Short-term exposure to carbamazepine causes oxidative stress on common carp (*Cyprinus carpio*)

Eloy Gasca-Pérez[a,b,*], Marcela Galar-Martínez[b,**], Sandra García-Medina[b], Isabel A. Pérez-Coyotl[b], Karina Ruiz-Lara[b], Selene Cano-Viveros[b], Ricardo Pérez-Pastén Borja[d], Leobardo M. Gómez-Oliván[c]

[a] *Cátedra CONACYT, Laboratory of Aquatic Toxicology, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Unidad Profesional Adolfo López Mateos, AV. Wilfrido Massieu s/n y cerrada Manuel Stampa, Col. Industrial Vallejo., Ciudad de México, CP, 07700, Mexico*
[b] *Laboratory of Aquatic Toxicology, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Unidad Profesional Adolfo López Mateos, AV. Wilfrido Massieu s/n y cerrada Manuel Stampa, Col. Industrial Vallejo., Ciudad de México, CP, 07700, Mexico*
[c] *Laboratory of Environmental Toxicology, Facultad de Química, Universidad Autónoma del Estado de México, Paseo Colón intersección Paseo Tollocan s/n. Col. Residencial Colón, 50120, Toluca, Estado de México, Mexico*
[d] *Laboratory of Molecular and Cellular Toxicology, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Unidad Profesional Adolfo López Mateos, AV. Wilfrido Massieu s/n y cerrada Manuel Stampa, Col. Industrial Vallejo., Ciudad de México, CP, 07700, Mexico*

A R T I C L E   I N F O

A B S T R A C T

The aim of this research was to determine the bioconcentration factor and if subacute exposure to carbamazepine ($2\,mg\,L^{-1}$) modifies the oxidative state of liver, gills and brain of *Cyprinus carpio*. This was measured through the following biomarkers: hydroperoxide and protein carbonyl content, lipid peroxidation degree, as well as superoxide dismutase, catalase and glutathione peroxidase activity. Carbamazepine concentration in carp's tissue was also determined by liquid chromatography with a diode arrangement detector. An increase in lipid peroxidation degree, hydroperoxide and protein carbonyl content, and a decrease in the activity of the antioxidant enzymes ($P < 0.05$) with respect to control was observed. Also, there is an increase in the concentration of carbamazepina present in the organs with respect to the water in the system, which denotes bioconcentration of the drug. In conclusion, carbamazepine is bioconcentrated and produces oxidative stress on the common carp (*C. carpio*).

1. Introduction

Pharmaceutical products and their metabolites arrive through sewage waters to treatment plants (Ternes, 1998; Metcalfe et al., 2003; Bartelt-Hunt et al., 2009; Writer et al., 2013; Estrada-Arriaga et al., 2016; Kot-Wasik et al., 2016); where their elimination can be incomplete or none, due to the fact that removal processes are not designed to eliminate these kinds of organic compounds. In consequence, these emerging pollutants are then discharged into treatment plants effluents and later detected in superficial (Ternes, 1998; Metcalfe et al., 2003; Bartelt-Hunt et al., 2009) and subterranean waters (López-Serna et al., 2013), as well as water destined for human consumption (Kot-Wasik et al., 2016). A group of pharmaceutical products is neuroactive

compounds. These have special relevance because they are biologically active prescription synthetic organic compounds. These drugs at very low concentrations can produce modifications to metabolism and central nervous system communications through a variety of mechanisms of action or toxicity. (Ramírez et al., 2009). An example of this pollutants is carbamazepine (CBZ, 5H-dibenzo [b,f] azepine-5-carboxamide); it is a tricyclic compound consisting of three aromatic rings connected by an azepine ring (seven-member unsaturated heterocyclic ring with a nitrogen atom) (Wishart et al., 2018). CBZ is a prodrug anticonvulsant that inhibits voltage-dependant sodium channels upon biotransformation in the liver (Mesdjian et al., 1999; Lipkind and Fozzard, 2010). It is utilized in the treatment of partial and tonic-clonic crisis, neuropathic pain, trigeminal neuralgia, manic-depressive disorders and aggressive

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behavior due to dementia (Tomson et al., 1980; Fritze et al., 1991; Quinn et al., 2004; Samara et al., 2016). Being one of the majorly prescribed pharmaceuticals – with a total volume of consumption of 1014 tons globally– it is the most commonly found neuroactive in aquatic environments (Zhang and Geißen, 2010; Hamer et al., 2012; Brandão et al., 2013). Thus, Ternes (1998) detected concentrations that ranged from 25 up to 110 ng L^{-1} de CBZ in rivers and streams of central Germany. Also, two rivers and a lake in Sweden showed quantities between 10 and 400 ng L^{-1} (Tixier et al., 2003). A more recent study showed concentrations between 0.1 and 2.6 ng L^{-1} in twenty-four Minnesota rivers (Writer et al., 2013). This leads to thinking of CBZ as a marker of anthropogenic activity in water bodies (Clara et al., 2004; Hai et al., 2018). In the other hand, the removal of CBZ in treatment plants using common processes - such as activated sludge, nitrification-denitrification and oxidation pits - has been found to be less than 40% (Heberer and Feldmann, 2005; Zhang and Geißen, 2010; Writer et al., 2013; Samara et al., 2016; Bo et al., 2017). Estrada-Arriaga et al. (2016) measured the presence of diverse pharmaceutical families in two Mexico residual waters treatment plants effluents, founding CBZ concentrations between 29.5 to 244 ng L^{-1}, and a maximal remotion efficiency of 33%. However, there are reports that indicate CBZ reaching concentrations close to 1 mg L^{-1} (Lester et al., 2013).

Oxidative stress – which can be induced by pharmaceuticals – is defined as the loss of balance between ROS and the antioxidant system in an individual, leading to macromolecular damage by increasing lipids peroxidation (LPOX) and the proteins degradation, as well as enzymatic inactivation (Gómez-Oliván et al., 2014; Galar-Martínez et al., 2015). *Cyprinus carpio* is a teleost species used as bioindicator due to its wide global distribution, economical significant, sensitivity to xenobiotic exposure and laboratory conditions adaptability (Karan et al., 1998; Oruç and Uner, 2002; Saucedo-Vence et al., 2015; Galar-Martínez et al., 2015). Li et al. (2010a, b, c) showed that high concentrations between 0.2 and 2 mg L^{-1} cause an increase in LPOX degree and carbonylated proteins (CP), plus a reduction SOD, GR and GPx activity in *Cyprinus carpio* sperm, after two hours in vitro exposure, however, there are not study about the effects of CBZ on oxidative status in this teleost. Additionally, the most recent review of the occurrence and effects of CBZ in fish trials is limited to zebrafish and rainbow trout, so our study will provide data on the potential risk of this drug to other aquatic organisms (Hai et al., 2018). Therefore, the objective of the present study is to establish the bioaccumulation of CBZ in liver, gills and brain, through subacute exposure to 2 mg L^{-1}, as well as the toxic effect produced by the drug in tissues using oxidative damage and antioxidant activity biomarkers.

2. Methods and materials

2.1. Test organisms acquisition and maintenance

The carps (*Cyprinus carpio)* used for the experiment had an average weight of 12.9 g. They were acquired from the Tiacaque Carp Center in Estado de México and later brought to the laboratory, where they were acclimated for two weeks. During that period, individuals were kept in 80 L glass aquariums equipped with a filtering system, a light:dark 12:12 cycle and constant aeration. Carps were fed three times a day with Nutripec (Purina®) pellets.

2.2. Test substance

CBZ (number CAS 298-46-4, > 99% purity) was proportioned by Armstrong Laboratories of Mexico (Lote 11010076) $C_{15}H_{12}N_2O$, 236.26 uma. 210 mg of CBZ were dissolved in DMSO (Sigma-Aldrich Corporation Mexico) in order to obtain the stock chemical solution for toxicological tests.

2.3. Determination of CBZ bioconcentration factor

2.3.1. Standard solution

The standard solution was prepared in acetonitrile. The mother solution concentration was 1000 µg mL^{-1}, while a 0.75 µg mL^{-1} one was used for the recovery tests. Concentrations of 0.25, 0.5, 1, 2.5, 5 y 10 µg mL^{-1} were used for the concentration curve. Linear regression coefficient (R^2) for CBZ was > 0.99.

