ORIGINAL ARTICLE

The effects of subchronic exposure to ciprofloxacin on zebrafish (*Danio rerio*)

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Abstract

OBJECTIVES: The aim of this study was to assess the impact of ciprofloxacin in *Danio rerio* on growth, on the development of histopathological changes in selected organs (gills, kidney, liver), and on the activity of some oxidative stress markers during a 28-day toxicity test.

METHODS: Juvenile growth tests were performed on *D. rerio* according to OECD guideline No. 215. Fish at the age of 30 days were exposed for 28 days to a range of concentrations of ciprofloxacin (0.7 $\mu g.L^{-1}$ – environmental concentration, 100, 650, 1 100 and 3 000 $\mu g.L^{-}1$).

RESULTS: There were no significant differences between the specific growth rates of fish from the test groups and from the control group. Histopathological examination revealed no pathological changes in organs of fish exposed to any ciprofloxacin concentration. The activity of glutathione S-transferase increased at the ciprofloxacin concentrations of 0.7 and 100 $\mu g.L^{-1}$ compared to the control. A significant decrease in glutathione reductase activity was obtained in fish exposed to ciprofloxacin at 1 100 and 3 000 $\mu g.L^{-1}$; a significant decrease in glutathione peroxidase activity was also found, but at all tested concentrations except for 100 $\mu g.L^{-1}$. A decrease in the concentration of thiobarbituric acid reactive substances was noted only at 100 $\mu g.L^{-1}$ compared to the control.

CONCLUSIONS: According to our results, all tested concentrations of ciprofloxacin, including the environmental concentration, had an influence on oxidative stress markers and detoxifying enzymes in exposed fish, but did not affect fish growth or cause the development of histopathological changes in the fish organism.

Abbreviations:

CAT	- catalase	NOEC	- no observed effect concentration
EC_{50}	- median effective concentration	LOEC	- lowest observed effect concentration
GPx	- glutathione peroxidase	OECD	- Organisation for Economic Co-operation and Development
GR	- glutathione reductase		
GST	- glutathione S-transferase	TBARS	- thiobarbituric acid reactive substances

INTRODUCTION

In general, pharmaceuticals are among the most important environmental contaminants, as they can have adverse effects on a large number of non-target organisms – in particular, on growth and development. Residues of pharmaceuticals have been detected in the aquatic environment, especially in sewage treatment plants, effluents, surface waters, seawaters, and groundwaters (Halling-Sørensen *et al.* 1998; Golet *et al.* 2002; Golet *et al.* 2003; Fent *et al.* 2006, Carlssson *et al.* 2009; Santos *et al.* 2009; Camacho-Munoz *et al.* 2010). High levels of pharmaceuticals are not only detected in water but also in sediments (Zhou *et al.* 2011; Gibs *et al.* 2013).

Ciprofloxacin is an antibiotic from the group of fluorinated quinolones, which are frequently used in both human and veterinary medicine in the Czech Republic and worldwide. Fluoroquinolones are broad-spectrum antimicrobial agents against a wide range of gram-positive and gram-negative bacteria and their mechanism of action is the inhibition of DNA replication in bacteria by means of the disruption of the normal function of two key enzymes, DNA topoisomerase II (i.e., gyrase) and DNA topoisomerase IV (Wolfson & Hooper 1989; Arnoldi *et al.* 2013; Cheng *et al.* 2013).

Residues of ciprofloxacin in the aquatic environment have been detected worldwide in relatively high concentrations ranging from $ng.L^{-1}$ to $mg.L^{-1}$. Watkinson *et al.* (2009) reported a maximum ciprofloxacin concentration of $1.30\,\mu g.L^{-1}$ in surface water in Australia; Yang *et al.* (2013) found a concentration of $304\,ng.L^{-1}$ in river water samples in China. According to Kolpin *et al.* (2002), ciprofloxacin was present in samples from 115 streams throughout the United States (the maximum concentration was $0.03\,\mu g.L^{-1}$). Also, Gibs *et al.* (2013) discovered residues of ciprofloxacin in water samples from sites downstream of wastewater treatment plant discharges ($0.077\,\mu g.L^{-1}$). In Switzerland, ciprofloxacin was detected in raw sewage at concentrations ranging from 313 to $568\,ng.L^{-1}$ and in final effluents from 62 to $106\,ng.L^{-1}$ (Golet *et al.* 2002). In Italy, Castiglioni *et al.* (2008) found ciprofloxacin concentrations in river waters ranging from 17.4 to $558.5\,ng.L^{-1}$. Even higher concentrations of ciprofloxacin and other fluoroquinolones were detected in areas with insufficient wastewater management: in India, for example, Fick *et al.* (2009) found $14\,mg.L^{-1}$ of ciprofloxacin in the effluent of a treatment plant and $6.5\,mg.L^{-1}$ in lakes. Similarly, an extremely high level of ciprofloxacin ($31\,mg.L^{-1}$) was reported by Larsson *et al.* (2007) in the effluent of a treatment plant serving drug manufacturers in Patancheru (also in India).

