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Acesulfame potassium: Its ecotoxicity measured through oxidative stress biomarkers in common carp (*Cyprinus carpio*)

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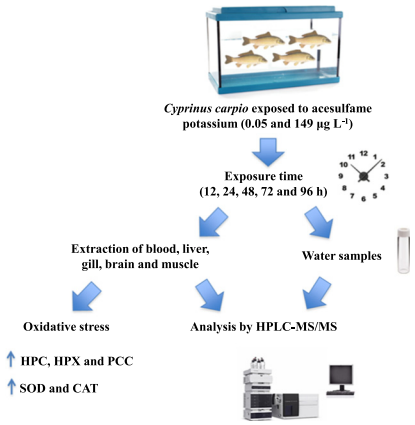
HIGHLIGHTS

- Acesulfame potassium was quantified in water system (12, 24, 48, 72 and 96 h).
- Acesulfame potassium was detected and quantified in different organs of *Cyprinus carpio*.
- Acesulfame potassium induces the SOD and CAT activity in blood, liver, gill, brain and muscle of *Cyprinus carpio*.
- Acesulfame potassium induces damage to lipids and proteins in blood, liver, gill, brain and muscle of *Cyprinus carpio*.

GRAPHICAL ABSTRACT



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ABSTRACT

Acesulfame potassium (ACS) is a widely-used sweetener worldwide. Its presence has been demonstrated in diverse bodies of water. However, the deleterious effects this causes in aquatic organisms has not yet been identified, which generates controversy concerning the risks that ACS represents after its disposal into the bodies of water. Thus, the objective of this work was to evaluate if the exposure of ACS in environmentally-relevant concentrations was capable of producing oxidative stress in blood, liver, gill, brain and muscle of common carp (*Cyprinus carpio*). With this finality, the carp were exposed to two environmentally-relevant concentrations (0.05 and 149 μg L^{-1}) at different exposure times (12, 24, 48, 72 and 96 h), having controls in the same conditions for each exposure time. Posteriorly, the following biomarkers of damage were evaluated: hydroperoxide content

Abbreviations: ACS, acesulfame potassium; ANOVA, analysis of variance; CAT, catalase; CYC, cyclamate; CHP, cumene hydroperoxide; DNPH, di-nitro phenyl hydrazine; EDTA, ethylenediaminetetraacetic acid; ESI, electrospray ionization; HEPES, N-2-hydroxyethylpiperazine-N-2′-ethanesulfonic acid; HPC, hydroperoxide content; HPLC-MS/MS, high-performance liquid chromatography-tandem mass spectrometry; LPX, lipid peroxidation; MDA, malondialdehyde; MRM, multiple reaction monitoring; OS, oxidative stress; PBS, phosphate buffered saline; PCC, protein carbonyl content; PT, total proteins; ROS, reactive oxygen species; SAC, saccharin; SOD, superoxide dismutase; SUC, sucralose; TBA, thiobarbituric acid; TCA, trichloroacetic acid; WWTPs, wastewater treatment plants.

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(HPC), level of lipoperoxidation (LPX) and protein carbonyl content (PCC), as well as the activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). The results showed that ACS produces significant increase in damage biomarkers evaluated in all organs, mainly in gill, brain and muscle, as well as significant changes in the activity of antioxidant enzymes in the same organs. Thus, it is concluded that ACS is capable of producing oxidative stress in common carp (*Cyprinus carpio*).

1. Introduction

Currently, health institutions are emphasizing the importance of reducing the consumption of free sugars, monosaccharides and disaccharides as a preventive measure for illnesses that impact a large part of the population such as diabetes and obesity (Kokotou and Thomaidis, 2013; Zygler et al., 2009). In this sense, new food products are being developed, which offer less caloric intake to a diet. An alternative to these requirements is the use of sweeteners. Sweeteners are any substance, natural or artificial, those are used as food additives in order to give a sweet taste to a food or product (Bassoli and Merlini, 2003). These days, sweeteners are consumed by millions of people worldwide. However, there are some controversies about whether these products constitute a health risk or not, and more recently, about they constitute a risk to the environment. These compounds are recognized as a new class of environmental contaminants due to their extreme persistence and presence in diverse aquatic ecosystems. They are also resistant to water treatment processes. Until now, its environmental behavior and long-term ecotoxicological effects in water resources are still very much unknown (Sang et al., 2014).

