

Establishing and developing of an ecotoxicology platform in Serbia and Croatia: a focus on zebrafish (*Danio rerio*)

Joint research project (JRP) funded within the SCOPES 2009-2012 Programme of the Swiss National Science Foundation (SNSF)

Project Summary

This joint research project has been promoted by three partner groups from Switzerland (Prof. Dr. Karl Fent, University of Applied Sciences Northwestern Switzerland, School of Life Sciences, Muttenz and ETH Zürich, Department of Environmental Sciences), Croatia (Dr. Tvrtko Smital, Laboratory for Molecular Ecotoxicology, Ruđer Bošković Institute, Zagreb) and Serbia (Prof. Dr. Radmila Kovačević, Laboratory for Ecotoxicology, University of Novi Sad). The main project goal was capacity-building and promoting ecotoxicological research groups in Serbia and Croatia.

This overall goal has been fulfilled by performing two core project activities. The first part of the project was directed to the transfer of knowledge and expertise that will enable the Serbian and Croatian partners to improve their overall research capacity, performing part of the research using zebrafish (*Danio rerio*) and related cell lines as highly established and promising models in biomedical and ecotoxicological research. Graduate students from both countries were trained in Switzerland. A small scale zebrafish facility and equipment needed for maintenance of fish cell cultures were established at the Serbian partner laboratory. Both Serbian and Croatian partners acquired knowledge needed for performing classical zebrafish acute toxicity assays, as well as the recently established zebrafish embryotoxicity *DarT* test.

The research part of the project was directed to better understanding of critical, evolutionary conserved xenobiotic defence systems in aquatic organisms, using zebrafish as a model. Taking into account the complexity of cellular defence mechanisms contributing to the overall adsorption, disposition, metabolism, excretion, and finally toxicity of xenobiotics in aquatic organisms, in this project we were focused on the uptake of environmental chemicals and metabolites by specific transmembrane proteins, detoxification by phase I and II biotransformation enzyme systems; and finally, active elimination of xenobiotics and metabolites through another class of transmembrane proteins (ABC transporters). Our project covered the following specific research topics:

- Identification and phylogenetic analyses of zebrafish genes encoding for members of the organic anion transporting polypeptides (OATPs), phase I enzymes belonging to cytochrome P450 (CYP) 1A/3A family, phase II enzymes (glutathione-S-transferases (GSTs) and ABC efflux transporters;
- Determination of tissue and age specific expression pattern of corresponding genes in zebrafish *in vivo*, and in selected zebrafish cell lines *in vitro*;
- Development, standardization and subsequent use of research tools needed for cloning and molecular characterization of selected, highly expressed members of the OATP, CYP1A/3A, GST and ABC family;
- Evaluation of usability of acquired and/or developed *in vitro* and *in vivo* tools in determination of interactions of environmental contaminants with critical cellular defence systems.

Apart from its research context, an important novel value of this project was close collaboration of our laboratories. Thus, by using a well established model species (zebrafish) and a range of cellular model systems, we provided a unique multidisciplinary training opportunity across multiple areas of ecotoxicology. The research fields covered by this proposal include xenobiotic uptake, disposition and elimination, adverse drug and chemical effects, and finally, identification of the presence of potentially hazardous chemicals in complex environmental matrices. Therefore, by realizing this project through the described research activities, exchange of students and capacity building, we enabled a direct transfer of knowledge and expertise among involved laboratories, fostering a high quality and competitive ecotoxicological research in all partner countries.