2.3.2. Equipment

The HPLC system consisted of an Infinity HPLC 1260 Agilent unit (Santa Clara CA). A C18 Nova Pack chromatography column (3.9 x 300 mm, 4 µm, Waters), maintained at 25 °C, was used. The mobile phase was a mixture of water-acetonitrile in a 40:60 v/v proportion, a flow of 1.0 ml min^{-1}, a run time of 10 min and an injection volume of 40 µL. CBZ was identified and quantized via a diode arrangement detector (Agilent, Santa Clara CA) with a wavelength of 220 nm (Demirkaya and Kadioğlu, 2005). It was expressed as µg of CBZ g of tissue^{-1}. Detection limit (MDL) and quantification limit (MQL) of the method were 0.69 and 2.11 respectively using typic error of calibration curve.

2.3.3. Carbamazepine extraction and quantification

Six individuals were exposed to 2 mg L^{-1} of CBZ for 96 h. The organisms were then euthanized by immersion in water with clove essential oil (Yamanaka et al., 2011; Fernandes et al., 2017). After that, liver, gills and brain were extracted by dissection. A pool was made for each organ, to which 5 mL of deionized water were added, for later sonication for 10 min. 5 mL of chloroform and 50 µL of ammonium hydroxide at 25% were added to the resulting mixture. Samples were mechanically agitated for 20 min and centrifuged at 3000 rpm for 10 min (Dordević et al., 2009). Organic phase was separated in a falcon tube, to which 0.5 g of DisQue dispersion salt (dihydrate tribasic sodium citrate 15%, sodium chloride 15%, sodium hydrogen carbonate sesquihydrate 5–10%, magnesium sulfate 60–65%, Waters) were added. The tube was mechanically agitated and centrifuged at 3500 rpm for 5 min. The supernatant was then filtered using a nylon membrane with 0.22 µm porum and dried off with air flow. The final sample was reconstituted with a 100 µL of acetonitrile for subsequent injection in the HPLC equipment. A control group of organisms exposed to DMSO at 0.0001% was formed. This group was later tested on the same conditions as exposed fishes. Recovery percentage of tissue was 97.21%. Also, 0.5 mL of ammonium hydroxide and 10 mL of chloroform were added to 10 mL of water taken from the exposure systems. The mix was mechanically agitated and centrifuged at 3000 rpm for 10 min. The organic phases were retrieved, while the watery phase was extracted two more times. 0.5 g of DisQue dispersion salt (dihydrated tribasic sodium citrate 15%, sodium chloride 15%, sodium hydrogen carbonate sesquihydrate 5–10%, magnesium sulfate 60–65 %, Waters) were added to the 5 mL mix and concentrated organic phases. The tube was mechanically agitated and centrifuged at 3500 rpm for 5 min. The supernatant was then filtered using a nylon membrane with 0.22 µm porum and dried off with air flow. The final sample was reconstituted with 100 µL of acetonitrile for further injection into the HPLC equipment with a 101.04% recovery. The experiments were performed three times, while the chromatograph injections were done twice.

2.4. Subacute toxicity assays

The exposure systems consisted of 5 containers with a final volume of 20 liters (one for each exposure time). 6 carps were placed in each container. Each experiment was repeated three times. 2 mg of CBZ L^{-1} were used, concentration at which the *Cyprinus carpio* sperm oxidative state is modified, according to Li et al., 2010a, b, c. Exposure periods of 12, 24, 48, 72 and 96 h were used. An exposure control group per time was used. Once the exposure time elapsed, individuals were removed

off of the systems, and euthanized by immersion in 50 mg L^{-1} of clove essential oil (Yamanaka et al., 2011; Fernandes et al., 2017). The liver, gills and brain were then extracted. The tissues were individually weighted and placed in tubes with 1.5 mL of buffer phosphates pH 7.4. Samples were homogenized and a 1000 μL fraction was centrifuged at 12,500 rpm and 4 °C for 15 min. Both fractions were stored at -70 °C. The following biomarkers were tested with the supernatant of the centrifuged fraction: total proteins (PT), protein carbonyl content (PCC), SOD, CAT and GPx activity. On the other hand, hydroperoxide content (HPX) and LPOX degree was quantized with the homogenate. The results were normalized with the PT per g concentration of tissue.

All procedures were performed in accordance to the guide for care and use of animals in the laboratory by the National Institutes of Health (NIH No. 8023), as well as production, care and use of lab animals technical specifications according to the Official Mexican Norm (NOM-062-ZOO-1999, 2001). The protocol was submitted for evaluation and approved by the Bioethics Committee of the Escuela Nacional de Ciencias Biológicas, IPN.

2.4.1. Determination of lipid peroxidation degree

400 μL of phosphate solution pH 7.4 and 1 mL of TBA-TCA [thiobarbituric acid 0.375% (TBA, Sigma-Aldrich) en trichloroacetic acid at 15% (TCA, Sigma-Aldrich)] reagent were added to 100 μL of homogenate. The mix was vortex agitated and water bathed until ebullition for 15 min. Once tempered, the samples were centrifuged at 3500 rpm for 10 min. The supernatant (200 μL) was transferred to a 96 dredges plate, where absorbance was measured at 540 nm with respect to the reagent blank (Büege and Aust, 1978). The malondialdehyde concentration (MDA) was obtained by absorbance interpolation in a 1,1,3,3-tetraethoxypropane curve (TEP). The results were expressed in micromoles (μM) MDA/mg PT/g tissue (Karatas et al., 2002).

2.4.2. Determination of hydroperoxide content

200 μL of TCA at 15% were added to 200 μL of the homogenate. After 5 min at room temperature, the mix was centrifuged at 10 000 rpm for 10 min. 800 μL of reagent solution [hydroxytoluene butyrate 4 mM, xylenol orange 0.01 mM in methanol at 90% and Fe$_2$SO$_4$ 0.25 mM in HCl 250 mM (Sigma)] were added to 200 μL of supernatant. The mix was then brought for 60 min incubation at room temperature. Absorbance was measured against reagent blank, while the concentration was determined by data interpolation on a cumene pattern curve (cumene hydroperoxide, Sigma). Results were expressed as nanomoles (nM) of cumene mg^{-1} of PT (Gay et al., 1999).

2.4.3. Determination of protein carbonyls content

150 μL of dinitrophenylhydrazine 10 mM were added to 100 μL of supernatant in HCl 2 M (Sigma). The mix was incubated in darkness and at room temperature for 60 min. Later, 500 μL of cold TCA at 6% were added, and the mix was left to rest for 15 min. The precipitate was washed three times with an ethanol/ethyl acetate mix in 1:1 proportion, dissolved in 1 mL of guanidine 6 M (pH 2.3). Absorbance was measured at 366 nm. Results were expressed as nM of formed protein carbonyles (PCC) mg^{-1} PT g^{-1} tissue, based in a molar extinction coefficient (CEM) of 21 000 mM cm^{-1} (Levine et al., 1994; Parvez and Raisuddin, 2005; Burcham, 2007).

2.4.4. Quantification of superoxide dismutase activity

The commercial kit RANSOD (RANDOX) was used to determine SOD activity. 7.5 μL of sample, 200 μL of R1a reagent [Xanthine 0.05 mM and 2-(4-iodophenyl)-3-(4-nitrophenol)-5- phenyl tetrazolium chloride (I.N.T) 0.025 mM reconstituted in buffer R1b (CAPS 40 mM and EDTA 0.94 mM, pH 10.2)] and 40 μL of R2 (Xanthine oxidase 80U/I) were added in a 96 wells plate. The plate was mixed and absorbance was measured in an ELx800 reader (BioTek) at 490 nm at 30 s and 210 s. Readings were interpolated in a pattern curve using the calibrator included in the kit. Results were expressed in U SOD per mg of protein per g of tissue.

