

Histological Changes Induced in Gonads, Liver and Kidney of Zebra Fish (*Danio Rerio*) Under the Effect Octylphenol (OP)

Gabi Dumitrescu, Liliana Petculescu Ciochină, Sorin Voia , Dorel Dronca, Liliana Boca

Faculty of Animal Sciences and Biotechnologies, Timisoara, Romania

Abstract

World-wide researches during the last years are focused upon the negative effects caused by the natural chemical compounds and anthropogenic compounds, already being demonstrated that a great part of them change the endocrine control over reproduction at different species. The issue studied by our team is concerned with the histological changes that appear within liver, kidney and gonadal development at the zebra fish (*Danio rerio*), exposed to octylphenol from 21-115 days, and within 21-75 days of life. In order to achieve the histological preparations, for each period under study, fishes were divided into three groups of 30 individuals, namely: Lot 1 - Control, respectively lots 2 and 3, at which the administrated octylphenol concentrations were of $60 \mu\text{g L}^{-1}$, respectively $100 \mu\text{g L}^{-1}$. Fishes of the six groups were raised in 30-liters aquariums (30 fish / aquarium). Fragments of liver, kidney and gonads were processed histologically, colored with the Mallory trichromic staining method and examined with the optical microscope. Histopathological study revealed changes in the three organs, namely this fine granulation hepatocytes, peritubular edema and vascular ectasia in renal parenchyma and their differentiation in the gonads to sex predominantly female, issues that demonstrate the estrogenic effect of the octylphenol. Those microscopical aspects observed in the kidney and liver of the specimens exposed to action of octylphenol, suggest that the octylphenol is bind to the estrogenic receptors on the membrane of hepatic cells, inducing vitellogenin synthesis.

Key words: zebra fish, kidney, liver, gonads, histology, octylphenol

1. Introduction

One of the most important problem of the today's ecology is the disorder induced by endocrine disruptors. Despite the intense researches and the fact that this term "endocrine disruptor" is relative new, it becomes obvious that exposures to the chemical antropogenic substances which are presented in nature, has bad effects for animals and human. The most endocrine disorders appear in the acvatic species and to those animals which have an diet based on the acvatic organism. 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, alkylphenols, organo-chlorinated 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

* Corresponding author: Gabi Dumitrescu, 0256 277 115, gdumitrescu@animalsci-tm.ro

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. 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 (White, R., 1994). 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, Folmar L.C. et al., 1996, Le Guellec et al., 1988), reduction of testicular development, ceasing of the spermatogenesis and formation of the oviducts, and in females, reducing the number of eggs spawned and their quality, low rate of fertility, increased age of the submission of spawn. The reason of this sensibility is long term exposure and easy way in which this substances get in the blood stream of the fishes trough the gills sistem.

2. Materials and methods

In the context of research conducted internationally, the problems of our collective aims to investigate histological changes occurring in the gonads, liver and kidney from zebra fish (*Danio rerio*) exposed to octylphenol the complete life cycle and from 21 to 75 days. In order to achieve the histological preparations, for each period under study, fish were divided into three groups of 30 individuals, namely: Lot 1 - Control, that lots 2 and 3 of Octylphenol concentrations were administered $60 \mu\text{g L}^{-1}$, respectively $100 \mu\text{g L}^{-1}$. Fishes of the six groups were raised in 30-liter tanks (30 fish / aquarium), equipped with air-conditioning systems to maintain a constant temperature (26°C), with lighting (neon lamps), with vibrators for maintaining a concentration in O_2 for over 60% of air saturation and filter pumps. Physical and chemical parameters of water daily, were as follows: pH 8, dH0 - 6.5. For histopathological study, 10 fish each were sacrificed from each group, were settled in 10% neutral formalin and included in paraffin blocks, after a prior dehydration in increasing alcohol solutions (70° , 80° , 90° , 100°) and clarification two baths of benzene. Paraffin blocks were cut with

Leica manual rotary microtome at thickness of 5μ , and after soldering, histological sections were colored by the method were examined tricromică Mallory and research Olympus CX41 microscope equipped with digital camera and image analysis software .

3. Results and discussion

Microscopic analysis of histological sections reveals, for individuals under octylphenol action, aged between 21-115 days of life, that 70% of individuals from experimental groups present female gonads. The gonads development degree in experimental groups differs from individuals from control group. Thus in individuals from control group, gonads frequently presents large ovocytes, with slightly eccentric nucleus and cytoplasm loaded with tertiary vitellus, including the ovocytes with secondary vitellus and a reduced amount of small ovocytes, with primary vitellus (figure 1). In the experimental group, the three types of ovocytes, were found in gonads of only approx. 28.5% of females (Figure 2), most female presenting small size ovocytes in gonads, with primary vitellus and spherical central nucleus in which many small nucleolus disposed on front of the nuclear membrane are present. In the experimental group III, in which octylphenol was administered at a concentration of $100 \mu\text{g L}^{-1}$, was observed that only 14.1% of females presented large ovocytes loaded with secondary vitellus in gonads (figure 3), the rest having in gonads predominantly small ovocytes (figure 4).

