Evaluation of Octhylphenol Effect on Development and Survival on Zebra Fish (*Danio Rerio*) During Different Ontogenic Period

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Abstract

This paper is part of a complex study of our research collective that studies the toxic effect of the ethinylestradiolum, and some of the polyethoxylated alkylphenols on the growth and reproduction of the Zebra fish (*Danio rerio*) and of the common Carp (*Cyprinus carpio*). Our study aim was to evaluate the effect of octylphenol on growth and survival of zebra fish, from 21-115 days, and within 21-75 days of life. For this purpose, for each period under study, fishes were divided into three groups of 30 individuals, named: Lot 1 - Control**,** respectively lots 2 and 3, at which the administrated octylphenol concentrations were of 60 μg L^{-1}, respectively 100 μg L^{-1}. Fishes of the six groups were raised in 30-liters aquariums (30 fish / aquarium). The growth was measured by weighing and biometric measurements (total length, standard length, the length of the head, maximal height, minimal height and the mass of the body), while the surviving rate was established at the end of every period and at the end of the experiment, when we were able to calculate the total number of dead fish. Biometric study of the analysis performed in 75 days, 115 days respectively shows that octylphenol has negative influence on body development, and survival both, the highest percentage of mortality (46,66%) was registered at 100 μgL-1 concentration, between 21 -75 days.

Keywords: Danio rerio, octhylphenol, development, survival

1. Introduction

A big part of the 70,000 artificial chemical substances resulting from different industries enter the aquatic environment (Metzler, M., 2001, Routledge, E.J., 1998). Among them, there is a wide range of chemical substances with effects that are similar to natural or pharmaceutical estrogens, alkiphenols, organo-chorinated pesticides and phthalates, showing different bondage affinities of the estrogenic receptors, which are called endocrine disruptors (Sumpter, P. J., 2002**)**. In nature are found in surface waters and in sediments, both in rivers and marine habitats, air and soil. From here, they get to the food chain, passing to animals and eventually in humans. Chemical pollution is a danger in disrupting the food chain and damage caused by chemical elements. Polyetoxilated alkylphenols are chemicals included under disruptorilor endocrine, which widely used in agriculture (as emulsifiers in the production of liquid pesticides) in plastics and elastomers industry, textile industry, the production of paper, detergents, deodorized products etc.. have significant potential for release into the environment. The endocrine disruptors may interact with estrogenic receptors (Maitre J.L., 1985, Paris F. et al., 2002**)** modifying the synthesis, secretion, transportation, binding, action or release of the estrogen hormones

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and, consequently, they affect body homeostasis, development, reproduction and behavior (Denslow et al., 1999, Higashitani T., 2003).

2. Materials and methods

Under the context of the researches performed in the world, the problems approached by our team aims at the assessment of growth and development of zebra fish (*Danio rerio*) exposed to octylphenol from 21-115 days, 21 to 75 days respectively.

Thus, for each period under study were made 3 groups each of 30 individuals, namely: group I - control group, group II – we added, in water, octylphenol (OP) in concentration of 60 μg L^{-1} and group III – we added a concentration of 100 μg L^{-1} OP. Stock solutions of 60 g L^{-1} OP (Sigma) and 100 g L^{-1} OP were obtained by diluting octylphenol 96^0 (Merck) in the alcohol, after which they were stored at a temperature of 4 ^{0}C. Following solutions obtained from stock solutions were made by dilution in aerated water and stored at 4 ^{0}C. Note that in low concentrations, alcohol has not adverse effects on development and larval growth (Van den Belt, K. et al., 2002, Van der Ven, L.T., et. Al., 2003).

The fish in the six batch were breed in 30-litre aquariums (30 fish/aquarium), endowed with conditioners for a constant temperature (24^0C), with illumination systems (neon lamps), with vibrators to maintain the O_2 concentration over 60% of the air saturation volume and with filtering pumps. The physical-chemical parameters of the water, daily measured, were: pH-8, dH^0 – 6.5. In order to establish the octylphenol effect on zebra fish growth and development, at the age of 75 days of life, respectively 115 days of life, we killed 10 individuals from each batch and determined the following biometrical parameters: total length, standard length, head length, maximal height, minimal height and weight. The statistical analysis was performed with the help of the test Mann-Withney U Test. Also, at the end of each experiment the percentage of viability of fish in each group was determined.