2.4.5. Quantification of catalase activity

960 mL of isolation buffer solution pH 7.4 (sucrose 0.3 M, EDTA 1 mM, HEPES 5 mM and KH$_2$PO$_4$ 5 mM) and 200 μL of H$_2$O$_2$ 20 mM were added to 40 μL of supernatant. Absorbance was measured at 240 nm at 0 s and 60 s. The following equation was used to get the results: CAT activity = (A$_0$-A$_{60}$)/CEM (0.043 nM cm^{-1}). These were expressed as mM of H$_2$O$_2$ mg^{-1} PT g^{-1} (Radi et al., 1991).

2.4.6. Quantification of glutathione peroxidase activity

Commercial kit RANSEL (RANDOX) was used to determine GPx activity. 10 μL of sample, 500 μL of R1a [Glutathione 4 mM, Glutathione reductase ≥ 0.5 U L^{-1}, NADPH 0.34 mM reconstituted in buffer R1b (Phosphate buffer 0.05 mM, EDTA 4.3 mM, pH 7.2)], and 40 μL de R2 (Cumene hydroperoxide 0.18 mM) were mixed in a quartz cell. Reagents were mixed and absorbance was measured in a spectrophotometer (Metash) at 340 nm at 0, 60, 120 and 180 s. The ΔA/min was multiplied by a 8412 factor. Results were expressed as U L^{-1} of hemolyzed per mg of protein per g of tissue.

2.4.7. Determination of protein content

2.5 μL of supernatant and 7.5 μL of deionized water were mixed on a 96 wells plate. 250 μL of Bradford reagent were then dispensed (0.05 g Coomassie blue, 25 mL of ethanol at 96%, and 50 mL H$_3$PO$_4$, in 500 mL of deionized water). Once the reagent was added, the plate was agitated and incubated for 5 min at room temperature. Absorptivity was evaluated at 595 nm. This results were interpolated in a bovine albumin pattern curve (Bradford, 1976).

2.5. Statistical analysis

Biomarkers results for the subacute toxicity essays were statistically evaluated by a two way variance analysis. Differences between measures (P < 0.05) were compared using a Tukey *pos hoc* test. Statistical tests were elaborated with the SigmaPlot v14 software (Systat Software, Inc, Germany).

3. Results

3.1. Quantification of carbamazepine and determination of bioconcentration factor

Table 1 shows the bioconcentration factors (BCF) of CBZ on different organs of exposed carps. It is observed that the toxic has low BCF in liver, gills and brain of individuals exposed for 96 h.

3.2. Oxidative stress

3.2.1. Determination of lipid peroxidation degree

Fig. 1 shows LPOX degree expressed as MDA content per mg of protein. An important increase (p ≤ 0.05) in liver's LPOX degree can be noted at 12 h (70.12%) and 48 h (78.9%). Gills present a notable reduction (p ≤ 0.05%) of MDA concentration at 12 h (34.67%), with a

Table 1
CBZ concentrations in *Cyprinus carpio* tissues after 96 h. Results were expressed as mean of six samples ± S. E.

Type of sample	CBZ concentration (μg g^{-1})	Bioconcentration factor (ratio [tissue]/[water])
Water	147.420 ± 5.487[a]	–
Liver	41.44 ± 4.45	0.28
Gills	63.32 ± 3.47	0.43
Brain	42.25 ± 5.31	0.29

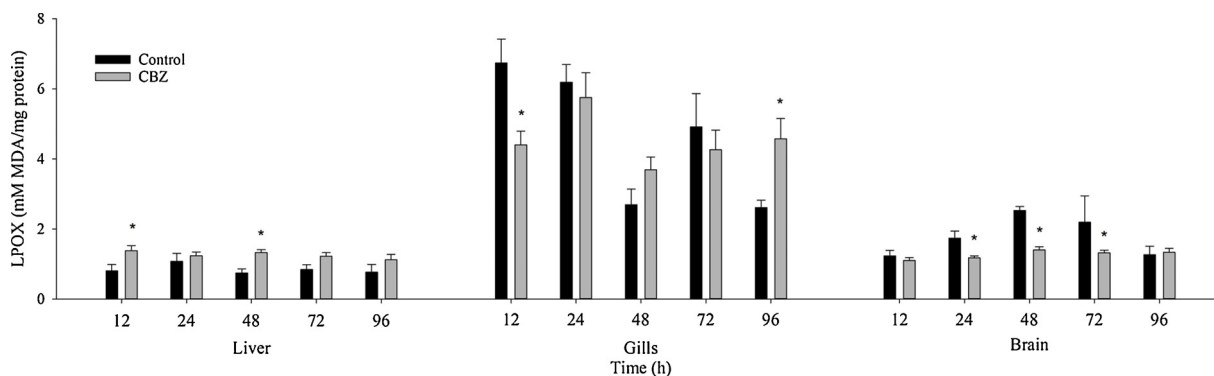[a] Concentration in water is expressed in μg mL^{-1}.

Fig. 1. Lipid peroxidation (LPOX) in liver, gills and brain of *C. carpio* exposed for 12, 24, 48, 72 and 96 h to carbamazepine concentration of 2 mg L^{-1} (CBZ). Values are the mean of three replicates ± SEM for each exposure time. N = 180. MDA = malondialdehyde. * Significantly different (p < 0.05) from the control group. Two-way ANOVA and Tukey test.

posterior increase that becomes significant at 96 h. At this time, LPOX degree reaches 75.25% with respect to control (p ≤ 0.05). On the other hand, an important reduction (p ≤ 0.05) in LPOX degree is observed in the brain at 24 (32.45%), 48 (44.50%) and 72 h (39.86%) with respect to the control sample.

3.2.2. Determination of hydroperoxide content

Fig. 2 shows the results for hydroperoxide content. The liver showed an increase in HPOX content, which was notable at 12 (233.16%), 48 (483.65%), 72 h (354.54%) and 96 h (209.26%) in comparison to control (p ≤ 0.05). In like manner, gills of exposed organisms showed an important increase (p ≤ 0.05) at 12 (571.14%), 48 (279.03%), 72 (132.11%) and 96 h (475.22%). Likewise, brains showed especially high LPOX levels at 12 (749.55%), 24 (193.18%) and 48 h (450.46%) with respect to control.

3.2.3. Determination of protein carbonyls content

Results for protein carbonyl content are presented in Fig. 3. All three organs suffered an increase in PCC levels. This was significant at 12 h for gills (111.74%) (p ≤ 0.05), while this biomarker showed a tendency to increase at 12 h for brain and 24 h for liver.

3.2.4. Quantification of superoxide dismutase activity

Results for SOD activity are shown in Fig. 4. An important reduction (p ≤ 0.05) in liver's enzymatic activity was registered at 12 (41.5%), 24 (57.81%), 48 (73.84%), 72 (66.28%) and 96 h (62.93%) in comparison to the control group. Gills also presented a reduction in SOD activity (p ≤ 0.05). This was notable at 12 h (78.91%), followed by a maximum decay at 24 h (90.67%), and then a constant decrease until the 96 h (88.84%). Similarly, in brain SOD activity decreases significantly,

starting at 12 h (63.89%), and then following a decaying tendency until inhibition of 72.65% at 96 h, with respect to control.

3.2.5. Quantification of catalase activity

Catalase activity is shown in Fig. 5. Comparably to SOD activity, there is an alarming decrease in CAT levels in all organs. Liver shows a significant reduction (p ≤ 0.05) that starts at 12 h (91.02%) followed by a decreasing tendency at 24 (82.9%), 48 (57.96%), 72 (89.81%) and 96 h (87.59%). Likewise, gills exhibit a significant reduction in the activity of this enzyme. This behavior starts at 12 h (73.52%), reaches a low of 72 h (69.35%) and continues to fall until 96 h (56.27%). CAT activity in the brain of exposed carps shows a similar behavior, presenting important reductions (p ≤ 0.05) at 12 (96.17%), 24 (91.89%), 48 (88.37%), 72 (90.25%) and 96 h (92.11%).

3.2.6. Quantification of glutathione peroxidase activity

GPx activity is shown in Fig. 6. This variable shows different tendencies between studied organs. Liver exhibited a significant increase (p ≤ 0.05) only at 48 h (84.09%), followed by a reduction at 72 h (41.27%). On the other hand, the activity of GPx in gills presented a reduction at all times, being important (p ≤ 0.05) at 12 (67.17%), 24 (71.54%) and 96 h (66.69%). Brain shows a similar behavior as that of gills. Important reductions were found at 12 (58.82%), 24 (56.61%) and 72 h (64.70%).

