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Small scale short-term embryo-toxicity tests for Copper(II) sulfate with olive flounder

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Aquatic toxicity tests have exhibited a trend toward shorter exposure duration that focus on key developmental or life history stages (Norberg-King, 1989). Short-term methods for estimating the chronic toxicity of effluents and receiving water with early-life stages of marine organism have generally utilized forigen species according to their standard labortory bioassay. For example, there are sheephead minnow, *Cyprinodon variegatus* in ASTM (American Society for testing and Materials), OECD (Organization for Economic Cooperation and Development) and USEPA (Unated States Environmental Protection Agency). These tests are typically conducted under continuous-flow conditions and, are generally not conducted on a routin basis. Consequently, there is a need for shorter term, sensitive, robust, and cost-effective tests with Korean species which are more likely to be used routinely. The application of copper sulfate in ponds is also very effective in reducing the abundance of phytoplankton, including Microcystis as well as other blue-green algae (Chen and Lin, 2001). However, copper sulfate is an important xenobiotic in aquatic ecosystem as well as a nondegradable and cumulative pollutant.

The purpose of this study was to develope a representation for toxicity test and to investigate the effects of Copper(II) sulfate ($CuSO_4$) according to the regional differences of habitate including different concentration in sensitivity ; hatching rate, egg and embryo-larvae survival rate and malformed rate of developing embryos of olive flounder, *Paralichthys olivaceus* collected from three regions of the Korean coast ; Cheju-Island, Yeosu and Chungnam.

Olive flounder, *P. Olivacus* were obtained as fertilized eggs from the hatcheries located in Cheju-Island, Yeosu and Chungnam in Korea. Fifty eggs that at around 7 hours post-fertilization (hpf) of each concentration were kept in the 50 mℓ glass beakers containing $CuSO_4$ solution of 0, 5, 10, 20, 40 and 80 $\mu g\ L^{-1}$ duplicately. Temperature was maintained at 20℃ and oxygen at 80% saturation. Fertilization took place in the laboratory. The experiment was designed that

embryos were sampled form 7 to 40 hpf. Embryonic development was monitored at specified time points every 3 h and mortality was recorded. After hatching, collected embryo-larvae were developed in $CuSO_4$ solutions as concentration of egg toxicity test. Then observation of embryo-larvae was extended until 72 h after the time of fertilization, the number of deformity and dead was counted. Malformation tendencies were pictured and described among the embryo and embryo-larvae using a microscope connected to a camera device (×400). Student's *t*-test was applied.

No discrepancy in the survival rates, hatching success and deformities of fertilized eggs and embryo-larvae collected from different regions. The survival rates and hatching ability of eggs significantly diminished in $CuSO_4$ treated groups; respectively 20 and 40 $\mu g\ L^{-1}$ with manner of concentration ($P<0.05$). Exposed to all of the concentrations significant malformation rate of egg was observed including various deformities; irregular eggs membrane, morphologically abnormal embryo formation, tail flexure and irregular yolk-sac. Notably, the reduction of embryo-larval survival rate significantly observed in $\geq$ 20 $\mu g\ L^{-1}$ treated groups in the same manner of the egg's malformation rates. The incidence of spinal cord deformation, abnormal eye and severly developmental delay were observed. There was a dose-dependent increase in toxic effects, and a similar type and sequence of lesions developed in embryos and newly hatched larvae after exposure $CuSO_4$.

Conclusively, the present study clearly shows that $CuSO_4$ negatively affects early life stage survival, development in olive flounder. Also. in this study, the obvious advantage include the large number of embryos that can be obtained the transparency of the fish embryo for morphological examination.

References

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