IRSTI 34.21.17; 34.21.19; 34.35.51

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Application of a zebrafish embryo toxicity assay for the study of surface water toxicity in the Lower Ile river

Abstract: The quality of surface waters of Lower Ile river, Kapchagay and Kutry reservoirs was assessed in zebrafish (*Danio rerio*) embriotoxicity test. The test was performed according to OECD guideline test No. 236, the exposure period was 5-72 h post fertilization, direct mutagen methylmethanesulfonate (MMS) was used as positive control to assess test system response. The standard visual mortality criteria of the test were applied for evaluation of possible lethal or teratogenic effect of surface waters. Exposure to MMS in concentration of 3.4 mg/L resulted in coagulation of 33.3% of embryos ($p \leq 0.01$) and almost 90% ($p \leq 0.01$) of survived embryos displayed various kinds of malformations to 72 hours post fertilization, which indicates test system susceptibility to the mutagens. It was established that none of surface water samples possess significant embryo toxic effects but all induce the spectrum of malformations related to axial skeleton (scoliosis, end tail malformation), water/salt balance and chorion permeability (oedema) and growth patterns (growth retardation) in different incidences. The lowest rate of teratogenicity was observed in embryos incubated in samples from Kapchagay bay (28.2%, $p \leq 0.05$) and Ile river at Bakanas region (site 2, 27.2%, $p \leq 0.05$). The teratogenic effect of water samples from Kurty reservoir and Ile river (site 1) was commensurable – 33.3% ($p \leq 0.05$) and 36.0% ($p \leq 0.01$) respectively. Among all tested sites only the samples of surface waters form Kurty pond produced multiple phenotypic effects in zebrafish *Danio rerio* embryos congruous to MMS exposure and especially, relatively high level of growth retardation, proposing the presence of disrupting or alkylating compounds in surface water samples.
Key words: bioassay, zebrafish embryos, teratogenicity, mutagen, malformations.

Introduction

The assessment of the surface water quality is of highest importance for a great deal of potentially hazardous contaminants like heavy metals, polycyclic aromatic hydrocarbons (PAH) or polychlorinated biphenyls (PCBs) or polychlorinated dibenzodioxins and furans (PCDD/PCDF), and many other compounds unwanted in the environment may present in aqueous environment. Chemical analysis is very expensive and has the main disadvantage that exclusively the target compounds are detected thus the method is blind for unexpected chemicals or even unknown compounds. Bioassays allow analyzing unwanted effects on organisms of all contaminants present in the water sample within an integrated process, addition of toxic effects and even possible synergistic effects of multiple compounds are taken into account [1].

Current approaches for water quality assessment apply a battery of standardized bioassays using all kind of aquatic organisms such as algae (*Desmodesmus subspicatus*), bacteria (*Vibrio fischeri, Arthrobacter globiformis*), invertebrates (*Daphnia magna, Caenorhabditis elegans, Lumbriculus variegates, Diporeia spp.; Hyalella azteca, Chironomus riparius, Potamopyrgus antipodarum*), yeast (*Saccharomyces cerevisiae*), plants (*Myriophyllum aquaticum*) and zebrafish embryos (*Danio rerio*) [2]. In order to better address potential toxicity of aqueous contaminants, the German joint research project DanTox – Development and application of a method for the measurement of specific toxicity and molecular effect mechanisms of sediment-bound environmental pollutants using the zebrafish (*Danio rerio*) – was realized for estimation of teratogenicity, neurotoxicity, genotoxicity, mutagenicity, and subcellular mechanistic effects in embryos of the zebrafish [3]. Use of

Printed in Kazakhstan