In the study conducted in the period 21-75 days was found that 75% of individuals from experimental group II, and 80% among individuals experimental group III respectively, had female gonads, consisting only of small ovocytes, with cytoplasm loaded with primary vitellus (figure 5). Individuals from experimental group III died at percentage of 46.66%.

The study reveals that octylphenol induces feminization of zebra fish populations in both concentrations tested, though he caused stagnation in the ovocytes evolution, thus increasing the age of filing of the first spawn.

Also, at young age, concentrations of $100 \mu\text{g L}^{-1}$ octylphenol, negatively influence sustainability.

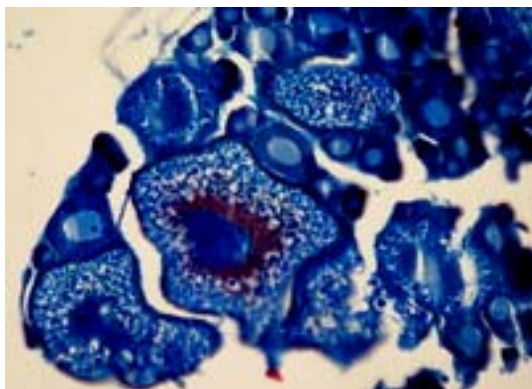


Fig. 1. LM 21-115 days: female gonads frequently formed of large ovocytes, with slightly eccentric nucleus and cytoplasm loaded with tertiary vitellus, trough which ovocytes with secondary vitellus and a small amount of small ovocytes, with primary vitellus are found (Trichromic Mallory st. 200x).

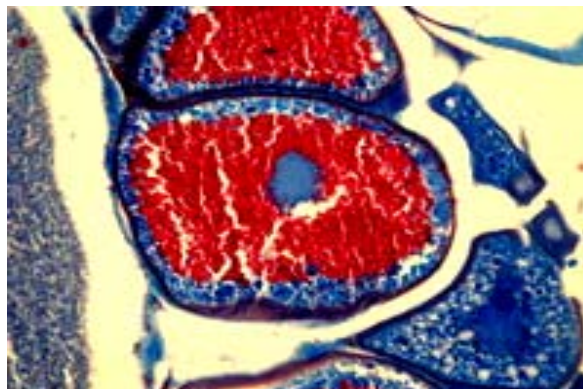


Fig. 2. L II – 21 – 115 days: the three types of ovocyte are found only in gonads of approx. 28.5% of female - predominantly oocytes with tertiary vitellus (Trichromic Mallory st. 200x).

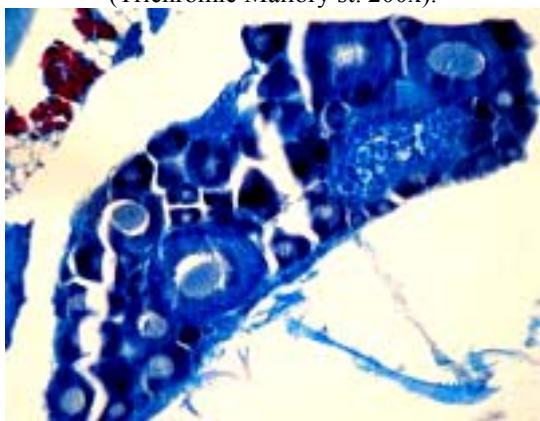


Fig. 3. L III – 21 – 115 days: only 14.1% of females had in gonads large ovocytes loaded with secondary vitellus (Trichromic Mallory st. 200x).

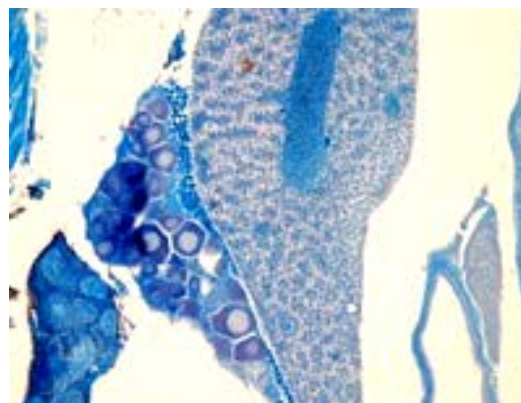


Fig. 4. L III – 21-115 days: 85,9 % of female gonads are formed predominantly of small ovocytes, with primary vitellus (Trichromic Mallory st. 200x).



Fig. 5. L III – 21-75 days - female gonads formed only of small ovocytes with primary vitellus (Trichromic Mallory st. 200x).

