

## Evaluation of octylphenol effect on embryo development in zebra fish (*Danio rerio*) and common carp (*Cyprinus carpio*)

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### SUMMARY

Worldwide, the scientific researches performed during the last years are focused on the determination of the negative effects caused by natural and antropogeneous chemical compounds on aquatic species; these species are more exposed to most pollutants than the land species, for the simple reason that the aquatic environment is the last destination for most residues. Our research team proposed to test the toxic effect caused by octylphenol, a substance belonging to the category of polyetoxy alkylphenols, on embryo development in zebra fish (*Danio rerio*) and common carp (*Cyprinus carpio*). Zebra fish embryos were obtained in laboratory, using for this 6 fish families (6 females and 12 males). Common carp embryos were purchased from the fish farm S.C. Acva Prod S.R.L. Cefa, Bihor country; these ones were obtained by artificial reproduction. After taking and selection, the fecundated spawns were introduced in 10 Nunk culture plates of 45 ml, where we introduced 40 ml water, too. For each species, we created 3 batches, with two replications, namely: batch I – control, batch II – in water, we added octylphenol (OP) in concentration of  $1.5 \mu\text{g L}^{-1}$  and batch III – we added in water a concentration of  $60 \mu\text{g L}^{-1}$  OP. During the incubation, the Nunk plates were kept in breeding aquariums, at a temperature of  $28^{\circ}\text{C}$  for zebra fish, respectively  $24^{\circ}\text{C}$  for carp. Embryo supervision was performed with the research microscope Olympus CX41, endowed with digital photo camera and software for image analysis. Beginning with 12 hours post-fecundation, we observed a higher sensibility of zebra fish under octylphenol action, so that, between 23 and 27 hours post-fecundation, the percentage of mortality increases in batches II and III to 30%, respectively 40%. 73 hours post-fecundation, 60% of the zebra fish embryos belonging to the control batch are already eclosed, while embryos belonging to batches II and III stagnate in the advanced faringula stage and in the eclosion period. 77 hours post-fecundation, all embryos belonging to the control group are eclosed, being in the larva stage; this stage in

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reached only by 80%, respectively 60% of the embryos belonging to batches II and III, and only after 82 hours. In carp, embryo eclosion occurred 100% in the control group after 85 hours post-fecundation, while in batches II and III it was carried out in a proportion of 80, respectively 70%. In these two batches, the other embryos, up to 100%, stagnated in the advanced faringula stage, when they died.

Key words: *Danio rerio*, *Cyprinus carpio*, embryos, octylphenol, development

## INTRODUCTION

Endocrine disruptors and the disorders caused by them represent today one of the stringent ecological problems. Despite the intense current researches and the fact that the term „endocrine disruptor” was introduced relatively recently, it is obvious that the exposure to antropogeneous chemical substances, present in the environment, have negative effects for the wild animals and implicitly for human. Most endocrine disorders appear in the organisms from the aquatic environment and in the species feeding on aquatic organisms. A big part of the about 70,000 artificial chemical substances resulting from different industries enter the aquatic environment. Among them, there is a wide range of chemical substances with effects that are similar to natural or pharmaceutical estrogens, alkylphenols, organo-chlorinated pesticides and phthalates, showing different bonding affinities of the estrogenic receptors, which are called endocrine disruptors. In nature, they can be found in surface water and in its sediments, and also in running waters, marine habitats, air and soil. From here, they pass into the trophic chain, getting to animals and finally to human. Chemical pollution represents a real danger because it disturbs the trophic chain, and also because of the chemical elements generated by deterioration. Polychlorinated alkylphenols are chemical substances included in the category of endocrine disruptors, which, used widely in agriculture (as emulsifiers in the production of liquid pesticides), in the industry of plastic and elastomers, in textile industry, in the production of paper, detergents, perfume products, etc. have a significant potential of release in the environment. The endocrine disruptors may interact with estrogenic receptors modifying the synthesis, secretion, transportation, binding, action or release of the estrogen hormones and, consequently, they affect body homeostasis, development, reproduction and behavior. With their lipophilic-nature quality, alkylphenols accumulate and deposit predominantly in fat tissues, liver, bile and kidneys, being possible to become available for human consumption. A major consequence of endocrine disruptors' action on fish is represented by the process of male effemination, caused by the induction and increase of the release of vitellogenin (Arukwe et al., 2000, Denslow et al., 1999, Folmar L.C. et al., 1996, Maitre et al., 1985, Le Guellec et al., 1988).