Many studies have dealt with the acute effects of pharmaceuticals on organisms, especially on aquatic organisms (Halling-Sørensen *et al.* 2000; Robinson *et al.* 2005; Nie *et al.* 2009). Ciprofloxacin may pose a risk to the most sensitive aquatic organisms (Martins *et al.* 2012; Santos *et al.* 2013). Ciprofloxacin is toxic to the green algae *Selenastrum capricornutum* (EC_{50} was $2.97\,mg.L^{-1}$) and highly toxic to the cyanobacteria *Microcystis aeruginosa* (EC_{50} was $5–60\,\mu g.L^{-1}$) (Halling-Sørensen *et al.* 2000). For fish, ciprofloxacin has low acute toxicity (Halling-Sørensen *et al.* 2000; Zaleska-Radziwill *et al.* 2011; Martins *et al.* 2012).

It is necessary to learn more about the chronic effects of this and other fluoroquinolones on fish organisms and on early developmental stages in order to evaluate the range of the problem accurately. In many studies, biomarkers of oxidative stress have been used to evaluate toxic effects, because the large amounts of xenobiotics including pharmaceuticals in the aquatic environment have the potencial to induce oxidative stress in fish organisms (i.e. through the production of free radicals and reactive oxygen species) (Valavanidis *et al.* 2006; Brandao *et al.* 2013; Bartoskova *et al.* 2014; Nava-Alvarez *et al.* 2014).

The aim of this study was to assess the impact of ciprofloxacin in surface waters on fish under experimental conditions. In detail, the aim was to assess the effects of subchronic exposure to ciprofloxacin on fish growth and the development of histopathological changes in selected organs (gills, kidney, liver), and on the activity of some oxidative stress markers. Thus, the activities of the enzymes gluthathione S-tranferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), and catalase (CAT), and the products of the lipid peroxidation (thiobarbituric acid reactive substances – TBARS) were determined.

MATERIAL AND METHODS

Experimental fish

Ciprofloxacin toxicity tests were performed on *D. rerio*, which is one of the model organisms most commonly used in toxicity tests to determine the effects of chemicals and pharmaceuticals on fish in the aquatic environment (Hill *et al.* 2005; Scholz *et al.* 2008; Carlsson *et al.* 2009; Segner 2009; Plhalova *et al.* 2012; Bartoskova *et al.* 2013). Experimental procedures were in compliance with national legislation (Act No. 246/1992 Coll., on the Protection of Animals Against Cruelty, as amended, and Decree No. 419/2012 Coll., on the Protection, Breeding and Use of Experimental Animals, as amended).

The subchronic toxicity test

Tests were performed on *D. rerio* at the age of 30 days, according to OECD guideline No. 215 Fish, Juvenile Growth Test. Aqueous testing solutions of ciprofloxacin were prepared from the active compound produced by Sigma–Aldrich (St. Louis, MO, USA; chemical purity – 98.0%).

The fish were randomly distributed into 30 liter glass aquaria, 50 specimens per each. The experiment was conducted in a flow-through system and the volume of each test solution was replaced twice a day. The fish were exposed to a range of ciprofloxacin concentrations

(0.7 μg.L^{-1} – environmental concentration, 100, 650, 1 100 and 3 000 μg.L^{-1}) for 28 days. Each test on a ciprofloxacin-treated group was performed in duplicate. The average initial weight of fish used in the experiment was 9±0.8 g and the average initial total length of fish was 10.71±2.48 mm. The fish were fed with dried *Artemia salina* without nutshells to the amount of 8% of their body weight per day. The food ration was based on initial fish weights and was recalculated after 14 days. At the end of the tests, the fish were euthanized (using anaesthetic MS 222) and weighed, and their tank-average specific growth rates determined. Food was withheld from the fish 24 h prior to weighing.