Study of liver histology sections, for individuals in the control group, reveals the presence of hepatocytar uniform cordons (figure 6), which converge to centrolobular vein. Hepatocytes were polygonal with central spherical nucleus slightly

eccentric or eccentric, with evident nucleolus and chromatic material.

The cytoplasm, predominantly clear, had vacuolar aspect. At the vascular pole of hepatocytes filled, sinusoidal capillaries are arranged. In case of individuals from lots II and III, the hepatocytes,

slightly hypertrophic, present fine granulations in cytoplasm, extended on large territories in individuals from group III. In individuals from these group, in the liver parenchyma, capillary ectasia were observed wide sinusoidal capillaries are large, filled (figure 7).

All these microscopic liver aspects were observed in experimental groups in both periods studied, could indicate that octylphenol, like other xenoestrogens, binds to estrogen receptors from the membrane of hepatocytes, stimulating the synthesis of vitellogenin (White et al., 1994, Jobling et al., 1996, Routledge et al., 1998). Vitellogenin is a phospholipoglycoprotein (yellow

protein precursor) synthesized in the liver of female fish in response to increased blood concentrations of estrogen (Arukwe et al., 2000), and is passing the bloodstream being subsequently incorporated into ovocytes. Male fish normally don't produce vitellogenin but have receptors for estrogen and liver genes for coding vitellogenin (Maitre et al., 1985, Le Guellec et al., 1988). When male fish are exposed to estrogen compounds, they may act on receptors inducing hepatic synthesis of vitellogenin. For this reason, vitellogenin is recognized as a good biomarker of the feminization of fish, the effect of estrogen (Denslow et al., 1999).

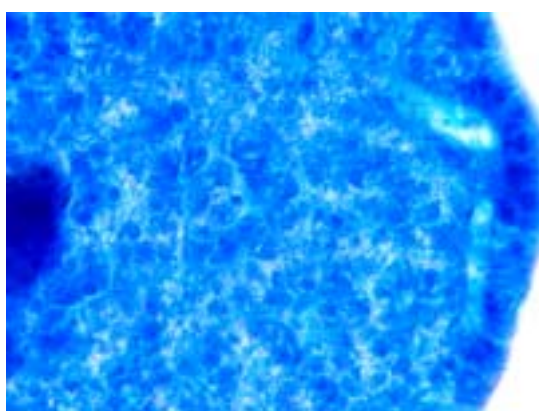


Fig.6. LM 21 – 115 days – section through liver (Trichromic Mallory st. 200x)..

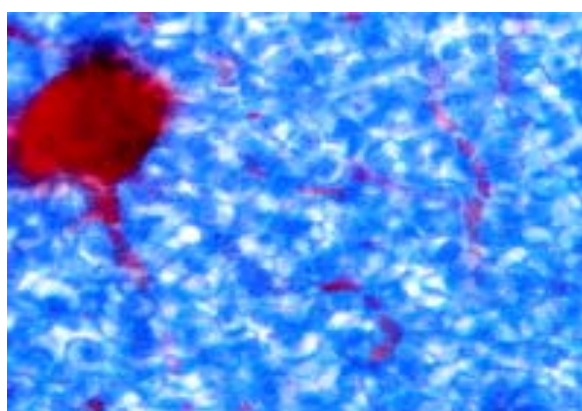


Fig. 7. L III -21 – 115 days - section through the liver: hypertrophy hepatocytes and capillary ectasia (Trichromic Mallory st. 200x)..

Microscopic sections taken from kidneys reveal the presence of renal corpuscles, of uriniferous tubules and peritubular capillary network. Renal corpuscles are formed from the glomerular capillaries and the Bowman capsules formed of two thin epithelial layers that delimited a small capsular space. The uriniferous tubules are lined up with cubic and prismatic nephrocytes with microvillia at the apical pole (figure 8). In the case of the individuals from group II and III, in both age periods, nephrocytes that line uriniferous tubules have fine granular cytoplasm, submit hyaline content of tubular lumen and in the peritubular interstitial there are large areas with numerous leucocitary infiltrates (figure 9). Also, the individuals from the experimental groups, have leucocitary interepithelial infiltrates (figure 10) both in uriniferous tubules, and at glomerular level. In large areas, in the

experimental groups, the disruption processes of the nephrocytes epithelium (figure 11), a large number of cells become hypertrophic, with vacuolar-type cytoplasm and basal membrane lose. These atrophic nephrocytes processes are more common in individuals from experimental group III, of both trials and especially in individuals exposed to octylphenol action in the interval 21-75 days. Numerous studies show the negative effect of endocrine and disruptors and octylphenol on the histological structure of various organs in aquatic species. Thus, Herman and Kincaid (1988) report severe kidney dysfunction, resulting in death, in different species of fish. Lye et al. (1997) found abnormalities of reproductive function and Jobling et al. (1996), Gimeno et al. (1996) observes the inhibition of testicular development.