## MATERIAL AND METHODS

Under the context of researches performed in the world, the problems approached by our team are focused on the toxic effect caused by octylphenol upon embryo development in zebra fish (*Danio rerio*) and common carp (*Cyprinus carpio*). The zebra fish was selected as experimental model because it has a quick growth, being recommended in ecotoxicology, for toxicity tests. In addition, our researches were extended on embryos belonging to the common carp (*Cyprinus carpio*), and economically important species.

Zebra fish embryos were obtained in laboratory, using for this 6 fish families (6 females and 12 males). The aquariums were flooded two days before the introduction of reproducers, and water temperature was drawn to 26 – 28°C. For deposition, we used a 3 mm-loop net. The reproducers were separated depending on sex for one week, when they were fed with live food at discretion (*Daphnia magna* and *Grindall*). They were introduced in the reproduction aquariums in the evening, 2 families/compartment, assuring a sex percentage of 2 males/1 female. The next day, after spawn lying around 10 a.m., the reproducers were removed. The fecundated spawns were taken with a microdropper in Petri dishes, then they were selected at the Optika-type stereomicroscope, and after that, for a facile handling and visualization, each ten of them were introduced in 45 ml-Nunk culture plates, where we introduced 40 ml water, too. The common carp embryos (*Cyprinus carpio*) were purchased from the fish farm S.C. Acva Prod S.R.L. Cefa, Bihor county, being obtained by artificial reproduction. For each species, we made 3 batches, with two replications, namely: batch I – control, batch II – in water, we added octylphenol (OP) in concentration of 1.5 µg L<sup>-1</sup> and batch III – we added in water a concentration of 60 µg L<sup>-1</sup> OP. During the incubation, the Nunk plates were kept in breeding aquariums, at a temperature of 28°C. Embryo supervision was performed with the research microscope Olympus CX41, endowed with digital photo camera and software for image analysis. During the process of embryo supervision, we applied the main characteristics of the embryo development stages in Cyprinidae as described by Kimmel et al. (1995).

## RESULTS AND DISCUSSION

By analyzing the data achieved after the supervision of zebra fish and common carp, we may draw the conclusion that the first stages of embryo development, respectively the period of zygote, cleavage and blastula, do not differ significantly between the experimental batches and the control batch.

After 9 ½ hours, in all three batches, the zebra fish embryos were in different gastrula stages. So, in the control batch, 20% embryos were in the stage of epiboly 90%, 70% in epiboly 80%, and 10% in epiboly 70%. An almost similar situation may be met in the case of the experimental batches, with the difference that, in the experimental batch II, 20% embryos were in a more

advanced gastrula stage, respectively the stage when the tail bud appears, and 10% embryos died (fig. 1a,b,c,d).