Tank-average specific growth rates were calculated using the following formula according to OECD No. 215:

$$r = \frac{\overline{\log_e W_2} - \overline{\log_e W_1}}{t_2 - t_1} * 100$$

r - tank-average specific growth rate
W_1, W_2 - weights of a particular fish at times t_1 and t_2 respectively
$\overline{\log_e W_1}$ - average of the logarithms of the values W_1 for the fish in the tank at the start of the study period
$\overline{\log_e W_2}$ - average of the logarithms of the values W_2 for the fish in the tank at the end of the study period
t_1, t_2 - time (days) at the start and end of the study period

During the tests, living conditions were checked at 24-hour intervals and the number of dead fish was recorded in each concentration. The mean values for water quality were: temperature, 25±1 °C; oxygen saturation above 60% (ranging from 77% to 94%); and pH from 8.53 to 8.71. The basic chemical parameters of dilution water used were: COD_{Mn} (chemical oxygen demand), 1.1–1.2 mg.L^{-1}; total ammonia below the limit of determination (<0.04 mg.L^{-1}); NO_3^-, 15.5–16.8 mg.L^{-1}; NO_2^- below the limit of determination (<0.02 mg.L^{-1}); Cl^-, 18.2–18.9 mg.L^{-1}; Σ Ca±Mg, 12.3 mmol.L^{-1}.

Determination of ciprofloxacin concentrations

The measurement of ciprofloxacin concentrations was based on high-performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry (LC-ESI-MS/MS) (Babic *et al.* 2010). Samples were filtered through a 0.45-μm nylon filter (Millipore, Billerica, MA) and used for LC-ESI-MS/MS analysis. A Thermo Scientific UHPLC Accela 1250 system was connected to a Thermo Scientific TSQ Quantum Access MAX Triple Quadrupole Instrument (Thermo, San Jose, CA, USA) equipped with a heated electrospray ionization (HESI-II) probe. A Thermo Scientific Hypersil C_{18} (2.1 mm × 50 mm, 1.9 μm) column was used at a constant flow rate of 300 μL.min^{-1}. The mobile phase consisted of water containing 0.1% formic acid (v/v) (solvent A) and acetonitrile containing 0.1% formic acid (solvent B). The gradient used was: 0–2 min linear gradient from 30 to 70% of B; 2–2.5 min held at 70% of B; 2.5–3 min from 70 to 30% of B, and 3–3.1 min held at 30% of B in order for the column to re-equilibrate before the next injection. The full loop injection volume of the tissue extract was set at 10 μL. The heated electrospray ionization was operated in positive-ion mode under the following conditions: Capillary Temperature, 325.0 °C; Vaporizer Temperature, 300.0 °C; Sheath Gas Pressure, 35.0 psi; Auxiliary (drying) gas, 10 a.u.; and Spray Voltage, 3 300 V. Standards of ciprofloxacin as well as trichloroacetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents were residual analysis purity (Chromservis, PCL, CZ).

Histopathological examination

The fish (10 specimens from each concentration) were prepared for histopathological examination (of selected organs – gills, kidney, liver), fixed in buffered 10% neutral formalin, dehydrated, embedded in paraffin wax, sectioned on a microtome at a thickness of 4 μm, and stained with haematoxylin and eosin. Five sections from each fish were examined at different levels.

Fish sampling and homogenisation

At the end of the test, the fish were immediately frozen, and stored at –85 °C until analyses. Whole body samples were weighed and homogenised (1:10 w/v) using phosphate buffer (pH=7.2). The homogenate was divided into two portions, one for measuring TBARS and a second centrifuged (10 500 × *g*, 4 °C, 20 min) to obtain a supernatant fraction for the determination of GST, GPx, GR and CAT activities.

Measurement of oxidative stress parameters and detoxifying enzyme

The total catalytic concentration of GST was determined by measuring the conjugation of 1-chloro-2,4-dinitrobenzene with reduced glutathione at 340 nm (Habig *et al.* 1974). The specific activity was expressed as the nmol of the formed product per min per mg of protein. The catalytic concentration of GR was determined spectrophotometrically by measuring NADPH oxidation at 340 nm (Carlberg & Mannervik 1975). The catalytic concentration of GPx was calculated from the amount of NADPH oxidation to $NADP^+$ produced by the reaction with GR at 340 nm (Flohe & Gunzler 1984). The specific activities of GR and GPx were expressed as the nmol of NADPH consumption per min per mg of protein. The activity of CAT was determined spectrophotometrically by measuring the breakdown of H_2O_2 at 240 nm. The specific activity was expressed as the μmol of decomposed H_2O_2 per min per mg of protein (Aebi 1984). Protein concentrations were determined by a Bicinchoninic Acid Protein Assay Kit (Sigma-Aldrich, St. Louis, MO, USA) using bovine serum albumin as the standard (Smith

et al. 1985). To check for lipid peroxidation, malondialdehyde was measured by the TBARS method as described by Lushchak *et al.* (2005) at 535 nm. The concentration was expressed as nmol per gram wet weight of tissue.