4. Discussion

It is a fact that the drugs detected in various environmental matrices are in contact with aquatic species, generating harmful effects on their physiology, because although the concentrations in ecosystems do not
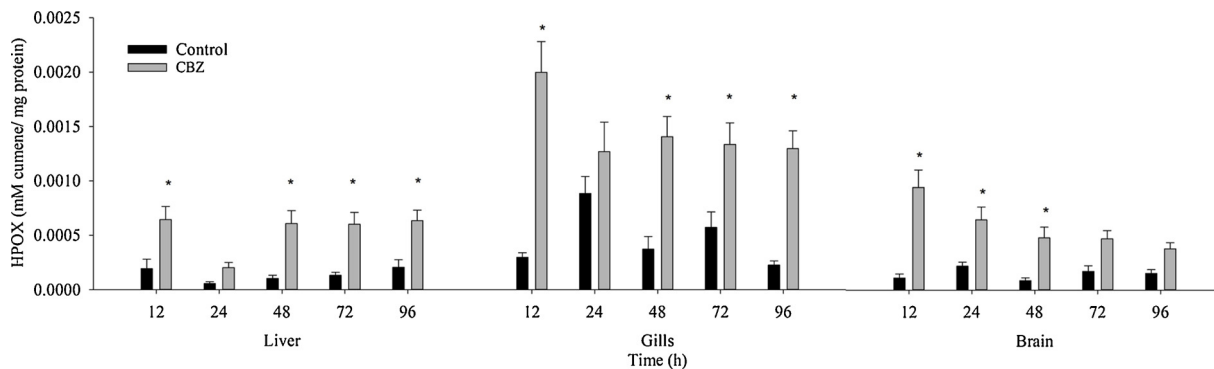

Fig. 2. Hydroperoxide content (HPOX) in liver, gills and brain of *C. carpio* exposed for 12, 24, 48, 72 and 96 h to carbamazepine concentration of 2 mg L^{-1} (CBZ). Values are the mean of three replicates ± SEM for each exposure time. N = 180. * Significantly different (p < 0.05) from the control group. Two-way ANOVA and Tukey test.
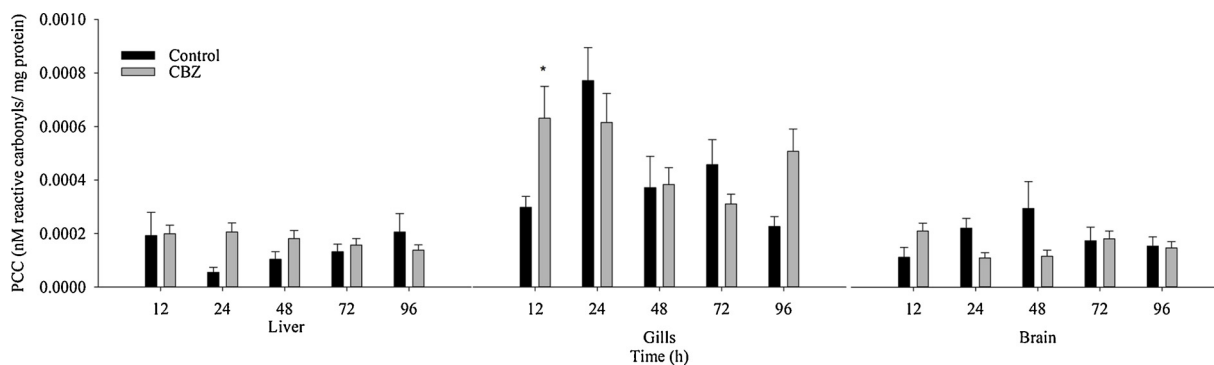
Fig. 3. Protein carbonyl content (PCC) in liver, gills and brain of *C. carpio* exposed for 12, 24, 48, 72 and 96 h to carbamazepine concentration of 2 mg L^{-1} (CBZ). Values are the mean of three replicates ± SEM for each exposure time. N = 180. * Significantly different (p < 0.05) from the control group. Two-way ANOVA and Tukey test.

exert toxic effects in humans can exert it in non-target species, such as fish. (Hoeger et al., 2008). CBZ is highly persistent in water and therefore represents a risk for aquatic species, since this can be absorbed by the organisms and bioconcentrate (Hai et al., 2018). Bioaccumulation is defined as the relationship between the concentration of a xenobiotic in tissues and the aquatic medium in steady state or equilibrium, which is influenced by the lipophilicity of the toxic, the concentration of oxygen in water, and the amount of lipids in the target organs (van der Oost et al., 2003). CBZ has a log K$_{OW}$ of 2.45 (Dal Pozzo et al., 1989), so it is classified as a molecule with low affinity to fatty tissues, however, it has been established that certain chemical compounds with log K$_{OW}$ < 5 have high values of bioconcentration and bioaccumulation (Arnot and Gobas, 2006; Valdés et al., 2016). Thus, García et al. (2012) found that in plasma, liver, brain and muscle of *Pimephales notatus* and *Ictalurus punctatus* exposed to CBZ, bioconcentration factors between 1.6 and 7.1 were present in both species under laboratory conditions; while in field studies with *Oreochromis niloticus*, bioaccumulation between 2.8 and 3.8 was observed. Our results are consistent with those obtained in tests performed on *Oncorhyncus mykiss* exposed to concentrations of 1.4 and 35.4 µg L-1 CBZ per 10 d where plasma bioconcentration factors of 0.4 and 0.3 were observed, respectively (Lahti et al., 2011). On the other hand, in wild *Carassius carassius* and *Cyprinus carpio* obtained from treatment plant streams in Japan, a bioaccumulation factor for CBZ of between 0.42–1.15 was observed for plasma, liver, kidney, muscle and gills (Tanoue et al., 2015). Variations in the distribution of CBZ in various tissues between studies depend on the conditions and time of exposure, as well as the bioindicator species used in each experiment. In addition, Huggett et al. (2004) concluded that CBZ is rapidly bioconcentrated (24 h) in plasma and then decreased (96 h), due to biotransformation processes of this drug in *Oncorhynchus mykiss*, which would explain the poor

accumulation of CBZ in the studied tissues.

ROS play a key role in maintaining the homeostasis of organisms, since they function as second cellular messengers mediating cascades of signaling, gene expression, maintenance of vascular integrity, spermatogenesis and others (Forman et al., 2010). When there is an over production of ROS and a failure in the antioxidant defense systems, the so-called oxidative stress is generated. Organic hydroperoxides and peroxides are common products of this process and they interact with cellular components, such as membrane lipids, proteins and genetic material (Gay et al., 1999). Our research determined that HPOX increases significantly in all three organs assessed, while LPOX shows this increasing behavior only in liver and gills with respect to control. This behavior was already observed in diverse studies performed on *Cyprinus carpio*, but using different drug families such as non-steroidal anti-inflammatories (Islas-Flores et al., 2013), antibiotics (Elizalde-Velázquez et al., 2017) and antihypertensives (Cortes-Diaz et al., 2017). This is associated to the biotransformation process of each xenobiotic, as well as biotic and abiotic degradation processes. The biotransformation of CBZ is carried out in the liver through the intervention of certain families of cytochrome P450 (CYP1, CYP2 and CYP3) that have the capacity to bioactivate the drug, but also to oxidize lipids by generating epoxyheicosatrienoic acid, leucotoxins, thromboxanes or prostacyclins (Massey and Nicolaou, 2011; Ayala et al., 2014). On the other hand, some cytochrome families like CYP1A, CYP2C, CYP2D and CYP3A, can form 10 and 11-epoxide adducts from CBZ. These can induce the formation of organic peroxides and later membrane lipids oxidation (Mesdjian et al., 1999). Gills show an increase in HPOX content starting at 12 h, and of the LPOX degree at 96 h, which matches studies performed on *Oncorhynchus mykiss*, where concentrations of 19.9 mg L^{-1} (CL$_{50}$) produce a similar behavior in exposed organisms gills (Li et al., 2011). This may be because this organ is committed to the exchange of
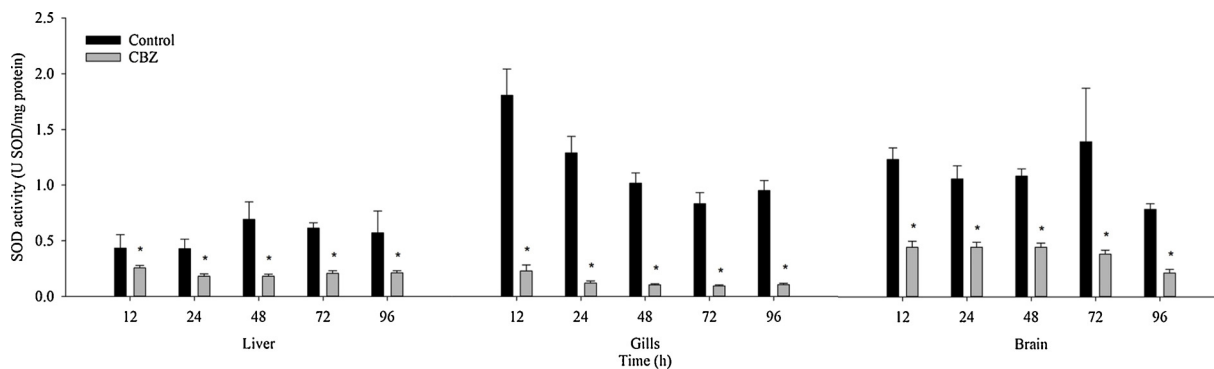