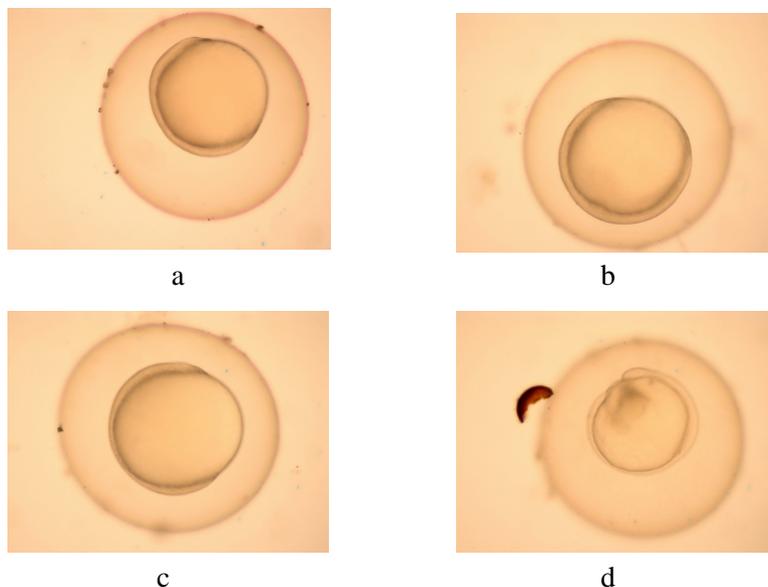
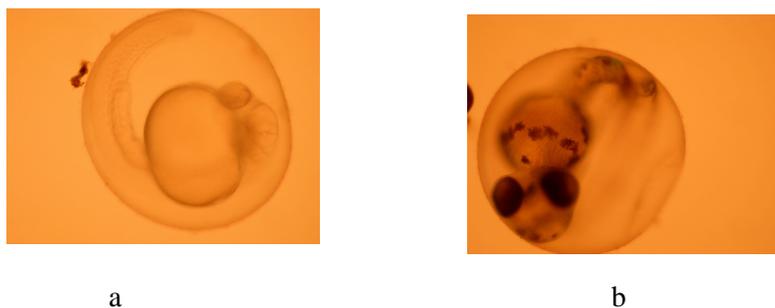


Fig. 1. Zebra fish embryos in different gastrula stages: a – epiboly 70%; b – epiboly 80%; c – epiboly 90%; d – tail bud

Beginning with 12 hours post-fecundation, we observed a higher sensibility of embryos under octylphenol action, so that, between 23 and 27 hours post-fecundation, the percentage of mortality increases in batches II and III to 30%, respectively 40%. 30 hours post-fecundation, the very most (90%) embryos in the control group were in an advanced faringula stage, and 10% were in an incipient faringula, while, in batch II, only 20% embryos were in an advanced faringula, 30% were in incipient faringula stages and 40% were less developed, respectively in their segmentation period – the stage of 18 somites. In the case of batch III, during the same time interval, embryos were in incipient and intermediary faringula stages (40%) (fig. 2 a, b, c, d).



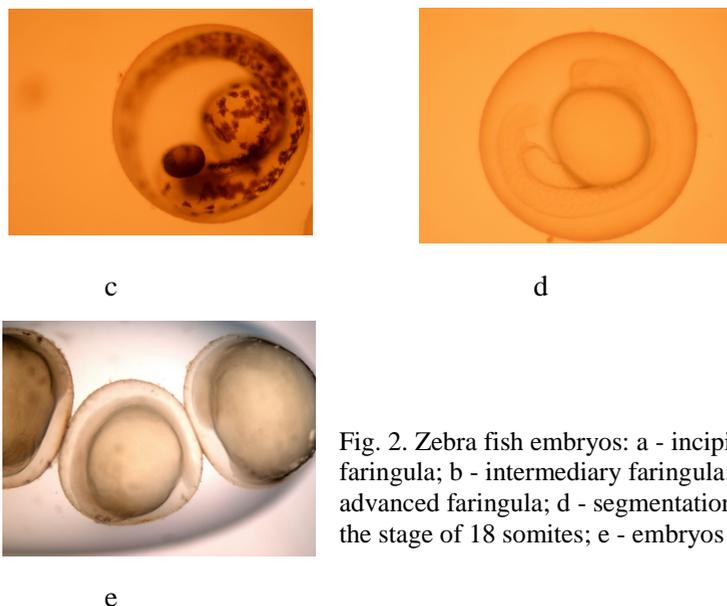


Fig. 2. Zebra fish embryos: a - incipient faringula; b - intermediary faringula; c - advanced faringula; d - segmentation period – the stage of 18 somites; e - embryos died

51 hours post-fecundation, in the control group 80% embryos entered the process of eclosion, the difference of 20% remaining in the advanced faringula stage. In batch II, 30% embryos are in eclosion, 60% in advanced faringula, while in batch III 20% embryos entered the process of eclosion and only 40% were recorded in the advanced faringula stage.