Statistical analysis and estimation of NOEC and LOEC

All data were tested for normal distribution using the Shapiro-Wilk test. After testing for homogeneity of variance across groups (the Levene test), an analysis of variance (one-way ANOVA) was used. The differences between test groups with different concentrations and the control group were assessed using Dunnett`s test with $p<0.05$ chosen as the level of significance.

Estimation of the LOEC (lowest observed effect concentration) and NOEC (no observed effect concentration) was based on the identification of the lowest concentration at which differences in specific growth rates and the activities of oxidative stress markers were/were not significant at a 0.05 probability level, and, further, on the results of histopathological examination and the assessment of changes in fish behaviour.

RESULTS

Mortality and behaviour

In all ciprofloxacin-exposed groups and in the control group, mortality did not exceed 5% during the 28-day experimental period (no mortality was observed in the control group). Fish in all treated groups and control exhibited normal feeding behaviour during the experiment.

Growth rate

The initial body weights were not significantly different ($p>0.05$) between groups; at the end of the trial, average values of body weights and total lengths in all ciprofloxacin-exposed tanks were compared to the control group.

No significant differences ($p>0.05$) in specific growth rate between fish from the various ciprofloxacin concentrations and those from the control group were found.

Histopathological examination

Histopathological examination did not reveal any pathological lesions in any ciprofloxacin-exposed fish or in fish from the control group.

Oxidative stress markers and detoxifying enzyme

The effects of subchronic exposure to ciprofloxacin on selected oxidative stress markers and the activity of the detoxifying enzyme are presented in Table 1.

GST exhibited significantly higher activity ($p<0.01$) in the 0.7 and 100 $\mu g.L^{-1}$ groups compared to the control group. In the other experimental groups, the GST activity was lower than in the control group, but none of these differences were significant ($p>0.05$).

A decrease in GR activity was observed in fish exposed to ciprofloxacin at 650, 1 100 and 3 000 $\mu g.L^{-1}$, but only in the groups exposed to ciprofloxacin at 1 100 and 3 000 $\mu g.L^{-1}$ were the decreases significant ($p<0.05$).

Significantly lower GPx activity ($p<0.05$, $p<0.01$) was found in fish exposed to ciprofloxacin concentrations of 0.7, 650, 1 100 and 3 000 $\mu g.L^{-1}$ compared to the control group; however, no significant difference in GPx activity between the 100 $\mu g.L^{-1}$ group and the control group was observed.

No significance differences ($p>0.05$) between the control group and any experimental group were found with respect to CAT activity.

The TBARS level was significantly lower ($p<0.01$) in fish exposed to ciprofloxacin at a concentration of 100 $\mu g.L^{-1}$ compared to the control, while in the other experimental groups no significant differences compared to the control group ($p>0.05$) were observed.

Validity of the tests

Our tests met all conditions required by the OECD – mortality in the control groups was below 10%, the final weight of control fish was higher than 150% of the initial weight, the dissolved oxygen concentrations were at least 60%, the water temperature did not differ by more than ± 1 °C among test aquariums, and test substance concentrations were above 80% of the initial measured concentration.

Tab. 1. Comparison of the activities of glutathione S-transferase (GST, nmol.min^{-1}.mg protein^{-1}), glutathione reductase (GR, nmol.min^{-1}.mg protein^{-1}), glutathione peroxidase (GPx, nmol.min^{-1}.mg protein^{-1}), and catalase (CAT, µmol.min^{-1}.mg protein^{-1}) and the levels of thiobarbituric acid reactive substance (TBARS, nmol.g ww tissue^{-1}) after ciprofloxacin exposure between different treatment groups and control.

Indices	Concentration of ciprofloxacin (µg.L^{-1})					
	Control	0.7	100	650	1100	3000
GST	114.02±10.94	140.52±23.42**	132.73±14.70**	107.69±8.94	107.56±14.48	108.83±7.23
GR	10.15±1.07	10.11±1.80	10.79±0.71	8.97±1.71	8.35±1.39*	8.38±1.36*
GPx	41.89±10.89	26.82±7.19**	49.44±14.55	30.80±5.77*	28.43±6.46**	25.59±4.77**
CAT	88.01±4.98	78.86±2.36	86.33±3.02	83.89±2.98	85.61±5.66	92.66±4.07
TBARS	20.51±5.22	19.53±2.45	14.99±2.15**	19.10±3.03	22.14±3.22	19.64±3.56

(mean ± standard error of the mean) (*$p<0.05$), (**$p<0.01$).