Of the variety of artificial sweeteners, which are currently used worldwide, only acesulfame potassium (ACS), cyclamate (CYC), sucralose (SUC) and saccharin (SAC) have been identified in effluents of wastewater treatments plants, surface and ground waters (Arbeláez et al., 2015; Lange et al., 2012).

Of the aforementioned compounds, one of special interest is ACS since it is one of the most consumed worldwide. It is known that by 2001, the demand for ACS in tons in different parts of the world was 2500 tons, of which Asia consumed 375 (15%), America consumed 1175 (47%), Africa and Oceania 100 (4%) and Europe 850 (34%) (Bahndorf and Kienle, 2004). By 2005, the demand for this sweetener had increased worldwide by 62.5%, reaching 4000 tons (Celanese, 2014). It is estimated that the high-intensity sweetener market (in which ACS is found) reached US$ 1.9 billion by 2017 (Leatherhead Food Research, 2014).

As previously mentioned, studies of ACS occurrence have demonstrated its presence in bodies of water. A study carried out in Spain demonstrated the presence of this sweetener in concentrations ranging from 49 to 149 μg L^{-1} in an influent of a wastewater treatment plant. After treatment, concentrations of 48–88 μg L^{-1} were found; these same authors determined concentrations of 0.12–1.62 μg L^{-1} in rivers (Arbeláez et al., 2015). Other studies report concentrations from 28 ng L^{-1} to 2 mg L^{-1} of ACS in diverse bodies of water (Scheurer et al., 2014; Kokotou et al., 2012; Lange et al., 2012; Müller et al., 2011; Buerge et al., 2009) and in groundwater, ranging from 4.7 to 34 μg L^{-1} (Perkola and Sainio, 2014; Kokotou et al., 2012; Lange et al., 2012; Buerge et al., 2011).

Little is known of the toxicity that ACS has caused in aquatic species, and from this lack of knowledge rises the interest in the present study to demystify or corroborate the effects this compound may have on aquatic life. However, there are some studies that have demonstrated that other sweeteners such as SUC, have generated deleterious effects in aquatic organisms. For example, Wiklund et al. (2014) demonstrated that at SUC concentrations ranging from 0.0001 to 5 mg L^{-1}, an increase in the swimming speed of *Daphnia magna* was produced, and that in the family of gammarid amphipods, an increase in the time required to reach food and shelter was seen. These authors demonstrated that sucralose can induce neurologic and oxidative mechanisms with potentially important consequences in the behavior and physiology of *Daphnia magna*. More recently, Saucedo-Vence et al. (2017) observed alterations in antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), also in cell oxidation biomarkers among which are hydroperoxide content (HPC), lipid peroxidation (LPX) and protein carbonyl content (PCC) in brain, gill and muscle of common carp (*Cyprinus carpio*) exposed to ecotoxicologically-relevant concentrations of SUC, demonstrating that this sweetener is capable of inducing oxidative stress. This biomarker has been considered as a significant mechanism of toxicity over the organisms it affects and has allowed for its use as a diagnostic tool, with the predictive capacity of evidencing the impact of the environmental contaminants over the organisms (Kelly et al., 1998).

The main result of this phenomenon of oxidative stress is seen in organisms as the oxidation of biomolecules such as lipids, proteins and nucleic acids, and alteration of the cell redox status (Marcon and Filho, 1999), as well as modification in antioxidant enzyme mechanisms of defense (Valavanidis et al., 2006).

Fish are ideal indicator organisms, since they are usually abundant in aquatic ecosystems, easy to capture and to identify and are well-studied (Nelson, 1994). They are also considered good indicators of water quality (Huidobro et al., 2000). Common carp (*Cyprinus carpio*) are commonly used as a "bioindicator" species (Huang et al., 2007), since cyprinids are quantitively the most important group of teleost fish in the world for commercial purposes. It has adapted to different environments, tolerates low concentrations of oxygen, very high temperatures and significant organic contamination, is an organism easy to obtain, maintain, and has a relevant response to toxic compounds (sensibility to low concentrations, to a wide variety of toxins).

Thus, the objective of this study was to evaluate if the exposure to ACS at two environmentally-relevant concentrations (0.05 and 149 μg L^{-1}) produces oxidative stress in blood, liver, gills, brain and muscle of common carp (*Cyprinus carpio*).