Fig. 4. Superoxide dismutase (SOD) activity in liver, gills and brain of *C. carpio* exposed for 12, 24, 48, 72 and 96 h to carbamazepine concentration of 2 mg L^{-1} (CBZ). Values are the mean of three replicates ± SEM for each exposure time. N = 180. * Snificantly different (p < 0.05) from the control group. Two-way ANOVA and Tukey test.
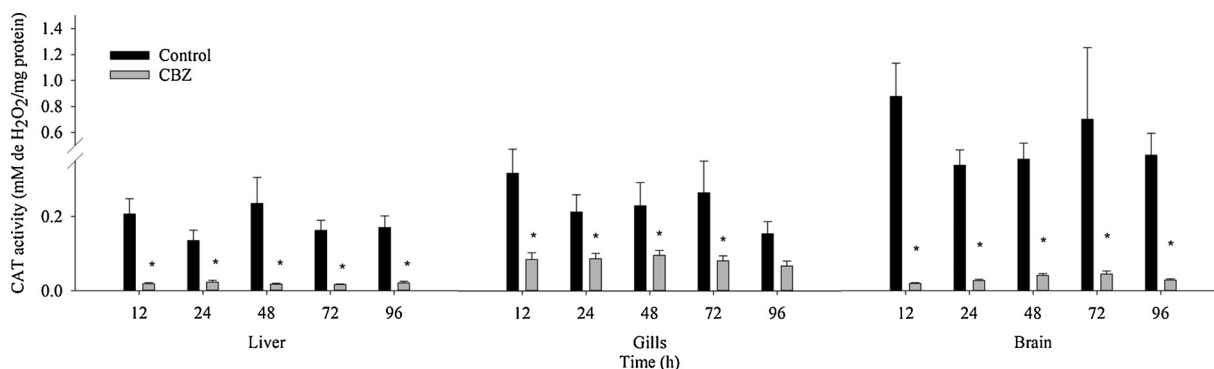
Fig. 5. Catalase (CAT) activity in liver, gills and brain of *C. carpio* exposed for 12, 24, 48, 72 and 96 h to carbamazepine concentration of 2 mg L^{-1} (CBZ). Values are the mean of three replicates ± SEM for each exposure time. N = 180. * Significantly different (p < 0.05) from the control group. Two-way ANOVA and Tukey test.

gases and the regulation of the concentration of ions such as Na$^+$, K$^+$ and Cl-, as well as being the first organ to come into contact with pollutants in water (Neri-Cruz et al., 2015). HPOX can be increased due to the high production of metabolites as epoxides, catechols and quinones derived from the biotransformation of CBZ (Williams and Naisbitt, 2002). These have the ability to interact with the oxidative environment derived from energy metabolism in gaseous exchange in the gills, generating ROS that may interact with lipids, proteins or nucleic acids (German and Kinsella, 1986). In our study, HPOX was increased from 12 to 48 h, followed by a decay of the time-dependent concentration, this could be due to the biotransformation processes of the drug or adaptive processes of brain tissue. Additionally, the degree of LPOX was significantly reduced between 24 h and 72 h with respect to control in the brain, which is an organ containing high concentrations of easily oxidized fatty acids (Yin et al. 2011). In a study conducted in *Oncorhynchus mykiss*, where brain homogenate was exposed to concentrations of 2 mg L^{-1} CBZ, an increase in the LPOX level was observed at 120 min of exposure. (Li et al., 2010c). Subsequently, another study was carried out showing the same behavior in brains of complete organisms of the same species exposed 96 h to 19.9 mg L^{-1} of CBZ (Li et al., 2011), in contrast to our results. However, there are differences between experimental designs that involve the route and time of exposure, the concentration of the toxicant and the variability between species, which is known to modify the toxicant response (Valdés et al., 2016; Eaton and Gilbert, 2013). On the other hand, CBZ action mechanism as antidepressant is related to changes in the arachidonic acid cascade, by repressing cytosolic phospholipase A2. This prevents the phospholipids hydrolysis and diminishes the amount of arachidonic acid available for the oxidative metabolism (Vernouillet et al., 2010; García et al., 2012). This could explain our findings.

Free radicals such as HO• can react with chain amino acids like valine and leucine, generating the corresponding hydroperoxides.

These react with other amino acids, like alanine, causing fragmentation and rearrangement processes that result in carbonyls and alcohols (Headlam and Davies, 2003). These subproducts are capable of joining thiol groups, which generates a loss of sulfhydryl groups and changes in amino acid resonance. This alters the structure and function of proteins (Gómez-Olivan et al. 2014; Galar-Martínez et al., 2015). Our results show that in the carps exposed to CBZ an increase is observed during the first exposure times in the three organs evaluated (12 h and 24 h). This can be associated with the rapid bioconcentration of CBZ in the first hours, followed by a decay related with biotransformation processes in the organisms (Hugget et al. 2004). However, PCC increase in given times can be related to the formation of high affinity CBZ abducts by these type of macromolecules (Pearce et al., 2005; Kang et al., 2008). These results are consistent with studies in *Oncorhynchus mykiss*, where no significant changes in PCC content were observed in individuals exposed by 7, 21 and 42 d (Li et al., 2010a), suggesting that HPOX content and LPOX degree were more sensitive biomarkers in the assessment of oxidative damage (Li et al., 2010b).

To the stimulus of ROS, organisms respond with defense mechanisms involving low molecular weight antioxidants (such as ascorbate, glutathione, and tocopherol) and enzymatic antioxidants such as SOD is a metalloenzyme that catalyze the conversion of the O$_2$$^{•-}$ anion to H$_2$O$_2$, which is subsequently degraded by CAT and GPx. (Gaté et al., 1999; Deavall et al., 2012). In this study the activity of SOD, CAT and GPx was significantly reduced with respect to the control group in the three organs examined, which is consistent with what was observed in spermatozoa of *Cyprinus carpio* exposed to a concentration similar to that used in our experiments (2 mg L^{-1}), concluding that there is a negative correlation between the activity and exposure time for the antioxidant enzymes activity (Li et al., 2010b). Under physiological conditions, the genes of the antioxidant enzymes are over expressed in response to oxidative stress, however, the damage to macromolecules
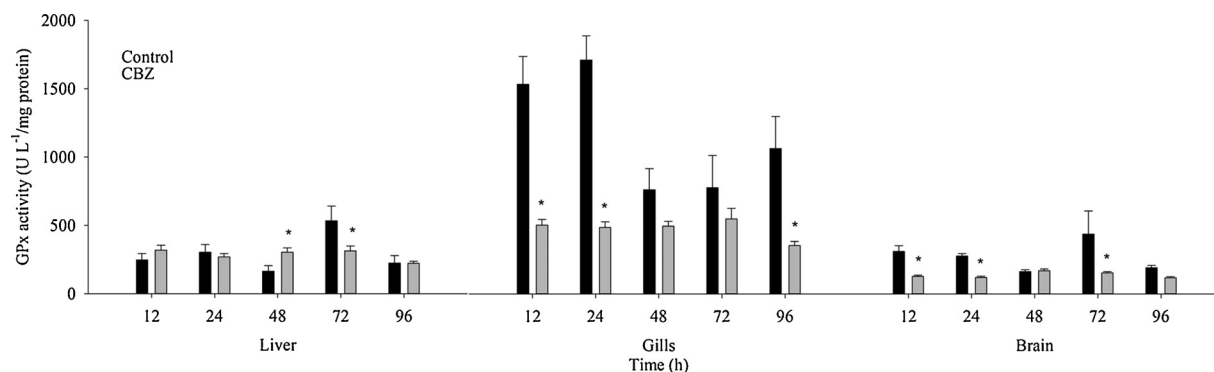