DISCUSSION

Pharmaceuticals are among the most important contaminants of the environment. While the acute effects of many pharmaceuticals on aquatic organisms are known, there is little information about the chronic impacts of residues of ciprofloxacin or other fluoroquinolones on fish. Although ciprofloxacin is not acutely toxic to fish with respect to survival (Halling-Sørensen *et al.* 2000; Zaleska-Radziwill *et al.* 2011; Martins *et al.* 2012), exposure to antibiotics in the aquatic environment might pose a risk. In our experiment, mortality did not exceed 5%, which corresponds with the results of Robinson *et al.* (2005), who investigated the effects of seven fluoroquinolones on fish larvae. Six of these antibiotics (ciprofloxacin, lomefloxacin, ofloxacin, levofloxacin, enrofloxacin and flumequine) caused less than 8% mortality at a concentration 10 $mg.L^{-1}$, in contrast to clinafloxacin, which caused 100% mortality at the same dose.

Zaleska-Radziwill *et al.* (2011) found ciprofloxacin to have an impact on growth – specifically, the stimulation of growth in juvenile *D. rerio.* Robinson *et al.* (2005) also described an increase in weight in the larval stage of fathead minnows (*Pimephales promelas)* after 7 days of exposure to ciprofloxacin (10 $mg.kg^{-1}$). The same effect on weight they also observed in fish after treatments with levofloxacin and ofloxacin (10 $mg.L^{-1}$). However, in our study, we did not find any significant differences in growth rates in zebrafish exposed to any of the tested ciprofloxacin concentrations compared to the control group. Bartoskova *et al.* (2014) described the same results in a juvenile growth test with another fluoroquinolone, norfloxacin. Also, a study by Carlsson *et al.* (2009) showed no effect of fluoroquinolones on fish growth. They exposed embryos of zebrafish to diluted effluent (from a wastewater treatment plant in India) containing high levels of fluoroquinolones and other pharmaceuticals. The concentrations of 11 of the drugs (including 6 fluoroquinolones – lomefloxacin, norfloxacin, enoxacin, ofloxacin, enrofloxacin and ciprofloxacin) exceeded 100 $\mu g.L^{-1}$ Concentrations of ciprofloxacin ranged from 28 to 31 $mg.L^{-1}$ (Larsson *et al.* 2007; Carlsson *et al.* 2009). According to Carlsson *et al.* (2009), diluted effluent water (1–16% dilutions) had no effect on the body length of hatched zebrafish embryos or on hatching time. However, they recorded a significant decrease in the number of movements (at 8 and 16% dilution) with reduced or no pigmentation on the body and a reduced heart rate (at 16% dilution). Because the effluent water also contained other pharmaceuticals at high concentrations, these results cannot be associated only with the action of fluoroquinolones.

Oxidative stress biomarkers are used for evaluation of the effects of pharmaceuticals and other pollutants on fish organisms. Fish are endowed with defensive mechanisms to counteract the impact of reactive oxygen species resulting from the metabolism of various chemicals or xenobiotics (Nwani *et al.* 2010). Our results showed significant differences in the activities of oxidative stress markers in all experimental groups, even at the environmental concentration of ciprofloxacin compared to the control group.

Glutathione S-transferase is the detoxifying enzyme of phase II that provides cellular protection against the toxic effects of a variety of environmental and endogenous substances (Di Giulio & Hinton 2008). Significantly increased GST activity was observed only in groups exposed to ciprofloxacin concentrations of 0.7 and 100 $\mu g.L^{-1}$. Similarly, Bartoskova *et al.* (2014) found an increase in GST activity only at the environmental concentration of norfloxacin (0.1 $\mu g.L^{-1}$), while in the other tested groups (0.1, 1, 10 and 30 $mg.L^{-1}$), the activity was comparable to the control. This might indicate that low concentrations of fluoroqiunolones led to the activation of GST. By contrast, high levels caused the depletion of the detoxifying system or disorder of the enzyme synthesis. Wang *et al.* (2009) studied the effects of enrofloxacin in relation to density stress on *Pangasianodon hypophtalmus.* Fish were fed with pellets containing 1 $g.kg^{-1}$ enrofloxacin for 7 days. Enrofloxacin accumulates in the liver, where it is converted into ciprofloxacin by cytochrome P450 enzymes (during this experiment the maximum ciprofloxacin level was only 3% of the maximum of enrofloxacin concentration). They detected no effect on the activity of GST in the liver or brain; however, GST activity in gills was lower in the tank with low fish density (40 $fish.m^{-3}$) compared to the tanks with medium or high fish density (80 and 120 $fish.m^{-3}$, respectively).

