there is a need for basic research

and for the development of contact assays to measure specific effects caused by contaminated sediments. The aims of the present project are (a) to develop a suitable test system for the assessment of the bioavailable toxicants in sediments, (b) to investigate the molecular and cellular mechanisms of toxicity, and (c) to elucidate the causality of biological effects. The long-term objective of this project will be the development of a DNA-chip containing selected genes which will be a useful tool for environmental screenings (cf, Menzel et al. 2009; Yang et al. 2009). In the end, this concept should be tested for its suitability for daily use and commercial viability. Single milestones will be:

- The basic molecular and physiological fundamentals of different ecotoxicological endpoints will be investigated by exposure with contaminated sediments using *D. rerio*. Within this project, it will be clarified if and when particle-bound pollutants can be measured in embryos. In addition, the influence of polluted sediments on the molecular mechanism and time-dependent activation of the biotransformation system in *D. rerio* will be evaluated. Moreover, the time-dependent adaptation and detoxification of the embryo will be demonstrated by comprehensive expression analysis in order to clarify the mode of action of selected chemicals.
- The development of a sediment contact assay is essential for a clarification of the above-mentioned questions. At the end of the project, a suitable test concept will be available for the comprehensive detection of the bioavailable, ecotoxicological potential of particular matter using a eukaryotic test system.
- Is it possible to identify certain substance classes in sediment samples which can change the gene expression in fish embryos using a "sediment-profiling"? To answer this question, a comparison of the gene expression profile of relevant pollutants with sediment samples will be conducted.
- In order to clarify how much of the bioavailable fraction is responsible for the overall hazard potential, the different exposure routes will be compared. Based on results from sediment contact assays together with gene expression analyses, the bioavailable hazard potential of native sediments will be demonstrated. Furthermore, these results will serve the possibility to extrapolate laboratory based observations to field situations.
- Statistical comparisons (Keiter et al. 2009b) with data/sediments of other projects (SeKT joint research project; Feiler et al. 2005) will verify if sediment contact assays and gene expression analyses correlate with other effect parameters. In addition, the present project will clarify if this combination of test systems provides a prospective contribution for a holistic understanding of the hazard of aquatic ecosystems.

These milestones of the joint research project are embedded in four modules with different working packages:

1. Module bioassay: Within this part of the project, specific effects (embryotoxicity, teratogenicity, genotoxicity, mutagenicity, AhR-mediated toxicity, neurotoxicity, calcium-oscillation and histopathology) will be tested using native sediments, sediment extracts, and selected chemicals.
2. Module gene expression: Relevant genes will be identified after exposure of *Danio rerio* to sediment extracts, native sediments and fractions of an effect-directed analysis using DNA-microarrays. The expression of the identified genes will be verified by a RT-PCR in order to create a specific microarray sensor for an "expression-profiling." In a further validation step changes in the gene expression will be measured after exposure to artificial sediments spiked with mono-substances.
3. Module data assessment: The objective of this part of the project is to identify certain pollutants via biomarker responses and the expression profile using different statistical methods. Therefore, data from bioassay and gene expression investigations will be compared by, e.g., cluster- and principle-component analyses.
4. Module practical application and commercial viability: Sediment samples will be investigated by an enterprise using a battery of standardized aquatic test systems. Data from these classical test batteries provide the possibility to evaluate the sensitivity and results from specific test systems.

3 The DanTox consortium

The DanTox consortium consists of five partners including representatives from two Universities, a Research Center, two small to medium-sized enterprises, and a federal institute for waterway regulation.

- Institute for Environmental Research (Biology V), Department of Ecosystem Analysis, RWTH Aachen University: Coordination embryotoxicity, teratogenicity, genotoxicity, mutagenicity, AhR-mediated toxicity, RT-PCR and "sediment profiling"
- Aquatic Ecology and Toxicology Section, Institute of Zoology, University of Heidelberg: Live Imaging of EROD induction, neurotoxicolgy, genotoxicity, and histopathology

- Karlsruhe Institute of Technology (KIT), Institute of Toxicology and Genetics (ITG): DNA-Microarray, data assessment and "sediment-profiling"
- GAIAC—Research Institute for Ecosystem Analysis and Assessment: Data assessment and statistical support, Meta-analyses
- Hydrotox GmbH: Standardized aquatic test battery, fish egg assay, and practical application
- German Federal Institute of Hydrology (BfG) as associated partner: Evaluation of project-related products for a possible implementation within water legislation and river basin management plans. The BfG supports the project as advisory partner and on sediment sampling.

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