THE STUDY OF TOXIC EFFECT OF NITRATES ON EMBRYOGENESIS OF ZEBRAFISH (*DANIO RERIO*)

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Recently, the number of nitrates used in the world has increased dramatically, which is the main factor in nitrogen imbalance in the biosphere. For example, nitrates in the composition of chemical fertilizers have become a frequent cause of environmental pollution, and the use of ammonium nitrate (NH_3) as a pesticide in large quantities and in conditions that do not meet the standards, has led to pollution of ecosystems. Therefore, monitoring, screening studies, as well as biotesting of the ground and aquatic environment is an important part of the conservation of bioresources and biodiversity.

One of the methods for testing the aquatic environment, validated by OECD (Organization for Economy Cooperative and Development), is ZFET (Zebrafish Embryo Toxicity test). Zebrafish or zebra danios (*Danio rerio*) is a good test object, because the development of their embryos is similar to the development of amphibians of natural populations, and their transparency contributes to the histological analyzes to detect developmental abnormalities. In addition, a large number of embryos in one spawning and their transparency is a good opportunity to conduct experiments with high statistical confidence. Therein, *D. rerio* became the standard model not only in toxicology but also in other areas of biology, such as genetics, neurobiology, etc.

In the conducted experiments, adult zebrafishes were kept in aquariums at a temperature of $23 \pm 1\ ^0$ C. At the same time, males and females were kept separately and fed by dry and alive food (*Artemia spp*.). To ensure optimal water quality remaining food should be removed daily. The day before spawning, they did not feed to avoiding the water pollution. In the evening of the same day, all mature fish were planted in aquariums for spawning at a temperature of $26 \pm 1\ ^0$ C with a special separating net on the bottom (to prevent the parents from eating the eggs). Plant imitations made of green glass are used as spawning substrate. On the morning of the next day, only fertilized eggs were collected. A single mature female lays 50-200 eggs per day. At the culture conditions described above, fertilized eggs undergo the first cleavage after approximately 15 min and consecutive synchronous cleavages form 4, 8, 16, and 32 cell blastomeres. At these stages (4 - 32 cells), eggs can be identified clearly as fertilized, and only these should be used for the experiments.

Selected fertilized eggs were placed in 3 containers with different concentrations of ammonium nitrate: 1) 0.04 mg / l, 2) 0.4 mg / l, 3) 4 mg / l and 4th with pure water, without ammonium nitrate (control). Observations of the development of embryos were made by using a digital stereo microscope (MoticDM 143) with an integrated camera and image display on a PC screen, that is why the embryos were photographed. In each group (a container with a different concentration of NH_3), the mortality of embryos were visually determined during 12, 24, 36, 48, 60 and 72 hours post fertilization (hpf). As a result of the conducted studies, the dose-dependent mortality of embryos at each stage of hpf were established. In 3rd container (4 mg / l), all embryos became extinct after 36 hpf, while in other containers (0.4 mg / l, 0.04 mg / l and control), the total share of all deaths after 72 hpf were 51.7%, 18.4 % and 5%, respectively. At the same time, various malformations were observed, such as: pericardium swelling, abnormal development of the yolk sac, curvature of the spine and tail tip, coagulation of embryos before and after hatching from the chorion.

In conclusion, increasing of the doses of ammonium nitrate in water, where eggs were contained, led to low survival and various deviations in the development of *Danio rerio* embryos, which confirms the toxic effect of this pollutant on hydrobionts.

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