2. Materials and methods

2.1. Test substances

All the reagents were obtained from Sigma-Aldrich (St. Louis, MO), unless indicated otherwise in the "material and methods" section of this work.

An analytical standard of ACS (6-Methyl-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide potassium salt) was purchased from Sigma-Aldrich (St. Louis, MO). The purity of the ACS standard was ≥99% ($C_4H_4KNO_4S$, molecular weight: 201.24 g mol^{-1} (CAS Number 55589–62-3)). All reagents were HPLC-grade. Nitrogen gas was sourced from INFRA, S.A. de C.V. (DF, Mexico). Ultrapure water was obtained using an ultrapure water purification system provided by Merck Millipore.

2.2. Collection and maintenance of common carp (Cyprinus carpio)

The carp employed in this study were obtained from the certified aquaculture center located in Tiacaque, State of Mexico. This center is one of the most important in the country dedicated to the massive breeding of common carp and the herbivore subtype. These are organisms free of contaminants and possess the ideal conditions in order to

Table 1
ACS concentrations in the exposure times.

Exposure concentration	Exposure time (h)	ACS in water system without carps ($\mu g\ L^{-1}$)	ACS in water system with carps ($\mu g\ L^{-1}$)	ACS in blood carp ($\mu g\ L^{-1}$)	ACS in gill carp ($\mu g\ g^{-1}$)	ACS in liver carp ($\mu g\ g^{-1}$)	ACS in brain carp ($\mu g\ g^{-1}$)	ACS in muscle carp ($\mu g\ g^{-1}$)
Control group	12	ND	ND	ND	ND	ND	ND	ND
	24	ND	ND	ND	ND	ND	ND	ND
	48	ND	ND	ND	ND	ND	ND	ND
	72	ND	ND	ND	ND	ND	ND	ND
	96	ND	ND	ND	ND	ND	ND	ND
0.05 $\mu g\ L^{-1}$	12	0.049 ± 0.0008	0.042 ± 0.005	0.0010 ± 0.0002	0.0002 ± 0.00002	0.00011 ± 0.000002	ND	ND
	24	0.049 ± 0.0001	0.038 ± 0.007	0.0010 ± 0.0002	0.0004 ± 0.00002	0.00017 ± 0.000003	ND	ND
	48	0.048 ± 0.0009	0.031 ± 0.006	0.0025 ± 0.0001	0.00017 ± 0.00002	0.00021 ± 0.000001	ND	ND
	72	0.048 ± 0.0003	0.027 ± 0.006	0.0032 ± 0.0001	0.00025 ± 0.00003	0.00029 ± 0.000002	0.00007 ± 0.000002	0.00003 ± 0.000001
	96	0.047 ± 0.0008	0.020 ± 0.005	0.0040 ± 0.0001	0.00037 ± 0.00001	0.00030 ± 0.000001	0.000011 ± 0.000002	0.00007 ± 0.000002
149 $\mu g\ L^{-1}$	12	148 ± 0.7	135 ± 3.5	5.1 ± 0.9	1.2 ± 0.8	0.92 ± 0.2	0.31 ± 0.07	0.34 ± 0.06
	24	147 ± 0.5	127 ± 4.2	6.1 ± 1.1	1.5 ± 0.5	0.97 ± 0.1	0.28 ± 0.08	0.37 ± 0.05
	48	145 ± 0.1	119 ± 2.8	5.8 ± 1.5	1.9 ± 0.6	1.3 ± 0.3	0.45 ± 0.05	0.42 ± 0.05
	72	144 ± 1.1	112 ± 1.9	7.3 ± 0.8	1.8 ± 0.7	1.4 ± 0.2	0.51 ± 0.04	0.49 ± 0.04
	96	143 ± 0.9	98 ± 4.7	11.3 ± 1.4	2.3 ± 0.9	1.7 ± 0.2	0.72 ± 0.03	0.63 ± 0.08

Values are the mean of five replicates ± SE; ND = Not detected.