Fig. 6. Glutathion peroxidase (GPx) activity in liver, gills and brain of *C. carpio* exposed for 12, 24, 48, 72 and 96 h to carbamazepine concentration of 2 mg L^{-1} (CBZ). Values are the mean of three replicates ± SEM for each exposure time. N = 180. * Significantly different (p < 0.05) from the control group. Two-way ANOVA and Tukey test.

by ROS generates accumulation of conformationally altered and inactive enzymes (Landis and Tower, 2005). This behavior may be due to damage to enzymes consistent with elevation of PCC content (Zhang et al., 2008; Galar-Martínez et al., 2015; Aguirre-Martínez et al., 2016), due to the formation of CBZ metabolites such as areno oxides, quinones, iminoquinones, catecholones or epoxides (Pearce et al., 2005; Kang et al., 2008). Results show that sublethal concentrations of CBZ can alter aquatic organisms physiology, situation that can arise in water bodies that have been impacted by emergent pollutants. This makes the organisms susceptible to inflammation processes, DNA damage, necrosis and apoptosis by oxidative processes, which can lead to death.

In conclusion, our results suggest that subacute exposure (96 h) to the concentration of CBZ (2 mg L^{-1}) can be bioconcentrate in the liver, gill and brain of *Cyprinus carpio*, also generate oxidative stress by increasing hydroperoxides content and lipoperoxidation degree as well as a dramatic reduction in antioxidant activity in the tissues analyzed, demonstrating that *Cyprinus carpio* is a good model to study the effect of emerging high persistence pollutants and generating a precedent for future studies on the dynamics of these in a species of ecological and economic value.

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References

Aguirre-Martínez, G.V., DelValls, T.A., Martín-Díaz, M.L., 2016. General stress, detoxification pathways, neurotoxicity and genotoxicity evaluated in *Ruditapes philippinarum* exposed to human pharmaceuticals. Ecotoxicol. Environ. Saf. 124, 18–31. https://doi.org/10.1016/j.ecoenv.2015.09.031.

Arnot, J.A., Gobas, F.A., 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in acuatic organisms. Environ. Rev. 14, 257–297. https://doi.org/10.1139/a06-005.

Ayala, A., Muñoz, M.F., Argüelles, S., 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hidroxi-2-nonenal. Oxid. Med. Cell. Longev. 2014, 31. https://doi.org/10.1155/2014/360438. Article ID 360438.

Bartelt-Hunt, S.L., Snow, D.D., Damon, T., Shockley, J., Hoagland, K., 2009. The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and Surface waters in Nebraska. Environ. Pollut. 157, 786–791. https://doi.org/10.1016/j.envpol.2008.11.025.

Bo, L., He, K., Tan, N., Gao, B., Feng, Q., Liu, J., Wang, L., 2017. Photocatalytic oxidation of trace carbamazepine in aqueous solution by visible-light-driven ZnIn$_2$S$_4$: Performance and mechanism. J. Environ. Manag. 190, 259–265. https://doi.org/10.1016/j.jenvman.2016.12.050.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Brandão, F.P., Rodrigues, S., Castro, B.B., Gonçalves, F., Antunes, S.C., Nunes, B., 2013. Short-term effects of neuroactive pharmaceuticals drugs on a fish species: biochemical and behavior effects. Aquat. Toxicol. 144-145, 218–229. https://doi.org/10.1016/j.aquatox.2013.10.005.

Burcham, P.C., 2007. Modified protein carbonyl assay detects oxidised membrane proteins: a new tool for assessing drug- and chemically-induced oxidative cell injury. J. Pharmacol. Toxicol. Methods 56 (1), 18–22. https://doi.org/10.1016/j.vascn.2006.02.015.

Clara, M., Strenn, B., Kreuzinger, N., 2004. Carbamazepine as a possible anthropogenic marker in the aquatic environment: investigations on the behavior of carbamazepine in wastewater treatment and during groundwater infiltration. Water Res. 38, 947–954. https://doi.org/10.1016/j.watres.2003.10.058.

Cortes-Diaz, J.A., Rodríguez-Flores, J., Castañeda-Peñalvo, G., Galar-Martínez, M., Islas-Flores, H., Dublán-García, O., Gómez-Oliván, L.M., 2017. Sublethal effects induced by captopril on *Cyprinus carpio* as determined by oxidative stress biomarkers. Sci. Total Environ. 605-606, 811–823. https://doi.org/10.1016/j.scitotenv.2017.06.208.

Dal Pozzo, A., Donzelli, G., Rodríquez, L., Tajana, A., 1989. "In vitro" model for the evaluation of drug distribution and plasma protein-binding relationship. Int. J. Pharm. 50, 97–101. https://doi.org/10.1016/0378-5173(89)90133-6.

Deavall, D.G., Martin, E.A., Horner, J.M., Roberts, R., 2012. Drug-induced oxidative stress and toxicity. J. Toxicol. 2012, 645460. https://doi.org/10.1155/2012/645460.

Demirkaya, F., Kadioğlu, Y., 2005. Determination of carbamazepine using RP-HPLC method in pharmaceutical preparations. FABAD. J. Pharm. Sci. 30, 78–82.

Dordević, S., Kilibarda, V., Stojanović, T., 2009. Determination of carbamazepine in serum and saliva samples by high performance liquid chromatography with

ultraviolet detection. Vojnosanit. Pregl. 66 (5), 347–352.

Eaton, E.L., Gilbert, S.G., 2013. Principles of toxicology. In: Klaassen, C.D. (Ed.), Toxicology The Basic Science of Poisons, 8th edn. Mc Graw Hill, Kansas, pp. 11–46.

Elizalde-Velázquez, A., Martínez-Rodríguez, H., Galar-Martínez, M., Dublán-García, O., Islas-Flores, H., Rodríguez-Flores, J., Castañeda-Peñalvo, G., Lizcano-Sanz, I., Gómez-Oliván, L.M., 2017. Effect of amoxicillin exposure on brain, gill, liver, and kidney of common carp (Cyprinus carpio): the role of amoxicilloic acid. Environ. Toxicol. 32 (4), 1102–1120. https://doi.org/10.1002/tox.22307.

Estrada-Arriaga, E.D., Córtes-Muñoz, J.E., Gónzalez-Herrera, A., Calderón-Mólgora, C.G., Rivera-Huerta, M.L., Ramírez-Camperos, E., Montellano-Palacios, L., Gelover-Santiago, S.L., Pérez-Castrejon, S., Cardoso-Vigueros, L., Martín-Domínguez, A., García-Sánchez, L., 2016. Assessment of full-scale biological nutrient removal system upgraded with physico-chemical processes for the removal of emerging pollutants present in wastewaters from Mexico. Sci. Total Environ. 571, 1172–1182. https://doi.org/10.1016/j.scitotenv.2016.07.118.

Fernandes, I.M., Bastos, Y.F., Barreto, D.S., Lourenço, L.S., Penha, J.M., 2017. The efficacy of clove oil as an anaesthetic and in euthanasia procedure for small-sized tropical fishes. Braz. J. Biol. 77 (3). https://doi.org/10.1590/1519-6984.15015. Epub Sep 26, 2016.

Forman, H.J., Maiorino, M., Ursini, F., 2010. Signaling functions of reactive oxygen species. Biochemestry 49 (5), 835–842. https://doi.org/10.1021/bi9020378.