If at 59 hours post-fecundation all embryos in the control group entered the process of eclosion, in batches II and III only 30% embryos entered this process. 73 hours post-fecundation, 60% of the zebra fish embryos belonging to the control batch are already eclosed, while embryos belonging to batches II and III stagnate in the advanced faringula stage and in the eclosion period (fig.3a, b).

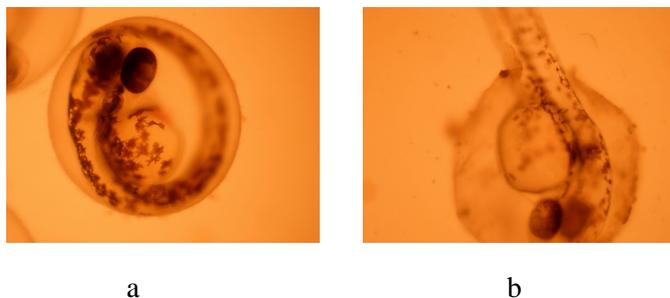


Fig. 3. Zebra fish embryos: a – advanced faringula; b – eclosion period;

77 hours post-fecundation, all embryos belonging to the control batch are eclosed, being in the larva stage; this stage is reached only by 80%, respectively

60% of the embryos belonging to batches II and III, and only after 82 hours (fig. 4).



Fig. 4. Zebra fish – larva stage

The analysis of the results achieved shows that, in zebra fish, octylphenol, even in small microgram-level doses, influences negatively embryo viability and also the process of eclosion, as well. Recent research (Dreze și colab., 2000, Gimeno și colab., 1998, Krisfalusi și Cloud, 1996, Tina H. Rasmussen și colab., 2002) are underling the negative effect, on grow and development of the zebra fish and other fish species, of endocrine disruptores generally and specially of the octhylphenol. Tina H. Rasmussen et. al., (2002) is noticing a significant grow of the mortality rate at a  $100 \mu\text{g L}^{-1}$  octhylphenol concentration, comparative to the control batch, at the *Zoarcetes viviparus* specia.

In carp, 7 hours post-fecundation, 70% embryos in the control group were in the stage of gastrula germinal ring, 20% in the stage of shield and 10% in epiboly 70%. During this period, there are not significant differences between the control batch and the two experimental batches. So, in batch II, 60% embryos were in the gastrula stage – the germinal ring stage, 30% in the shield stage and 10% in epiboly 70%. In batch III, 60% embryos were in the gastrula stage – the germinal ring stage, and 40% in the shield stage (fig. 5a,b,c).

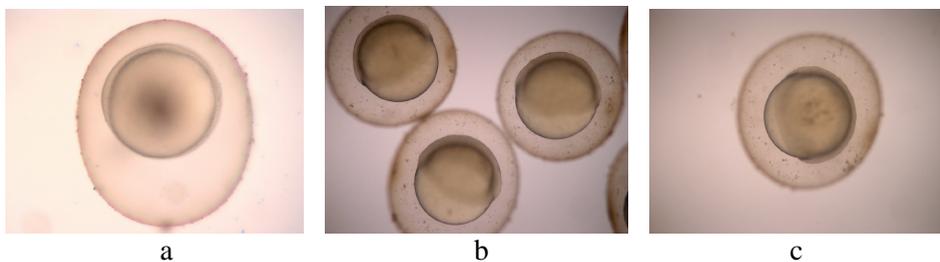


Fig. 5. Carp embryos in different gastrula stages. a - germinal ring; b – shield; c - epiboly 70%

After 24 hours post-fecundation, all embryos in the three batches left the gastrula stage and entered the period of segmentation, in the stage of 20 somites for 100% embryos in the control batch, for 60% embryos in the batch II and for 50% embryos in the batch III. At this age, 20% embryos in the batch II,

respectively 50% embryos in the batch III entered the stage of 18 somites. Moreover, in batch II, 20% embryos were in the stage of 25 somites (fig. 6a, b).