guarantee reproducible, repeatable and reliable results. The carps utilized had a length of 19.3 ± 0.3 cm and a weight of 51.4 ± 5.3 g. Once collected, the carps were placed in polyethylene bags, which contained previously oxygenated water and were transported to the laboratory. In the aquarium, the carps were placed in fish tanks with a 120 L capacity, which contained previously dechlorinated tap water. They were maintained in said conditions for 45 days prior to their use. During this period, the carps were fed with Pedregal Silver Corp^{MR} special fish feed. Within the fish tanks, each day, three quarters of water were replaced in order to maintain the environment in good conditions. The characteristics of the water during the period of acclimatization were always the same: temperature of 21 ± 2.1 °C, oxygen concentration of 80–90%, pH of 7.0 to 8.0, total alkalinity of 18.3 ± 6.5 mg L^{-1} and total water hardness of 19.1 ± 0.5 mg L^{-1}. The light-darkness photoperiod was normal.

2.3. Exposure procedure the common carp (Cyprinus carpio) to ACS

All the test systems contained water with the same physicochemical characteristics, and conditions utilized during the acclimatization and maintenance periods described in section 2.2 were used. The systems were static, with no renovation of the medium and no feeding of the fish was done during the exposure periods of the test organisms.

Two concentrations of ACS (0.05 and 149 $\mu g\ L^{-1}$) were employed in the different test systems. These concentrations were deemed "environmentally relevant" considering the study carried out by Sang et al. (2014) and Arbeláez et al. (2015), and that in Mexican bodies of water, no presence of sweeteners has been reported in scientific literature. The exposure periods used were 12, 24, 48, 72 and 96 h, and a ACS free control system was set up for each exposure time. Each test system contained six fish and the tests were performed in triplicate (360 carps were employed for the oxidative stress tests).

After the exposure times, the organisms were anesthetized with clove oil at a concentration of 50 mg L^{-1} for a period of 2 to 5 min and blood samples were obtained via puncture of the caudal vein of the carp with a heparinized 1-mL hypodermic syringe. The carps were also dissected and gill, muscle, liver and brain samples were obtained. The organs and tissues were placed in phosphate buffered saline (PBS) with a 7.4 pH and centrifuged at 12,500× *g* for 15 min at a temperature of 4 °C.

It was decided that the study would involve work with blood, organs and tissues, considering the following aspects: gills, because they play an important role in the transport of oxygen and other ions such as Na^+, K^+ and Cl^-, in order to maintain the acid-base equilibrium, osmotic pressure of the body and regulation of the entry and exit of ions and being the first contact organ; the liver was selected due to the fact that this organ plays a determinant role in the biotransformation of xenobiotics; muscle was selected because of its high protein content; brain was selected because it is one of the most important organs, where motor activity is determined and because of its high lipid content; blood was selected because it is the main reservoir of the contaminants in the organism. Also, all of the aforementioned organs have demonstrated to be sensible to oxidative damage.

All procedures were performed in accordance with ethical protocols for the maintenance, use and handling of test animals approved for use in the Universidad Autónoma del Estado de México (México). Provisions in the pertinent official Mexican norm were also taken into account (NOM-062-ZOO-1999, Technical specifications for the production, care and use of laboratory animals).

2.4. Water

Water samples (10 mL) were collected from the different test systems in glass vials. Samples were acidified with 1 M HCl and ACS was extracted from 1 mL water samples with 5 mL of methanol: water (1:1). The mixture was centrifuged at 1800× *g* for 10 min, and the upper organic layer was used for the analysis by HPLC–MS/MS.

2.5. Plasma

5 mL of ice-cold acetone were added to the plasma samples and then centrifuged at 2500× *g* for 5 min. The supernatant was separated and analyzed for ACS by HPLC–MS/MS.

2.6. Tissues

0.2 g of tissue were homogenized in 4 mL of methanol: water (1:1), followed by centrifugation at 2500× *g* for 5 min. The top layer was carefully placed into a clean 10 mL glass vial for its further analysis by HPLC–MS/MS.