Fritze, J., Unsorg, B., Lanczik, M., 1991. Interaction between carbamazepine and fluvoxamine. Acta Psychiatr. Scand. 84, 583–584.

Galar-Martínez, M., García-Medina, S., Gómez-Oliván, L.M., Pérez-Coyotl, I., Mendoza-Monroy, D.J., Arrazola-Morgain, R.E., 2015. Oxidative stress and genotoxicity induced by ketorolac on the common carp Cyprinus carpio. Environ. Toxicol. 31 (9), 1035–1043. https://doi.org/10.1002/tox.22113.

García, S.N., Foster, M., Constantine, L.A., Huggett, D.B., 2012. Field and laboratory fish tissue accumulation of anti-convulsant drug carbamazepine. Ecotox. Environ. 84, 207–211. https://doi.org/10.1016/j.ecoenv.2012.07.013.

Gaté, L., Paul, J., Ba, G., Tew, K.D., Tapiero, H., 1999. Oxidative stress induced in pathologies: the role of antioxidants. Biomed. Pharmacother. 53, 169–180. https://doi.org/10.1016/S0753-3322(99)80086-9.

Gay, C., Collins, J., Gebicki, J.M., 1999. Hydroperoxide assay with ferric-xylenol orange complex. Anal. Biochem. 273, 149–155. https://doi.org/10.1006/abio.1999.4208.

German, J.B., Kinsella, J.E., 1986. Hydroperoxide metabolism in trout gill tissue: effect of glutathione on lipoxygenase products generated from arachidonic acid and docosahexaenoic acid. Biochim. Biophys. Acta 879 (3), 378–387.

Gómez-Oliván, L.M., Galar-Martínez, M., García-Medina, S., Valdés-Alanís, A., Islas-Flores, H., Neri-Cruz, N., 2014. Genotoxic response and oxidative stress induced by diclofenac, ibuprofen and naproxen in Daphnia magna. Drug Chem. Toxicol. 37 (4), 391–399. https://doi.org/10.3109/01480545.2013.870191.

Hai, F.I., Yang, S., Asif, M.B., Sencadas, V., Shawkat, S., Sanderson-Smith, M., Gorman, J., Xu, Q., Yamamoto, K., 2018. Carbamazepine as a possible anthropogenic marker in water: occurrences, toxicological effects, regulations and removal by wastewater treatment technologies. Water 10 (2), 107. https://doi.org/10.3390/w10020107.

Hamer, H.M., Dodel, R., Strzelczyk, A., Balzer-Geldsetzer, M., Reese, J.P., Schöffski, O., Graf, W., Schwab, S., Knake, S., Oertel, W.H., Rosenow, F., Kostev, K., 2012. Prevalence, utilization and costs of antiepileptic drugs for epilepsy in Germany – nationwide population-based study in children and adults. J. Neurol. 259, 2376–2384. https://doi.org/10.1007/s00415-012-6509-3.

Headlam, H.A., Davies, M.J., 2003. Cell-mediated reduction of protein and peptide hydroperoxides to reactive free radicals. Free Radic. Biol. Med. 34 (1), 44–55.

Heberer, T., Feldmann, D., 2005. Contribution of effluents from hospitals and private households to the total loads of diclofenac and carbamazepine in municipal sewage effluents-modeling versus measurements. J. Hazard. Mater. 122, 211–218. https://doi.org/10.1016/j.jhazmat.2005.03.007.

Hoeger, B., Dietrich, D.R., Schmid, D., Hartmann, A., Hitzfeld, B., 2008. Distribution of intraperitoneally injected diclofenac in brown trout (Salmo trutta f. fario). Ecotoxicol. Environ. Saf. 71 (2), 412–418. https://doi.org/10.1016/j.ecoenv.2007.10.020.

Huggett, D.B., Ericson, J.F., Cook, J.C., Williams, R.T., 2004. Plasma concentrations of human pharmaceuticals as predictors of pharmacological responses in Fish. In: Kümmerer, K. (Ed.), Pharmaceuticals in the Environment, 2nd edn. Springer, Freiburg, pp. 373–386.

Islas-Flores, I., Gómez-Oliván, L.M., Galar-Martínez, M., Colín-Cruz, A., Neri-Cruz, N., García-Medina, S., 2013. Diclofenac-induced oxidative stress in brain, liver, gill and blood of common carp (*Cyprinus carpio*). Ecotoxicol. Environ. Saf. 92, 32–38. https://doi.org/10.1016/j.ecoenv.2013.01.025.

Kang, P., Liao, M., Wester, M.R., Leeder, J.S., Pearce, R.E., Correira, M.A., 2008. CYP3A4-mediated carbamazepine (CBZ) metabolism: formation of a covalent CBZ-CYP3A4 adduct and alteration of the enzyme kinetic profile. Drug Metab. Dispos. 36 (3), 490–499. https://doi.org/10.1124/dmd.107.016501.

Karan, V., Vitorović, S., Tutundžić, V., Poleksić, V., 1998. Functional enzymes activity and gill histology of carp after copper sulfate exposure and recovery. Ecotoxicol. Environ. Saf. 40 (1), 49–55. https://doi.org/10.1006/eesa.1998.1641.

Karatas, F., Karatepe, M., Baysar, A., 2002. Determination of free malondialdehyde in human serum by high-performance liquid chromatography. Anal. Biochem. 311, 76–79. https://doi.org/10.1016/S0003-2697(02)00387-1.

Kot-Wasik, A., Jakimska, A., Śliwka-Kaszyńska, M., 2016. Occurrence and seasonal variations of 25 pharmaceutical residues in wastewater and drinking wáter treatment plants. Environ. Monit. Assess. 188, 661. https://doi.org/10.1007/s10661-016-5637-0.

Lahti, M., Brozinski, J.M., Jylhä, A., Kronberg, L., Oikari, A., 2011. Uptake from water, biotransformation, and biliary excretion of pharmaceuticals by rainbow trout. Environ. Toxicol. Chem. 30 (6), 1403–1411. https://doi.org/10.1002/etc.501.

Landis, G.N., Tower, J., 2005. Superoxide dismutase evolution and life span regulation.

Mech. Ageing Dev. 126 (3), 365–379. https://doi.org/10.1016/j.mad.2004.08.012.

Lester, Y., Mamane, H., Zucker, I., Avisar, D., 2013. Treating wastewater from a pharmaceutical formulation facility by biological process and ozone. Water Res. 47, 4349–4356. https://doi.org/10.1016/j.watres.2013.04.059.

Levine, R.L., Williams, J.A., Stadtman, E.R., Shacter, E., 1994. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol. 233, 346–357.

Li, H., Li, P., Rodina, M., Randak, T., 2010a. Effect of human pharmaceutical carbamazepine on the quality parameters and oxidative stress in common carp (Cyprinus carpio L.) spermatozoa. Chemosphere 80, 530–534. https://doi.org/10.1016/j.chemosphere.2010.04.046.

Li, Z.H., Li, P., Randak, T., 2010b. Effect of a human pharmaceutical carbamazepine on antioxidant response in brain of model teleost in vitro: an efficient approach to biomonitoring. J. Appl. Toxicol. 30, 644–648. https://doi.org/10.1002/jat.1534.

Li, Z.H., Velisek, J., Zlabek, V., Grabic, R., Machova, J., Kolarova, J., Randak, T., 2010c. Hepatic antioxidant status and hematological parameters in rainbow trout Oncorhynchus mykiss, after chronic exposure to carbamazepine. Chem. Biol. Interact. 183, 98–104. https://doi.org/10.1016/j.cbi.2009.09.009.

Li, Z.H., Zlabek, V., Velisek, J., Grabic, R., Machova, J., Kolarova, J., Li, P., Randak, T., 2011. Acute toxicity of carbamazepine to juvenile rainbow trout (Oncorhynchus mykiss): Effects on antioxidant responses, hematological parameters and hepatic EROD. Ecotoxicol. Environ. Saf. 74, 319–327. https://doi.org/10.1016/j.ecoenv.2010.09.008.