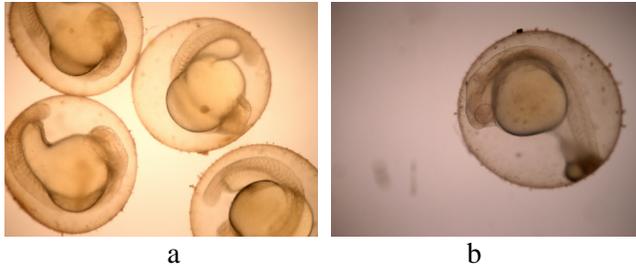


Fig. 6. Carp embryos in different segmentation stages: a - 20 somites; b - 25 somites

The stage of incipient faringula is reached by all embryos in the three batches after 35 hours. It must be mentioned that embryos in batches II and III stagnate in evolution, so that 48 hours post-fecundation they are in the same stage of development, while embryos in the control batch enter the intermediary faringula stage (fig. 7a,b).

78 hours post-fecundation, 90% embryos in the control batch were in the eclosion period, and in batches II and III only 60% were in this stage. Embryo eclosion occurred almost in a proportion of 100% in the control batch after 85 hours post-fecundation, while in batches II and III it occurred in a proportion of 80%, respectively 70%. In the case of these two batches, the rest of embryos, up to 100%, stagnated in their advanced faringula stage and died (fig. 8a,b,c).

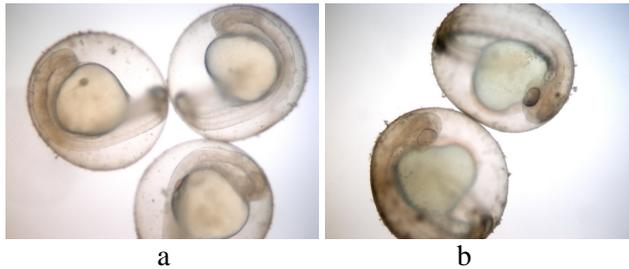


Fig. 7. Carp embryos: a - incipient faringula; b - intermediary faringula;

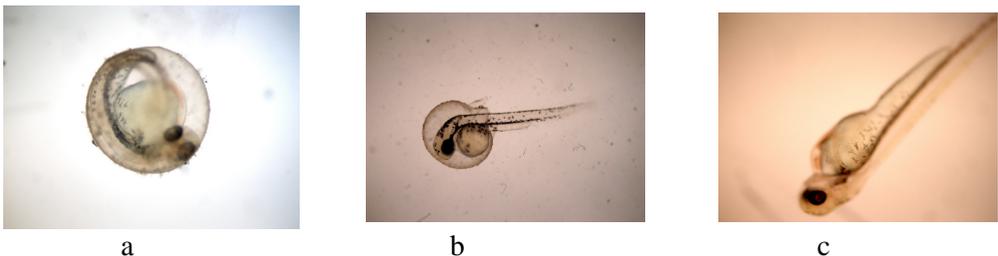


Fig. 8. Carp embryos: a - advanced faringula; b - eclosion period; c - larva stage

In carp, the analysis of the results achieved shows that octylphenol, in small microgram-order doses, influences negatively the process of development, and also embryo viability; in batches II and III mortality was 20%, respectively 30%.

#### CONCLUSIONS

In zebra fish and carp as well, in the case of the control batch, the embryo development characteristics are similar with the ones described by Kimmel et al. (1995).

In zebra fish, 77 hours post-fecundation, embryos in the control batch closed in a proportion of 100%, while in batches II and III the percentage of eclosion was 90%, respectively 60%; the difference of 10%, respectively 40% is represented by mortality.

In carp, embryo eclosion occurred in a proportion of 100% in the control batch after 85 hours post-fecundation, while in batches II and III it occurred in a proportion of 80%, respectively 70%. In the case of these two batches, the rest of embryos, up to 100%, stagnated in their advanced faringula stage and died.

The results achieved show that, in the two fish species studied, octylphenol causes delays in embryo growth and development, influencing negatively their viability.

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