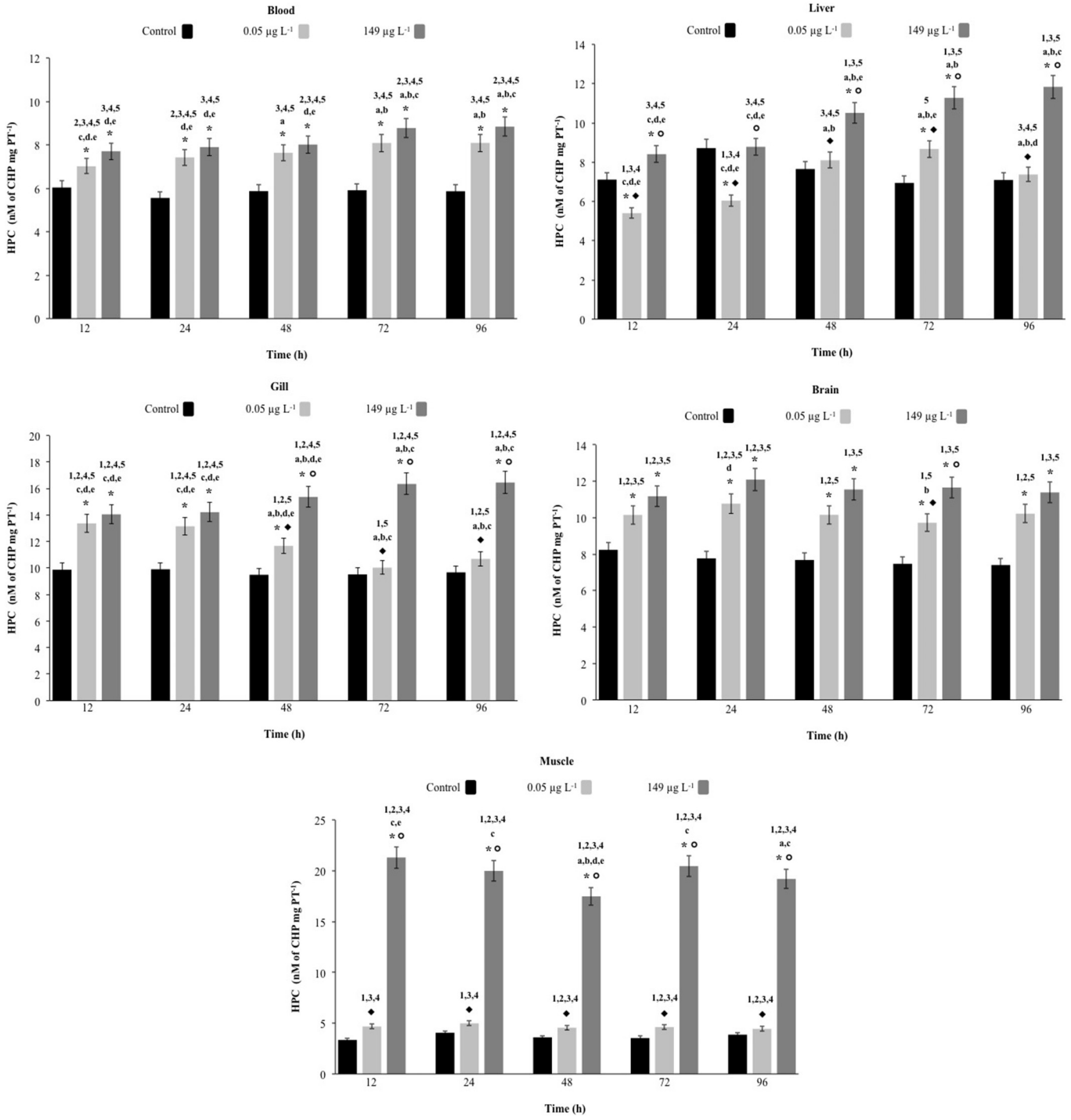

Fig. 1. HPC in blood, liver, gill, brain and muscle of *C. carpio* exposed for 12, 24, 48, 72 and 96 h to ACS concentrations of 0.05 μg L^{-1} and 149 μg L^{-1}. Values are the mean of three replicates ± SEM. CHP = cumene hydroperoxide. Significantly different ($p < 0.05$) from: *control group; [a]12 h; [b]24 h; [c]48 h; [d]72 h; [e]96 h; ♦149 μg L^{-1}; º0.05 μg L^{-1}; [1]blood; [2]liver; [3]gill; [4]brain; [5]muscle. ANOVA and Bonferroni *post-hoc* test.