more obvious in the case of individuals exposed to octylphenol from 21 until 115 days of life

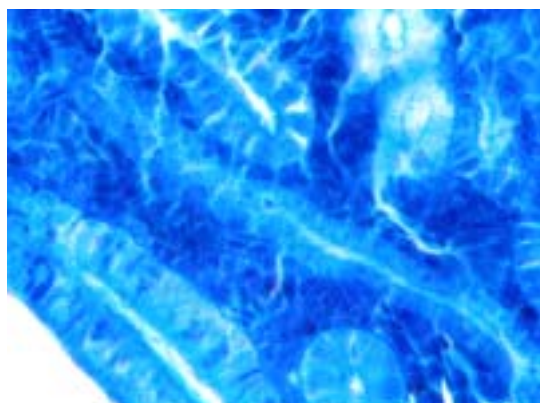


Fig. 8. LM – 21 – 115 days - section through kidney (Trichromic Mallory st. 200x).

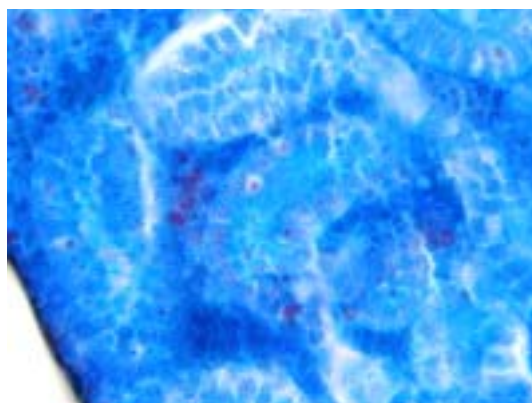


Fig. 9. L II – 21 – 75 days– section through kidney: many interstitial leukocitary infiltrates (Trichromic Mallory st. 200x).

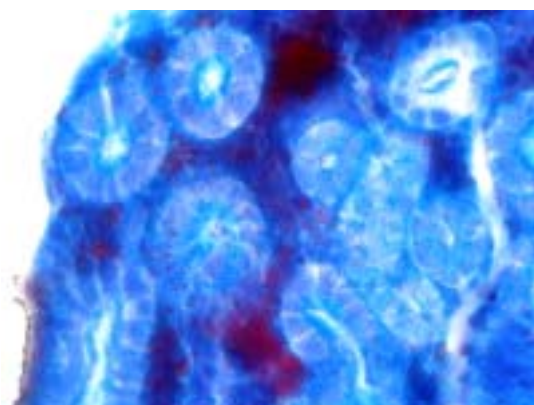


Fig. 10. L III 21-75 days –: section through kidney – leucocitary infiltrations (Trichromic Mallory st. 200x).

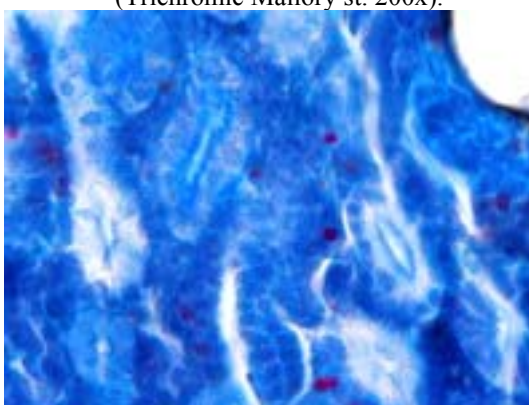


Fig. 11. L III – 21-75 days – section through kidney aspects of nephrocyte epithelial disruption (Trichromic Mallory st. 200x).

4. Conclusions

1. In both periods considered in the study, octylphenol induces differentiation of female gonads, at high concentrations of approx. $100 \mu\text{g L}^{-1}$ OP, inducing stagnation in the evolution of oocytes.

2. Histology study of liver done on sections reveal, in case of the individuals in the control group, the presence of uniform hepatocytes cords with polygonal appearance with central spherical nucleus, slightly eccentric or eccentric, with evident nucleolus and chromatic material, and cytoplasm, predominantly clear with vacuolar aspect.

3. In individuals from lots II and III, the hepatocytes present in cytoplasm numerous fine-grain, capillaries ectasia, extended on larger territories in group III individuals. The issues are

4. Microscopic sections taken from the kidneys reveals, in individuals from group II and III, the presence of nephrocytes with fine granular cytoplasm, of the tubular lumen with hyaline content, and leucocitary infiltrates present both interstitial and interepithelial.

5. In case of experimental lots, in both periods considered in the study, at the renal parenchyma level atrophic nephrocitar processes was observed.

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