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Project title: Linking uptake and effects of environmental pollutants in the zebrafish embryo model

Applicant

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1. Introduction

Nowadays an enormous variety of chemical compounds is used by humans, which, often unintended, end up **in the environment as contaminants** such as industrial chemicals, pesticides, biocides or pharmaceuticals (El-Amrani et al., 2012). Exposure to these substances, even at low concentrations, can affect the **exposed organisms**. The potential to **bioaccumulate** provides an important measure for the putative toxicological risk associated with the exposure to a chemical (El-Amrani et al., 2013). Determining the internal concentration, i.e., the amount of a chemical accumulated in tissue, thus helps to understand the **chemical´s toxicity**. The uptake of substances by an organism depends on toxicokinetic processes, such as rates of diffusion or metabolic modification, which then determine time point and extent of biochemical reactions that may eventually lead to an effect. Various factors can be the cause for differences in bioaccumulation between substances and species (Escher B, Hermens J, 2004). In this project I will explore bioaccumulation of **environmentally relevant chemicals** and relate the findings with data on **toxicological effects** of the respective compounds. Experiments will be performed with embryos of zebrafish (*Danio rerio*), small tropical fish in which about 75% of the genes are similar to those of humans (ZFIN, 2011).

2. Scientific knowledge

The zebrafish embryo is a common model system in **ecotoxicology** and used to study toxicological modes of action of chemicals (Busch et al.). The uptake and **bioconcentration** potential is an important parameter which is required to be obtained for many potentially **environmentally relevant chemicals** (Escher B, Hermens J, 2004). The **OECD** guideline OECD 236 is established for toxicological tests to determine the acute or lethal effects of chemicals on embryonic stages of zebrafish. Freshly fertilized zebrafish eggs are placed in water containing the test compounds and exposed for a period of up to 96 hrs. Effects are determined after certain periods of time (24, 48, 72, 96 hrs post fertilization). Concentration of those compounds in the aquatic environment, their known **effects and toxicokinetic** studies in adult fish are an integral part of **risk assessment** also in regard to other aquatic organisms. Generally, the severity of effect „cumulates“ during exposure, i.e., effects after longer times of exposure are more severe than after shorter exposure times.

This can be related to the **toxicokinetics of the test chemical**, i.e., toxic effects occur when the internal concentration, i.e, the tissue levels, reach a certain threshold (and or cannot be counteracted by detoxification or elimination mechanisms).

Zebrafish embryos are proposed as **animal-friendly alternative model** to adult fish, for instance to determine the **bioconcentration factor** (BCF) of a chemical which currently needs to be determined using adult fish requiring at least 100 animals per test . Beyond including animal testing this procedure is costly (Scholz et al. 2013). Although it is advantageous that the procedure **can be down-scaled** requiring less material and consuming **less time** when using fish embryos, it is a challenge that much **less tissue material** is available for the analytics. Nevertheless, only recently various studies were published, in particular also from the Department of Bioanalytical Ecotoxicology at the UFZ, that show the feasibility of quantitative analytics with fish embryo tissue (Brox ,S., Ritter, A.P. et al. 2014). For obtaining sufficient material for analytics **only up to 10 embryos** need to be pooled. The obtained data reveal uptake and elimination kinetics of the tested compounds (Brox ,S., Ritter, A.P. et al. 2014)

3. Aim of the project

In this research project, uptake and **elimination kinetics** of environmentally relevant chemicals used as pharmaceuticals or pesticides will be analyzed in zebrafish embryos. The rate of the chemical´s uptake by organisms, its biotransformation and excretion are important parameters **determining the effects** observed (Scholz S et al. 2008). Chemical effects have been frequently studied in zebrafish embryos, but **there are only few data on internal concentration** as the measurement of tissue levels of chemicals has remained a challenge. Therefore, occurrence of effects in fish embryos **has not been related to toxicokinetics** so far. The aim of this project is to determine toxicokinetics of chemicals in zebrafish embryos and to relate those data to the occurrence of toxic effects. Information on toxicokinetics is **important to understand occurrence of toxic effects** in fish embryos and to extrapolate to adult fish test results in the BCF tests.

4. **Approach and Methods**

As a first step, test compounds that were identified as environmentally highly relevant will be selected based on a recent study on occurrence of chemicals in European rivers and their potential biological risk (Busch et al., 2016).