2.7. Quantification of ACS by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS)

The stock solution of the ACS standard was prepared by dissolving the compound in methanol and ultrapure water (50:50) at a 1000 μg L^{-1} concentration. Posteriorly, it was stored at −20 °C until analysis. The high-performance liquid chromatography (HPLC)–MS/MS system used was an Agilent 1290 Infinity HPLC unit coupled to an Agilent 6430 Triple Quadrupole MS equipped with electrospray ionization (ESI). The RRHD Eclipse Plus C18 chromatography column (2.1 × 50 mm, 1.8-μm) was maintained at 40 °C. The mobile phase was composed of ultrapure water (A) and acetonitrile (B), both containing 5 mM ammonium acetate and 1 mM TRIS. The mobile phases were filtered before use. Gradient elution was carried out at a flow rate of 0.4 mL min^{-1}, and the mobile phase gradient was ramped linearly from 0% to 75% B over 8 min, held for 1 min, returned back to 10% B over 1 min, ramped again to 70% B within 0.5 min, and held for 1.9 min. Eluent B was then lowered back to 0% within 0.5 min, and the system was allowed to equilibrate for 10 min before the next injection. The injection volume was 20 μL, and injection was performed by an

Table 2
Increases and decreases in oxidative stress biomarkers comparing 0.05 and 149 μg L^{-1} against the control group.

Oxidative damage					Antioxidant activity				
Biomarkers	Organs	Time (h)	Concentration		Biomarkers	Organs	Time (h)	Concentration	
			0.05 μg L^{-1}	149 μg L^{-1}				0.05 μg L^{-1}	149 μg L^{-1}
HPX	BLOOD	12	↑16[a]	↑28[a]	SOD	BLOOD	12	↑96[a]	↑80[a]
		24	↑34[a]	↑42[a]			24	↑11	↑**112**[a]
		48	↑30[a]	↑37[a]			48	↑**108**[a]	↑20
		72	↑37[a]	↑48[a]			72	↑96[a]	↑80
		96	↑**38**[a]	↑**51**[a]			96	↑51[a]	↑87[a]
	LIVER	12	↓24[a]	↑18[a]		LIVER	12	↑**89**[a]	↓11
		24	↓**31**[a]	↑1			24	↑36	↑19
		48	↑6	↑37[a]			48	↑18	↑**79**[a]
		72	↑25[a]	↑62[a]			72	↑84[a]	↑67[a]
		96	↑4	↑**67**[a]			96	↑25	↑**79**[a]
	GILL	12	↑**35**[a]	↑42[a]		GILL	12	↑233[a]	↑57[a]
		24	↑33[a]	↑43[a]			24	↑93[a]	↑**164**[a]
		48	↑23[a]	↑62[a]			48	↑18	↑2
		72	↑5	↑**72**[a]			72	↑**253**[a]	↑77[a]
		96	↑10	↑70[a]			96	↓35	↑18
	BRAIN	12	↑23[a]	↑36[a]		BRAIN	12	↓5	↑40[a]
		24	↑**39**[a]	↑**56**[a]			24	↑1	↑**92**[a]
		48	↑32[a]	↑50[a]			48	↓7	↓11
		72	↑30[a]	↑**56**[a]			72	↓16	↓40[a]
		96	↑38[a]	↑54[a]			96	↓**27**	↓10
	MUSCLE	12	↑**40**	↑**537**[a]		MUSCLE	12	↑65[a]	↑37[a]
		24	↑23	↑393[a]			24	↑48[a]	↑**76**[a]
		48	↑26	↑385[a]			48	↑129[a]	↑61[a]
		72	↑31	↑479[a]			72	↑**141**[a]	↑38[a]
		96	↑15	↑396[a]			96	↑10	↑4
LPX	BLOOD	12	↑72[a]	↑132[a]	CAT	BLOOD	12	↑33[a]	↑37[a]
		24	↑75[a]	↑36			24	↑31[a]	↑30[a]
		48	↑**160**[a]	↑**162**[a]			48	↑31[a]	↑**41**[a]
		72	↑63[a]	↑86[a]			72	↑32[a]	↑36[a]
		96	↑119[a]	↑101[a]			96	↑**66**[a]	↑40[a]
	LIVER	12	↑**61**[a]	↑40		LIVER	12	↓9	↓**21**[a]
		24	↑17	↓3			24	↓5	↓9
		48	↑17	↑**128**[a]			48	↑5	↑9
		72	↑6	↑54[a]			72	↑4	↓4
		96	↑22	↑76[a]			96	↑**11**[a]	↓3
	GILL	12	↑257[a]	↑309[a]		GILL	12	↑31[a]	↑**34**[a]
		24	↑284[a]	↑254[a]			24	↑**44**[a]	↑**34**[a]
		48	↑322[a]	↑319[a]			48	↑23[a]	↑21[a]
		72	↑**429**[a]	↑424[a]			72	↑31[a]	↑22[a]
		96	↑397[a]	↑**425**[a]			96	↑35[a]	↑14[a]
	BRAIN	12	↑47[a]	↓15		BRAIN	12	↓9	↑14[a]
		24	↑39	↑53[a]			24	↓3	↑13[a]
		48	↑33	↑19			48	↓11	↑8
		72	↑**75**[a]	↑14			72	↑**31**[a]	↑**47**[a]
		96	↑53[a]	↑**159**[a]			96	↑1	↑**47**[a]
	MUSCLE	12	↑35[a]	↑21		MUSCLE	12	↑18[a]	↑25[a]
		24	↑**49**[a]	↑30[a]			24	↑**33**[a]	↑22[a]
		48	↑44[a]	↑5			48	↑29[a]	↑35[a]
		72	↑27	↓5			72	↑28[a]	↑30[a]
		96	↑1	↑**122**[a]			96	↑30[a]	↑**56**[a]
PCC	BLOOD	12	↑**43**[a]	↑**47**[a]					
		24	↑42[a]	↑**47**[a]					
		48	↑38[a]	↑38[a]					
		72	↑41[a]	↑44[a]					
		96	↑28[a]	↑36[a]					
	LIVER	12	↓3	↓4					
		24	↑23[a]	↑24[a]					
		48	↑16	↑**59**[a]					
		72	↑43[a]	↑47[a]					
		96	↑**60**[a]	↑41[a]					
	GILL	12	↑32[a]	↑30[a]					
		24	↑**65**[a]	↑72[a]					
		48	↑33[a]	↑66[a]					
		72	↑20[a]	↑**80**[a]					
		96	↑35[a]	↑46[a]					
	BRAIN	12	↑14	↑10					
		24	↑44[a]	↑34[a]					
		48	↑47[a]	↑59[a]					
		72	↑55[a]	↑**66**[a]					
		96	↑**67**[a]	↑**66**[a]					

