

## Abstract

The dynamic development of civilization generates more and more physical and chemical pollutants in the environment. Among the latter, estrogens, relatively difficult to remove during wastewater treatment, and therefore widespread in surface waters and bottom sediments, are of particular importance. These compounds are characterized by high biological activity, which can have a particularly intense effect on fish - exposed to constant contact with these substances. These hormones have a negative impact on the development of secondary sexual characteristics of animals, on the circulatory, reproductive and immune systems, they disrupt metabolic pathways and lead to carcinogenesis. The aim of the study is to investigate the effect of estriol, 17- $\beta$ -estradiol and estrone on selected parameters of larvae pigmentation and on the expression of the *cyp1b1* gene (a marker of carcinogenesis in humans and fish) in the zebrafish model (*Danio rerio*). In order to determine the relative level of *cyp1b1* gene expression, the RT-PCR technique was used, while to determine the relative level of pigmentation, a proprietary method of larval pigmentation analysis was used using a microscope coupled with a high-resolution camera and GIMP software. The obtained results indicate a negative influence of 17- $\beta$ -estradiol on the staining of the larvae. Changes in the expression of the *cyp1b1* gene in fish exposed to the tested hormones were confirmed. A statistically significant effect of estrone and estriol has been demonstrated, which is a pioneering discovery for these two substances. Among the tested estrogens, at a concentration of 10 ng / l, a reduction in expression was observed in the research groups, with the highest level of relative expression in the control group, which significantly ( $p < 0.05$ ) differed from the groups exposed to 17- $\beta$ -estradiol and estrone and very significantly ( $p < 0.01$ ) from the group treated with estriol. At the concentration of 100 ng / L, the highest expression was observed in the group exposed to 17- $\beta$ -estradiol, while the lowest in the control group. Highly significant ( $p < 0.01$ ) differences were observed between all analyzed groups. In the case of the concentration of 1000 ng / l, the group exposed to oestrone was characterized by the highest normalized relative expression, while the control group was characterized by the lowest. Highly significant ( $p < 0.01$ ) differences were again observed between all analyzed groups. For the first time, a statistically significant positive correlation (0.745455) was found between the overall relative pigmentation of the tested fish and the expression of the *cyp1b1* gene, which suggests the potential usefulness of the developed method of analyzing fish color changes as a tool for assessing the effect of substances with high biological reactivity. The obtained results, against the background of the prevalence of various estrogens in the environment, also indicate that we should not focus on one of them, but to conduct extensive research on various estrogenic substances, as they may play an important role in carcinogenesis.

**Key words:** estrogens, pigmentation, qPCR, larvae, estrogen receptors, genetic marker, graphical analysis