Data on toxicity of those compounds for zebrafish embryos will be retrieved from the literature or experimentally determined with the standardized zebrafish embryo assay (OECD 236)

For determining uptake kinetics by zebrafish embryos freshly fertilized zebrafish eggs will be exposed to sublethal concentrations of selected test compounds (e.g. < LC10) and eggs/embryos and water will be sampled after 3, 6, 12, 24, 48, 60 and 72 hrs of exposure for analysis of water and tissue levels. Chemical analytics will be performed using HPLC/UV or FLD or GC/MS following so far established protocols (Kühnert, Brox). Occurrence of biotransformation products might be analyzed in addition to parent compounds (Brox et al.).

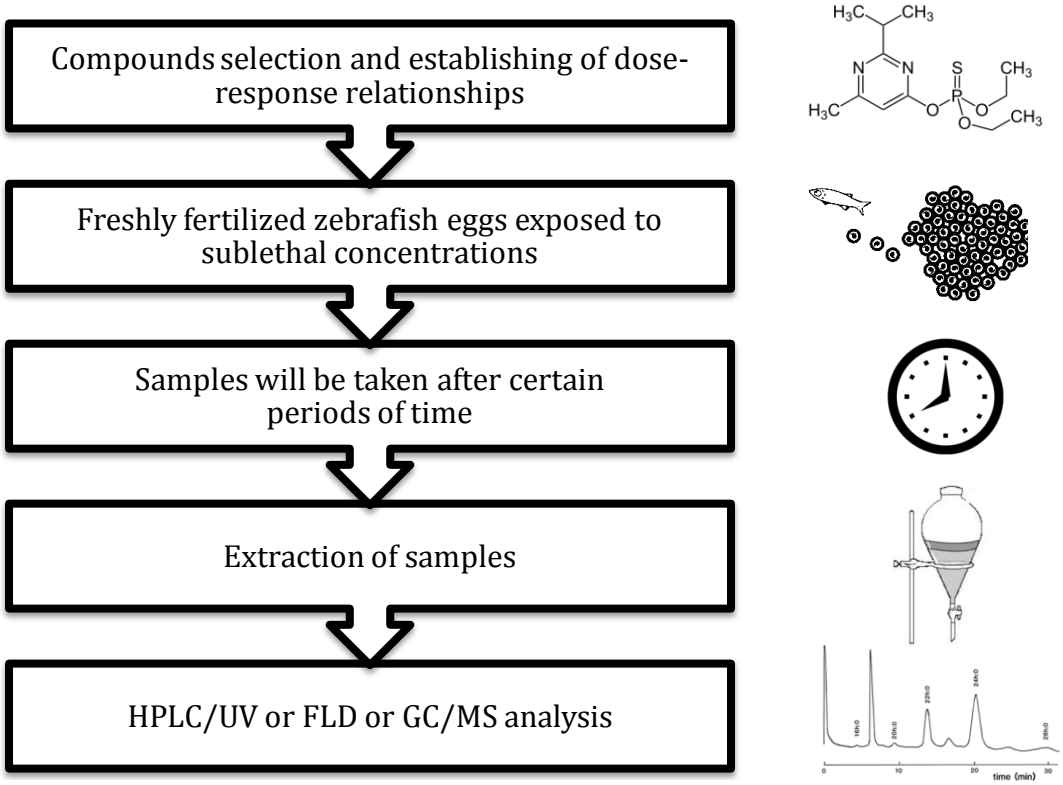

Fig. 1. Scheme of methodology of determination the internal concentration in zebrafish embryos.

5. **Relevant Literature**

Busch et al. (2016) Environ. Toxicol. Chem. 35 (8): 1887-1899;

Brox ,S. et al. (2014) Aquatic Toxicology, 157:134-140.

Brox ,S., Ritter, A.P. et al. (2014) Analytical and Bioanalytical Chemistry, 406 (20): 4831-4840.

Brox ,S. et al. (2016) Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology, 185-186, 20-28.

Kühnert et al. (2013) Environmental Toxicology and Chemistry, 32 (8), pp. 1819-1827.

El-Amrani et al. (2012) Sci. Total Environ. 425: 184–190.

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Petersen, G.I., Kristensen, P., (1998) Environ. Toxicol. Chem. 17: 1385–1395

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Scholz S et al. (2013) Regulalory Toxicology and Pharmacology 67 (3): 506-30.

Scholz S et al. (2008) Environ Sci Pollut Res (2008) 15:394–404

ZFIN Zebrafish Information Network, 2011