

APPLICATION OF THE FISH EMBRYO TOXICITY TEST FOR THE ASSESSMENT OF WASTE WATER TREATMENT PLANT EFFLUENTS

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Abstract: *Fish embryo toxicity test is a modern non-animal test. This test has been developed as an alternative to acute test with adult fish, and it is applied in Germany for the whole effluent toxicity testing of wastewater treatment plants (WWTP). Whole effluent toxicity testing protocols were developed in several countries like Canada, USA, the Netherlands, Australia, New Zealand, and Germany. Argument for application of those protocols is that monitoring of individual pollutants alone cannot ensure safe environmental conditions of the receiving water bodies. Several studies reported adverse effects at fish induced by the WWTP effluents such as reproductive toxicity, endocrine disruption, immunotoxicity or genotoxicity. The fish embryo toxicity test with zebrafish (*Danio rerio*) has been standardized for routine WWTP effluents testing by ISO. In the present investigation, we performed a screening study to evaluate the acute toxicity of effluents from seven WWTPs in the Czech Republic according to modified ISO norm. The results revealed no mortality revealing thus sufficient water quality according to ISO. Some sublethal effects were observed with the heart edema being the most pronounced.*

Keywords: wastewater treatment plant, whole effluent test, embryo test, zebrafish.

INTRODUCTION

The Fish embryo toxicity (FET) test is a modern test recently normalized as ISO norm for use in WWTP (ISO 2008). The test gains increasing scientific interest and several methods for acute and teratogenicity testing were developed (Nagel 2002). The FET test uses fish embryos instead of adult fish. This made the test an alternative method to animal testing, because embryos are not considered animals according to valid EU legislation (EC 1986). Further, this test gives comparable results as an acute fish test with adult specimen (Lammer et al. 2009; Braunbeck, Lammer 2005). It is therefore possible to replace the test with adults by the FET test. The ISO norm 15088 is adopted for a purpose of a whole effluent testing. The concept of whole effluent testing is based on knowledge that assessment of individual compounds cannot predict a biological

effect of the complex wastewater effluents (Grothe et al. 1996). Various effects as endocrine disruption, immunotoxicity and genotoxicity have been found even in highly diluted samples (Lyney et al. 2005). The standard ISO test studies only mortality as an endpoint (ISO 2008) and employs zebrafish (*Danio rerio*). Zebrafish is quite easy to maintain and it lays transparent eggs whole year, which brings several practical advantages (Braunbeck, Lammer 2005). The FET test with zebrafish is applied only in Germany by law (Nagel 2002). In other countries such as Canada, USA, Australia, and New Zealand acute fish tests with adult specimen are being used (Grothe et al. 1996). The whole effluent testing concept is not applied in the Czech Republic, and the effect of Czech WWTP effluents on zebrafish embryos remains unknown. Here we present some of the first results of the FET assessment of Czech WWTP effluents.

MATERIALS AND METHODS

Seven WWTP effluents from the Czech Republic were sampled in May 2010 and toxicity tested. The total sum of equivalent citizens of these WWTPs was 2.13 million. Sampling was possible due to kind support of the Veolia Voda Czech Republic. A screening test with zebrafish embryos (50 and 100 % effluent) was used to identify toxic samples. Two selected effluents with the most pronounced toxicity were further assessed using FET test according to OECD (2006) and ISO 15088. To determine the lowest ineffective dilution, simple mortality rule from ISO 15088 and the Williams test were used. The lowest ineffective dilution is the lowest tested dilution of the effluent without any toxic effect. Validation of the result was done with the standard compound 3,4-dichloroaniline according to OECD (2006).

RESULTS

No mortality was observed in any of the tested effluents (results not presented) and number of samples had no other apparent toxic effects. Some sublethal effects occurred at selected samples with the heart edema being the most frequent (fig. 1). The most toxic effluent sample had the lowest ineffective dilution for sublethal effects 17 % of the original effluent. The $EC_{50} = 2.6 \mu\text{g/L}$ of the validation experiment with the model compound 3,4-dichloroaniline is equal to published data (Braunbeck, Lammer 2005), thus we can consider the experiments valid.

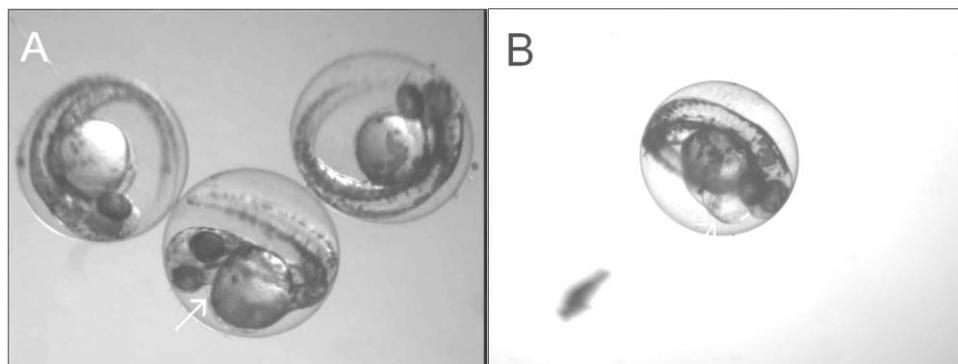


Fig. 1: No obvious effects in control (A), heart edema in 17 % dilution sample (B)

DISCUSSION

Czech WWTP effluents did not cause mortality in any of the tested samples. All tested WWTP would fulfill the requirements of sufficient water quality according to ISO 15088 (ISO 2008), which considers only mortality. The treatment technologies used in the studied WWTPs seems to keep water quality better than toxic to fish in acute mortality tests. Nevertheless, this was a first screening study and further assessment should address effects of more frequently collected samples.

Some sublethal toxic effects were observed in this study but the impact of the observed effects on wild populations is hardly predictable. Most of the sublethal effects observed during acute test would probably lead to lethality caused by inability to avoid the predation. It would be interesting to relate observed effects with responsible compounds or a mixture of compounds, and possible approaches such as effect-directed analysis (EDA) have been suggested (Kaisarevic et al. 2009). Further research on FET should also address nonlethal endpoints such as endocrine disruption. This could be done using the molecular methods such as quantitative RT-PCR or western blotting (Scholz 2008). Research of the Interestingly zebrafish is used as a model organism for human heart development (Mably, Childs 2010), and because the most pronounced effect in this study was the heart deformations, further research should also address parallels between fish and adult cardiotoxicity.

CONCLUSION

FET using zebrafish is a modern alternative method standardized according to ISO (2008), and it has been successfully used for assessment of seven Czech WWTP effluents. No mortality of fish embryos have been observed at any of the tested samples, which demonstrate sufficient quality of the treated waste waters at tested WWTPs. Some sublethal effects (heart edema)

have been observed, and further research should employ endpoints of chronic toxicity in the FET.

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