Glutathione peroxidase metabolizes H_2O_2 and also reduces fatty acid peroxides. This enzyme, which can act on a variety of organic peroxides, catalyzes the oxidation of reduced glutathione to glutathione disulfide (Chance *et al.* 1979; Di Giulio & Hinton 2008). The activity of GPx in our study with ciprofloxacin showed a significant decrease in contrast to Bartoskova *et al.* (2014), who reported an increased in GPx activity in all tested groups (except the environmental concentration of norfloxacin).

Glutathione reductase catalyzes the transformation of glutathione disulfide to reduced glutathione (Di Giulio & Hinton 2008). A significant decrease in GR activity was observed at the highest ciprofloxacin concentrations (1 100 and 3 000 $\mu g.L^{-1}$). This can be explained by the assumption that the highest concentrations led to a decrease in enzyme activity. In contrast, Bartoskova *et al.* (2014) found no differences in the activity of GR at any concentration of norfloxacin compared to the control.

Catalase is an enzyme that provides the conversion of H_2O_2 into water and oxygen (Wang *et al.* 2009). Even though the activity of CAT in all tested groups with ciprofloxacin was comparable to the control, Bartoskova *et al.* (2014) reported a significant increase in CAT activity in fish exposed to norfloxacin. Likewise, Wang

et al. (2009) reported changes in CAT activities in the gills and brain of fish after enrofloxacin treatment, but found no effect in the liver.

Many organisms, especially aquatic ones, contain high amounts of unsaturated lipids, which are a substrate for oxidation (their oxidation usually leads to the production of peroxides). To monitor lipid peroxidation, methods based on the measurement of end products are used (malondialdehyde) (Lushchak 2011). In the present study, a significant decrease in TBARS level was noted only at 100 μg.L^{-1} of ciprofloxacin. Similarly, in a study with norfloxacin, Bartoskova *et al.* (2014) did not find any effect of ciprofloxacin on lipid peroxidation. Wang *et al.* (2009) found significant differences in lipid peroxidation levels in the gills and brain of fish fed with pellets containing enrofloxacin compared to the control. In contrast, no effect was observed in the liver of these fish.

In the light of the above findings, it was concluded that even the lowest tested concentration of ciprofloxacin (the environmental concentration) caused significant changes in some oxidative stress markers (GST, GPx); hence, the NOEC value could not be detemined. The LOEC value for ciprofloxacin for juvenile *D. rerio* after 28 days of exposure was 0.7 μg L^{-1}.

CONCLUSIONS

According to our results, the subchronic exposure of *Danio rerio* to ciprofloxacin did not affect fish growth and did not cause the development of histopathological changes in the fish organism. However, we can conclude that ciprofloxacin (even at the environmental concentration) may have a negative impact on some biochemical processes which are connected with the production of reactive oxygen species and free radicals in the fish organism.