3. Results and discussion

The biometric study effectuated on individuals from the first study (21 – 115 days) reveal the fact that between group I (control group) and group II, whose individuals were exposed at a concentration of 60μg OP L^{-1}, are significant differences ($p<0.05$) regarding total length, maximum height and minimum height (table 1). The higher concentration of octyphenol administered to group III (100 μg L^{-1} OP) influence significantly the total weight of each individual from group III compared with those from control group (table 2). Also, between group II and group III are significant differences ($p<0.05$) in most of the biometrical parameters studied, except maximum and minimum height in which the differences were not significant ($p>0.05$) (table 3). Between experimental groups II and III the differences in biometrical parameters studied were not significant (table 3).

Between 21-75 days, the exposal of fishes at a higher concentration then 60μg OP L^{-1} determined significant ($p<0.05$) modifications compared with control group, regarding total length and standard length (table 4). Also, this two biometrical parameters differ between individuals of group I and III (table 5), and between group II and III (table 6).

At the end of every study the mortality percent of every group was determined. It was observed that this parameter was 46.66% for group III, 30% for group II, between 21 – 75 days, compared with control group which had 4% mortality. In case of experimental lots from first study, in which octyphenol was administered in age interval 21 – 115 days, mortality was 40% at experimental group III and 20% at experimental group II, compared with control group which registered 13.4% mortality.

Table 1. Significance of the differences between groups I and II, for the main biometrical parameters (21-115 days)

Specification	Group I control	Group II (60 μgL^{-1})	Differences B I –B II	Significance tests
	$x \pm Sx$	$x \pm Sx$		
Total length (cm)	2.25±0.08	2.03±0.06	0.22	0.034*
Standard length (cm)	1.61±0.05	1.43±0.05	0.018	0.058NS
Head length (cm)	0.43±0.01	0.40±0.01	0.03	0.073NS
Max height (cm)	0.44±0.01	0.40±0.02	0.04	0.022*
Min height (cm)	0.22±0.01	0.19±0.01	0.03	0.028*
Body weight (g)	0.10±0.01	0.08±0.01	0.02	0.112NS

Notă: p>0,05 - NS = insignifiant; * p<0.05 - signifiant

Table 2. Significance of the differences between groups I and III, for the main biometrical parameters (21-115 days)

Specification	Group I control	Group III (100 μgL^{-1})	Differences B I –B III	Significance tests
	$x \pm Sx$	$x \pm Sx$		
Total length (cm)	2,25±0,08	1,91±0,05	0,34	0,005*
Standard length (cm)	1,61±0,05	1,40±0,04	0,21	0,005*
Head length (cm)	0,43±0,01	0,38±0,01	0,05	0,003*
Max height (cm)	0,44±0,01	0,39±0,02	0,05	0,068NS
Min height (cm)	0,22±0,01	0,19±0,01	0,03	0,070NS
Body weight (g)	0,10±0,01	0,06±0,01	0,04	0,0065**

Note: * p<0,05 ; ** p<0,01; : p>0,05 - NS = insignifiant

Table 3. Significance of the differences between groups II and III, for the main biometrical parameters (21-115 days)

Specification	Group II	Group III (100 μgL^{-1})	Differences B I –B II	Significance tests
	$x \pm Sx$	$x \pm Sx$		
Total length (cm)	2.03±0.06	1.91±0.05	0.12	0.32NS
Standard length (cm)	1.43±0.05	1.40±0.04	0.03	0.57NS
Head length (cm)	0.40±0.01	0.38±0.01	0.02	0.080NS
Max height (cm)	0.40±0.02	0.39±0.02	0.01	0.76NS
Min height (cm)	0.19±0.01	0.19±0.01	0.00	0.61NS
Body weight (g)	0.08±0.01	0.06±0.01	0.02	0.32NS

Notă: p>0,05; NS = insignifiant

Table 4. Significance of the differences between groups I and II, for the main biometrical parameters (21-75 days)

