ESTROGENIC COMPOUNDS IN WATER: ESTABLISHMENT OF A FISH-BASED SCREENING SYSTEM

Katalin Bakos *, Zsolt Csenki, Dora Bencsik, Robert Kovacs, Dora Kanaine Sipos, Yavor Hadzhiev, Balazs Kovacs, Ferenc Müller, Bela Urbanyi

Department of Aquaculture Institute of Environmental
and Landscape Management Faculty of Agricultural
and Environmental Sciences Szent Istvan University
Pater K. Street 1. H-2100 Godollo (Hungary)
bakos.katalin@mkk.szie.hu

There is ever increasing demand for new test systems enabling the detection of pollutants in waters and for replacing animals with alternatives in pharmaceutical, toxicological and basic biology research. EDCs (Endocrine Disrupting Chemicals), mostly including estrogenic substances are natural or synthetic chemicals that interfere with or mimic the effects of natural hormones causing serious developmental and reproductive disorders. Therefore the presence of estrogenic compounds in wastewater is of international consideration as they pose a potential threat to exposed fishstock, wildlife and humans.
Zebrafish (*Danio rerio*) is an excellent model organism in basic biological research and becoming a popular test tool in pharmacology and environmental toxicology. They are able to detect low toxic agent levels and besides enabling to study toxic effects exerted on fish, their genes, receptors, molecular and physiological processes are analogous to those in mammals and so to humans as well. In addition, zebrafish embryos and larvae up to the free feeding age are not considered as animals by the Animals (Scientific Procedures) Act 1986 of the UK and the relevant Directive (86/609/EEC Art. 2b) of the European Union.

Our aim is to develop a transgenic zebrafish line in which the expression of the fluorescent protein is inducible by estrogenic compounds enabling the detection of their presence in vivo.

For this reason, a liver-specific, estrogen inducible gene, the zebrafish vitellogenin-1 was chosen. 3,5kb upstream region (promoter and cis regulatory elements) of the gene and the fluorescent protein (mCherry) coding region were cloned in two steps to the transposon-based multsite Gateway system. The construct was then coinjected with transposase mRNA into 1-2 cell stage wild type zebrafish embryos (AB line). The specificity and inducibility of the construct was tested using 100 ng/l 17-b-estradiol.

About 20% of the injected embryos showed fluorescent signal in the liver while transient, non-specific expression was detected in the eye, kidney and in the yolk. In the F1, only inducible liver-specific fluorescent signal was detected as a response to estrogenic exposure. The F2 generation shows Mendelian inheritance of the transgene.. The newly developed transgenic line would enable the in vivo detection of estrogenic compounds as well as the monitoring of other effects exerted on the most important organs and tissues for toxicology.

This work was supported by OTKA (NNF 78834) and KMOP-1.1.1-09/1-2009-0048, TÁMOP-4.2.2.B-10/1-2010-0011 projects.