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REFERENCES

1 Aebi H (1984). Catalase in vitro. Method Enzymol. **105**: 121–126.
2 Arnoldi E, Pan XS, Fisher LM (2013). Functional determinants of gate-DNA selection and cleavage by bacterial type II topoisomerases. Nucl Acis Res. **40**: 9411–9423.
3 Babic S, Pavlovic DM, Aperger D, Perisa M, Zrncic M, Horvat AJM, et al (2010). Determination of multi-class pharmaceuticals in wastewater by liquid chromatography-tandem mass spectrometry (LC-MS-MS). Anal Bioanal Chem. **398**: 1185–1194.
4 Bartoskova M, Dobsikova R, Stancova V, Zivna D, Blahova J, Marsalek P, et al (2013). Evaluation of ibuprofen toxicity for zebrafish (*Danio rerio*) targeting on selected biomarkers of oxidative stress. Neuroendocrinol Lett. **34**(Suppl 2): 102–108.
5 Bartoskova M, Dobsikova R, Stancova V, Pana O, Zivna D, Plhalova L, et al (2014). Norfloxacin – toxicity for zebrafish (*Danio rerio*) focused on oxidative stress parameters. BioMed Res Int. **2014**: 6 pages DOI:10.1155/2014/560235.
6 Brandao FP, Rodrigues S, Castro BB, Goncalves F, Antunes SC, Nunes B (2013). Short-term effects of neuroactive pharmaceutical drugs on a fish species: Biochemical and behavioural effects. Aquat Toxicol. **144**: 218–229.
7 Camacho-Munoz D, Martin J, Santos JL, Aparicio I, Alonso E (2010). Occurrence, temporal evolution and risk assessment of pharmaceutically active compounds in Doñana Park (Spain). J Hazard Mater. **183**: 602–608.
8 Carlberg I, Mannervik B (1975). Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J Biol Chem. **250**: 5475–5480.
9 Carlsson G, Örn S, Larsson DGJ (2009). Effluent from bulk drug production is toxic to aquatic vertebrates. Environ Toxicol Chem. **28**: 2656–2662.
10 Castiglioni S, Pomati F, Miller K, Burns BP, Zuccato E, Calamari D, et al (2008). Novel homologs of the multiple resistance regulator marA in antibiotic-contaminated environments. Water Res. **42**: 4271–4280.
11 Chance B, Sies H, Boveris A (1979). Hydroperoxide metabolism in mammalian organs. Physiol Rev. **59**: 527–605.
12 Cheng G, Hao H, Dai M, Liu Z, Yuan Z (2013). Antibacterial action of quinolones: From target to network. Eur J Med Chem. **66**: 555–562.
13 Di Giulio RT, Hinton DE, editors (2008). The Toxicology of Fishes. Boca Raton: CRC Press, Taylor and Francis Group.
14 Fent K, Weston AA, Camidada D (2006). Ecotoxicology of human pharmaceuticals. Aquat Toxico. **76**: 122–159.
15 Fick J, Söderström H, Lindberg RH, Phan C, Tysklind M, Larsson DGJ (2009). Contamination of surface, ground, and drinking water from pharmaceutical production. Environ Toxicol Chem. **28**: 2522–2527.
16 Flohe L, Gunzler WA (1984). Assays of glutathione peroxidase. Method Enzymol. **105**: 114–121.
17 Gibs J, Heckathorn HA, Meyer MT, Klapinski FR, Alebus M, Lippincott RL (2013). Occurrence and partitioning of antibiotic compounds found in the water column and bottom sediments from a stream receiving two wastewater treatment plant effluents in Northern New Jersey, 2008. Sci Total Environ. **458**: 107–116.
18 Golet ME, Alder AC, Giger W (2002). Environmental exposure and risk assessemnt of fluoroquinolone antibacterial agents in wastewater and river water of the Glatt Valley Watershed, Switzerland. Environ Sci Technol. **36**: 3645–3651.
19 Golet ME, Xifra I, Siegrist H, Alder AC, Giger W (2003). Environmental exposure assessment of fluoroquinolone antibacterial agent from sewage to soil. Environ Sci Technol. **37**: 3243–3249.
20 Habig WH, Pabst MJ, Jakoby WB (1974). Glutathione S-transferases. First enzymatic step in mercapturic acid formation. J Biol Chem. **249**: 7130–7139.
21 Halling-Sørensen B, Nors Nielsen S, Lanzky PF, Ingerslev F, Holten Lützhøft HC, Jørgensen SE (1998). Occurrence, fate and effects of pharmaceuticals substances in the environment – a review. Chemosphere. **36**: 357–393.
22 Halling-Sørensen B, Holten Lützhøft HC, Andersen HR, Ingerslev F (2000). Environmental risk assessment of antibiotics: comparison of mecillinam, trimetoprim and ciproloxacin. J Antimicrob Chemoth. **46**(Suppl. S1): 53–58.
23 Hill, AJ, Teraoka, H, Heideman, W, Peterson RE (2005). Zebrafish as a model vertebrate for investigating chemical toxicity. Toxicol Sci. **86**: 6–19.
24 Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, et al (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. Environ Sci Technol. **36**: 1202–1211.
25 Larsson DGJ, de Pedro C, Paxeus N (2007). Effluent from drug manufactures contains extremely high levels of pharmaceuticals. J Hazard Mater. **148**: 751–755.

26 Lushchak VI, Bagnyukova TV, Lushchak OV, Storey JM, Storey KB (2005). Hypoxia and recovery perturb free radical processes and antioxidant potential in common carp (*Cyprinus carpio*) tissues. Int J Biochem Cell Biol. **37**: 1319–1330.