Specification	Group I control	Group II (60 μgL^{-1})	Differences B I –B II	Significance tests
	$x \pm Sx$	$x \pm Sx$		
Total length (cm)	1,80±0,08	1,59±0,04	0,39	0,049*
Standard length (cm)	1,36±0,06	1,19±0,04	0,17	0,023*
Head length (cm)	0,36±0,02	0,41±0,09	0,05	0,54NS
Max height (cm)	0,35±0,02	0,33±0,01	0,02	0,30NS
Min height (cm)	0,17±0,01	0,25±0,10	-0,08	0,32NS
Body weight (g)	0,06±0,01	0,04±0,00	0,02	0,17NS

Notă: * p<0,05 - signifiant; p>0,05 - NS = insignifiant

Table 5. Significance of the differences between groups I and III, for the main biometrical parameters (21-75 days)

Specification	Group I control	Group III (100 μgL^{-1})	Differences B I –B II	Significance tests
	$x \pm Sx$	$x \pm Sx$		
Total length (cm)	1.80±0.08	1.56±0.07	0.24	0.049*
Standard length (cm)	1.36±0.06	1.09±0.09	0.27	0.023*
Head length (cm)	0.36±0.02	0.33±0.01	0.03	0.16NS
Max height (cm)	0.35±0.02	0.34±0.02	0.01	0.30NS
Min height (cm)	0.17±0.01	0.15±0.01	0.02	0.09NS
Body weight (g)	0.06±0.01	0.04±0.01	0.02	0.17NS

Notă: p<0,05 – signifiant; p>0,05 - NS = insignifiant

Table 6 . Significance of the differences between groups II and III, for the main biometrical parameters (21-115 days)

Specification	Group II (60 μgL^{-1})	Group III (100 μgL^{-1})	Differences B I –B II	Significance tests
	$x \pm Sx$	$x \pm Sx$		
Total length (cm)	1.59±0.04	1.56±0.07	0.03	0.028*
Standard length (cm)	1.19±0.04	1.09±0.09	0.10	0.023*
Head length (cm)	0.41±0.09	0.33±0.01	0.08	0.32NS
Max height (cm)	0.33±0.01	0.34±0.02	-0.01	0.44NS
Min height (cm)	0.25±0.10	0.15±0.01	0.10	0.08NS
Body weight (g)	0.04±0.00	0.04±0.01	0.00	0.19NS

Notă: * p<0,05 ; p>0,05; NS = insignifiant

Dates obtained in this experiment, regarding the negative impact of octyphenol on growth and development, are similar with dates from literature, both in ciprinides and other fish species including vivipars (Krisfalusi, M and Cloud, J.G., 1996; Gimeno, S. et al., 1998; Dreze, V., et al., 2000; Tina, H. Rasmussen et al., 2002). Also in our observations, we mention that octyphenol negatively influence the viability, in embryonic stage, in which the mortality was 30% at a concentration of 60μg L^{-1} (Dumitrescu G., et al., 2008) and in the first posteclosional stages when mortality was 47%.

4. Conclusions

1.From dates analysis, we conclude that the exposal of zebra fish in the two concentrations of octyphenol (60μg L^{-1} OP; 100μg L^{-1} OP) in the two periods studied, influence the total length and standard length in the two experimental groups, compared with control group. Moreover the concentration of 100μg L^{-1} OP determines significant differences in ody weight between control group and group III.

2.In high doses octyphenol has negative influence on the viability of zebra fish, mortality was 46,66% for group III and 30% for group II, in the period 21 – 75 days, compared with control group which had 4% mortality. In case of experimental lots from first experiment, in which octyphenol was administered between 21-115 days of life, mortality was 40% at experimental group III and 20% at experimental group II compared with control group which had a mortality of 13,4%.

References

1. Arukwe A., Celius T., Walther B.T., Goksoyr A., 2000, Effects of xenoestrogen treatment on zona radiata and vitellogenin expression in Atlantic Salmon (Salmo salariu). Aquat. Toxicol. 49: 159 – 170.

2. Denslow N.D., Chow M.M., Bonomelli S., Folmar L.C., Heppel S.A., Sullivan C.V., 1997, Development of biomarkers for environmental contaminants affecting fish, p. 73-86. In Rolland R.M., Gilbertson M., Petersen RE (ed.), Chemically induced alterations in functional development and reproduction of fishes. Setac, Pensacola, Fla., U.S.A.