(continued on next page)

Table 2 (*continued*)

Oxidative damage					Antioxidant activity				
Biomarkers	Organs	Time (h)	Concentration		Biomarkers	Organs	Time (h)	Concentration	
			0.05 μg L^{-1}	149 μg L^{-1}				0.05 μg L^{-1}	149 μg L^{-1}
		24	↑19[a]	↑27[a]					
		48	↑24[a]	↑39[a]					
		72	↑22[a]	↑35[a]					
		96	↑**38**[a]	↑**46**[a]					

HPC = hydroperoxide content, LPX = lipid peroxidation, PCC = protein carbonyl content, SOD = superoxide dismutase activity, CAT = catalase activity.
The highest increases or decreases are shown in bold.
[a] Significant differences.

autosampler. HPLC-MS/MS analyses were conducted in negative ionization mode using multiple reaction monitoring (MRM). The standard, without a column, was injected to optimize the conditions of ESI-MS/MS. These conditions were as follows: nebulizer pressure of 45 psi, drying gas (N_2) flow rate of 11 L min^{-1}, drying gas temperature of 350 °C and capillary voltage of 4000 V.

2.8. Oxidative stress evaluation

The biomarkers of oxidative stress that were evaluated in this work: HPC, LPX, PCC and the antioxidant activity of SOD and CAT enzymes. All bioassays were performed with the supernatant. Likewise, in order to normalize the results of the oxidative stress biomarkers evaluated and to express the results, the content of total proteins (PT) was determined via the Bradford (1976) method. The study was carried out in triplicate. To analyze oxidative stress in different organs and systems, the methodologies described next were employed.

2.8.1. Determination of HPC

The method described by Jiang et al. (1992) was used. One hundred microliters of supernatant were mixed with 900 μL of the reaction mixture, which contained 0.25 mM $FeSO_4$, 25 mM H_2SO_4, 0.1 mM xylenol orange and 4 mM butyl hydroxytoluene in 90% methanol (v/v). The resulting solution was mixed for 1 min using a vortex and was posteriorly left to rest for 1 h at room temperature and protected from the light. Once the rest period had concluded, absorbance was determined at 560 nm. The results were expressed in nm of cumene hydroperoxide (CHP) per mg of PT.