Lipkind, G.M., Fozzard, H.A., 2010. Molecular model of anticonvulsant drug binding to the voltage-gated sodium channel inner pore. Mol. Pharmacol. 78 (4), 631–638. https://doi.org/10.1124/mol.110.064683.

López-Serna, R., Jurado, A., Vázquez-Suñé, E., Carrera, J., Petrović, M., Barceló, D., 2013. Occurrence of 95 pharmaceuticals and transformation products in urban groundwaters underlying the metrópolis of Barcelona, Spain. Environ. Pollut. 174, 305–315. https://doi.org/10.1016/j.envpol.2012.11.022.

Massey, K.A., Nicolaou, A., 2011. Lipidomics of polyunsaturated fatty-acid-derived oxygenated metabolites. Biochem. Soc. Trans. 39 (5), 1240–1246. https://doi.org/10.1016/j.freeradbiomed.2012.08.565.

Mesdjian, E., Sérée, E., Charvet, B., Mirrione, A., Bourgarel-Rey, V., Desobry, A., Barra, Y., 1999. Metabolism of carbamazepine by CYP3A6: a model for in vitro drug interactions studies. Life Sci. 64 (10), 827–835.

Metcalfe, C.D., Koenig, B.G., Bennie, D.T., Servos, M., Ternes, T.A., Hirsch, R., 2003. Ocurrence of neutral and acidic drugs in the effluents of Canadian sewage treatment plants. Environ. Toxicol. Chem. 22 (12), 2872–2880. https://doi.org/10.1897/02-469.

Neri-Cruz, N., Gómez-Oliván, L.M., Galar-Martínez, M., Romero-Figueroa, M.S., Islas-Flores, H., García-Medina, S., Jiménez-Vargas, J.M., SanJuan-Reyes, N., 2015. Oxidative stress in Cyprinus carpio induced by hospital wastewater in Mexico. Ecotoxicol. 24, 181–193. https://doi.org/10.1007/s10646-014-1371-y.

NOM-062-ZOO-1999, 2001. Norma Oficial Mexicana. Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. DOF, Mexico.

Oruç, E.O., Uner, N., 2002. Marker enzyme assessment in the liver of Cyprinus carpio (L.) exposed to 2,4-D and azinphosmethyl. J. Biochem. Mol. Toxicol. 16 (4), 182–188. https://doi.org/10.1002/jbt.10040.

Parvez, S., Raisuddin, S., 2005. Protein carbonyls: novel biomarkers of exposure to oxidative stress-inducing pesticides in freshwater fish Channa punctata (Bloch). Environ. Toxicol. Pharmacol. 20 (1), 112–117. https://doi.org/10.1016/j.etap.2004.11.002.

Pearce, R.E., Uetrecht, J.P., Leeder, J.S., 2005. Pathways of carbamazepine bioactivation in vitro II. The role of human cytochrome P450 enzymes in the formation of 2-hydroxyiminostilbene. Drug Metab. Dispos. 33, 1819–1826. https://doi.org/10.1124/dmd.105.004861.

Quinn, B., Gagné, F., Blaise, C., 2004. Oxidative metabolism activity in Hydra attenuata exposed to carbamazepine. Fresenius. Environ. Bull. 13 (8), 1–6.

Radi, R., Turrens, J.F., Chang, L.Y., Bush, K.M., Crapo, J.D., Freeman, B.A., 1991. Detection of catalase in rat heart mitochondria. J. Biol. Chem. 266 (32), 22028–22034.

Ramírez, A.J., Brain, R.A., Usenko, S., Mottaleb, M.A., O'Donell, J.G., Stahl, L.L., Wathen, J.B., Snyder, B.D., Pitt, J.L., Perez-Hurtado, P., Dobbins, L.L., Brooks, B.W., Chambliss, C.K., 2009. Occurrence of pharmaceuticals and personal care products in fish: results of a national pilot study in the United States. Environ. Toxicol. Chem. 28 (12), 2587–2597. https://doi.org/10.1897/08-561.1.

Samara, M., Nasser, A., Mingelgrin, U., 2016. Mechanochemical removal of carbamazepine. Chemosphere 160, 266–272. https://doi.org/10.1016/j.chemosphere.2016.06.082.

Saucedo-Vence, K., Dublán-García, O., López-Martínez, L.X., Morachis-Valdes, G., Galar-Martínez, M., Islas-Flores, H., Gómez-Oliván, L.M., 2015. Short and long-term exposure to diclofenac alter oxidative stress status in common carp Cyprinus carpio. Ecotoxicology 24, 527–539. https://doi.org/10.1007/s10646-014-1401-9.

Tanoue, R., Nomiyama, K., Nakamura, H., Kim, J.W., Isobe, T., Shinohara, R., Kunisue, T., Tanabe, S., 2015. Uptake and tissue distribution of pharmaceuticals and personal care products in wild fish from treated-wastewater-Impacted streams. Environ. Sci. Technol. 49 (19), 11649–11658. https://doi.org/10.1021/acs.est.5b02478.

Ternes, T.A., 1998. Occurrence of drugs in German sewage treatment plants and rivers. Water Res. 32 (11), 3245–3260. https://doi.org/10.1016/S0043-1354(98)00099-2.

Tixier, C., Singer, H.P., Oellers, S., Müller, S.R., 2003. Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. Environ. Sci. Technol. 37 (6), 1061–1068.

Tomson, T., Tybring, G., Bertilsson, L., Ekbom, K., Rane, A., 1980. Carbamazepine therapy in trigeminal neuralgia – clinical effects in relation to plasma concentration. Arch. Neurol. 37, 699–703.

Valdés, M.E., Huerta, B., Wunderlin, D.A., Bistoni, M.A., Barceló, D., Rodriguez-Mozaz, S., 2016. Bioaccumulation and bioconcentration of carbamazepine and other pharmaceuticals in fish under field and controlled laboratory experiments. Evidences of carbamazepine metabolization by fish. Sci. Total Environ. 557-558, 58–67. https://doi.org/10.1016/j.scitotenv.2016.03.045.

Van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149.

Vernouillet, G., Eullaffroy, P., Lajeunesse, A., Blaise, C., Gagné, F., Juneau, P., 2010. Toxic effects and bioaccumulation of carbamazepine evaluated by biomarkers measured in organism of different trophic levels. Chemosphere 80, 1062–1068. https://doi.org/10.1016/j.chemosphere.2010.05.010.

Williams, D.P., Naisbitt, D.J., 2002. Toxicophores: groups and metabolic routes associated with increased safety risk. Curr. Opin. Drug Discov. Devel. 5 (1), 104–115.

Wishart, D.S., Feunang, Y.D., Guo, A.C., Lo, E.J., Marcu, A., Grant, J.R., Sajed, T., Johnson, D., Li, C., Sayeeda, Z., Assempour, N., Iynkkaran, I., Liu, Y., Maciejewski, A., Gale, N., Wilson, A., Chin, L., Cummings, R., Le, D., Pon, A., Knox, C., Wilson, M., 2018. DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic Acids Res. 46 (D1), D1074–D1082. https://doi.org/10.1093/nar/gkx1037.

Writer, J.H., Ferrer, I., Barber, L.B., Thurman, E.M., 2013. Widespread occurrence of neuro-active pharmaceuticals and metabolites in 24 Minnesota rivers and wastewater. Sci. Total. Eviron. 461-462, 519–527. https://doi.org/10.1016/j.scitotenv.2013.04.099.

Yamanaka, H., Sogabe, A., Handoh, I.C., Kawabata, Z., 2011. The effectiveness of clove oil as an anaesthetic on adult common carp, Cyprinus carpio l. J. Anim. Vet. Adv. 10 (2), 210–213. https://doi.org/10.3923/javaa.2011.210.213.

Zhang, Y., Geißen, S., 2010. Prediction of carbamazepine in sewage treatment plant effluents and its implications for control strategies of pharmaceutical aquatic contamination. Chemosphere 80, 1345–1352. https://doi.org/10.1016/j.chemosphere.2010.06.030.

Zhang, Y., Geißen, S., Gal, C., 2008. Carbamazepine and diclofenac: removal in wastewater treatment plants and occurrence in water bodies. Chemosphere 73, 1151–1161. https://doi.org/10.1016/j.chemosphere.2008.07.086.