3. Dreze, V., Monod, G., Cravedi, J.P., Biagianti-Risbourg, S., Le Gac, F., 2002, Effects of 4-nonylphenol of sex differentiation and puberty in mosquitofish (Gambusia holbrooki). Ecotoxicology 9: 93-103.
4. Gabi Dumitrescu, Liliana Petculescu Ciochină, Voia, S, Grozea A., Polen T., Liliana Boca, Bănățean-Dunea I, 2008, Evaluation of octhylpfenol effect on embryo development in zebra fish (Danio rerio) and common carp (Cyprinus carpio). Book of abstracts of the 7th International Symposium of Animal Biology and nutrition-IBNA, september 25-26, pag. 30.
5. Gimeno, S., Komen, H., Gerritsen, A.G.M., Bowmer, T., 1998, Feminisation of young males of the common carp, Cyprinus carpio, exposed to 4-tert-pentylphenol during sexual diferentiation. Aquat. Toxicol. 43: 77-92.
6. Higashitani T., Tamamoto H., Takahaschi A., Tanaka H., 2003, Study of estrogenic effect on carp (Cyprinus carpio) exposed to sewage treatment plant effluents. Pure appl. Chem., vol.75, nr. 11-12, IUPAC
7. Krisfalusi, M., Cloud, J.G., 1996, Effects of exogenus estradiol-17β on early growth and gonadal development of diploid and triploid female rainbow trout (Oncorhyncus mykiss). Dev. Genet. 19: 302-308.
8. Le Guellec K., Lawless K., Valotaire Y., Kress M., Tnniswood M. 1988, Vitellogenin gene expression in male rainbow traut (Salmo gairdneri). Gen. Comp. Endocrinol. 71: 359 – 371.
9. Maitre J.L., Mercier L., Dole L., Valotaire Y., 1985, Characterization of estradiol specific receptors and induction of vitellogenin mRNA in the rainbow trout liver (Salmo gairdneri). Biochemie 67: 215 – 225.
10. Metzler, M., Pfeiffer Erika, 2001, Chemistry of natural and anthropogenic endocrine active compounds. The Handbook of Environmental Chemistry, Vol 3, Part L, Endocrine Disruptors. Ed Springer – Verlag, Berlin Heidelberg.
11. Paris F., Balaguer P., Terouanne B., Servant N., Lacoste C., Cravedi J.P., et. al., 2002. Phenylphenols, bisphenols, bisphenol A and 4 – tert- octylphenol exhibit α and β estrogen activies and antiandrogen activity in reporter cell lines. Mol Cell Endocrinol. 193: 43 – 49.
12. Routledge, E.J., Sheahan, D., Desbrow, C., Brighty, G.C., Waldock, M., Sumpter, J.P., 1998. Identification of estrogenic chemicals in STW effluent. 2. In vivo responses in trout and roach. Environ. Sci. Technol., 32, 1559-1565.
13. Sumpter, P. J., 2002, Endocrine disruption in the Aquatic Environment. The Handbook of Environmental Chemistry, Vol 3, Part M, Endocrine Disruptors. Ed Springer – Verlag, Berlin Heidelberg.
14. Tina H. Rassmusen, Thomas, K., Andreassen, Soren, N. Pedersen, Leo, T.M. Van der Ven, Poul Bjerregaard, Bodil Korsgaard, 2002, Effects of waterborne exposure of octylphenol and oestrogen on pregnant viviparous eelpout (Zoarces viviparus) and her embryos in ovaryo. The Journal of Experimental Biology 205: 3857-3876.
15. White, R., Jobling, S., Hoare. S.A., Sumpter, J.P., Parker, M.G., 1994. Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology, 135, 175-182.
16. Van der Belt, K., Wester, P.W., van der Ven, L.T., Verheyen, R., Witters, H., 2002. Effects of ethinylestradiol on the reproductive physiology in zebrafish in zebrafish (Danio rerio): time dependency and reveribility. Environ. Toxicol. Chem. 21: 767-775.
17. Van der Ven, L.T., Wester, P.W., Vos, J.G., 2003. Histopatology as a tool for the evaluation of endocrine disruption in zebrafish (Danio rerio). Environ. Toxicol. Chem. 22: 908-913.