27 Lushchak VI (2011). Environmentally induced oxidative stress in aquatic animals. Aquat Toxicol. **101**: 13–30.

28 Martins N, Pereira R, Abrantes N, Pereira J, Goncalves F, Marques CR (2012). Ecotoxicological effects of ciprofloxacin on freshwater species: data integration and derivation of toxicity threshold for risk assessment. Ecotoxicology. **21**: 1167–1176.

29 Nava-Alvarez R**,** Razo-Estrada AC, Garcia-Medina S, Gomez-Olivan LM, Galar-Martinez M (2014). Oxidative stress induced by mixture of diclofenac and acetaminophen on common carp (*Cyprinus carpio*). Water Air Sol Polut. **225**: 1873, DOI: 10.1007/s11270-014-1873-5.

30 Nie X, Gu J, Lu J, Pan W, Yang Y (2009). Effects of norfloxacin and butylated hydroxyanisole on the freshwater microalga Scendesmus obliquus. Ecotoxicology. **18**: 677–684.

31 Nwani CD, Lakra WS, Nagpure NS, Kumar R, Kushwaha B, Srivastava SK (2010). Toxicity of the herbicide atrazine: Effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish *Channa punctatus* (Bloch). Int J Env Res Pub He. **7**: 3298–3312.

32 OECD Guidelines for testing chemicals, No. 215: Fish, Juvenile growth test. OECD, Paris, 2000.

33 Plhalova L, Stepanova S, Blahova J, Praskova E, Hostovsky M, Skoric M, et al (2012). The effects of subchronic exposure to terbuthylazine on zebrafish. Neuroendocrinol Lett. **33**(Suppl 3): 113–119.

34 Robinson AA, Belden JB, Lydy MJ (2005). Toxicity of fluoroquinolone antibiotics to aquatic organisms. Environ Toxicol Chem. **24**: 423–430.

35 Santos JL, Aparicio I, Callejón M, Alonso E (2009). Occurrence of pharmaceutically active compounds during 1-year period in wastewaters from four wastewater treatment plants in Seville (Spain). J Hazard Mater. **164**: 1509–1516.

36 Santos LHMLM, Gros M, Rodriguez-Mozaz S, Delerue-Matos C, Pena A, Barcelo D, et al (2013). Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters: Identification of ecologically relevant pharmaceuticals. Sci Total Environ. **461–462**: 302–316.

37 Scholz S, Fischer S, Gundel U, Kuster E, Luckenbach T, Voelker D (2008). The zebrafish embryo model in environmental risk assessment-applications beyond acute toxicity testing. Environ Sci Pollut Res Int. **15**: 394–404.

38 Segner H (2009). Zebrafish (*Danio rerio*) as a model organism for investigating endocrine disruption. Comp Biochem Physiol C Toxicol. Pharmacol. **149**: 187–195.

39 Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, et al (1985). Measurement of protein using bicinchoninic acid. Anal Biochem. 150: 76–85.

40 Valavanidis A, Vlahogianni T, Dassenakis M, Scoullos M (2006). Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxic Environ Safe. **64**: 178–189.

41 Wang N, Nkejabega N, Hien NN, Huynh TT, Silvestre F, Phuong NT, et al (2009). Adverse effects of enrofloxacin when associated with environmental stress in Tra catfish (*Pangasianodon hypophthalmus*). Chemosphere. **77**: 1577–1584.

42 Watkinson AJ, Murby EJ, Kolpin DW, Constanzo SD (2009). The occurrence of antibiotics in an urban watershed: From wastewater to drinking water. Sci Total Environ. **407**: 2711–2723.

43 Wolfson JS, Hooper DC (1989). Fluoroquinolone Antimicrobial Agents. Clin Microbiol Rev. **2**: 378–424.

44 Yang X, Chen F, Meng F, Xie Y, Chen H, Young K, et al (2013). Occurrence and fate of PPCPs and correlations with water quality parameters in urban riverine waters of the Pearl River Delta, South China. Environ Sci Pollut R. **20**: 5864–5875.

45 Zaleska-Radziwill M, Lebkowska M, Affek K, Zarzeczna A (2011). Environmental risk assessment of selected pharmaceuticals present in surface waters in relation to animals. Environ Prot. 37: 31–42.

46 Zhou LJ, Ying GG, Zhao JL, Yang JF, Wang L, Yang B, et al (2011). Trends in the occurrence of human and veterinary antibiotics in the sediments of the Yellow River, Hai River and Liao River in northern China. Environ Pollut. **159**: 1877–1885.