2.8.2. Determination of LPX

The method described by Büege and Aust (1978) was employed. Fifty microliters of supernatant were mixed with 450 μL of Tris-HCl solution (150 mM, pH 7.4). Posteriorly, 1 mL of a mixture of thiobarbituric acid (TBA) and trichloroacetic acid (TCA) in a 0.375% proportion of the first in 15% of the second, was added. The resulting solution was shaken for 1 min using a vortex. Afterwards, a thermal shock to the samples was induced by submerging the vials in boiling water for 15 min and immediately cooling them 5 °C for 3 min. Finally, the samples were incubated for 30 min at 37 °C and then centrifuged at 3500× *g* for 10 min. The samples were read at 535 nm. The results obtained were expressed as mM of malondialdehyde (MDA) per mg of PT.

2.8.3. Determination of PCC

The method described by Levine et al. (1994), modified by Parvez and Raisuddin (2005) and Burcham (2007), was used. One hundred microliters of supernatant were mixed with 150 μL of a solution of di-nitro phenyl hydrazine (DNPH) (10 mM dissolved in 2 M HCl). The samples were perfectly mixed for 1 min with a vortex and were left to rest at room temperature for 1 h, protected from light. One the rest period had concluded, 500 μL of a solution of 20% TCA were added to the samples and were left to rest for 15 min at 4 °C. The mixture was centrifuged at 1100× *g* for 5 min. The supernatant was disposed of and the button was rinsed three times with a mixture of ethanol: ethyl acetate (1:1). After the rinses, the button was dissolved using 2 mL of a solution of 6 M guanidine (pH 2.4) and the mixture was incubated at 37 °C for 30 min. Posteriorly, the absorbance was determined at 366 nm. The results were expressed as mM of reactive carbonyls (C═O) per mg of PT.

2.8.4. Determination of SOD activity

SOD activity was analyzed via the Marklund and Marklund (1974), as modified by Magnani et al. (2000).

2.8.4.1. Delipidation of the sample. To one hundred microliters of the sample, 30 μL of chloroform and 50 μL of methanol were added. The samples were mixed for 1 min using a vortex. Posteriorly, the samples were centrifuged at 6000× *g* for 15 min and the supernatant was removed.

2.8.4.2. Samples. To 100 μl of the delipidated sample, 2.8 mL of a Tris-EDTA buffer solution (pH 8.2) were added. The resulting solution was mixed with a vortex for 1 min. Next, 50 μL of a 0.2 mM Pirogallol solution were added and the difference in optical density (OD) at 10 and 60 s, at a wavelength of 420 nm, was determined. The results were expressed as UI of SOD per mg of PT.

2.8.5. Determination of CAT activity

In order to evaluate the activity of catalase, a method referred to by Radi et al. (1991) was employed. Twenty microliters of supernatant were mixed with 1000 μL of isolation buffer solution (0.3 M saccharose, 1 mM of EDTA, 5 mM of HEPES and 5 mM KH_2PO). Posteriorly, 200 μL of a solution of hydrogen peroxide (20 mM) were added. Finally, absorbance was read at 240 nm at 0 and 60 s. The results of CAT activity were expressed as μM of H_2O_2 per mg of PT.

2.8.6. Determination of PR

The method described by Bradford (1976) was employed. Twenty-five microliters of supernatant were mixed with 75 μL of deionized water and 2.5 mL of Bradford reagent. The mixture was placed over a vortex for 1 min and was left to rest for 5 min. The absorbance was read at 595 nm and the results were interpolated in a bovine serum albumin curve. The total protein content was expressed as mg protein wet tissue.

2.9. Statistical analysis

Results of the oxidative stress biomarkers were statistically evaluated by one-way analysis of variance (ANOVA) followed by the Bonferroni *post-hoc* multiple comparisons test, with P set at <0.05. Pearson's correlation analysis was used to find potential correlations between ACS concentrations and biomarkers of oxidative stress. Statistical determinations were performed with SPSS v10 software (SPSS, Chicago IL, USA).

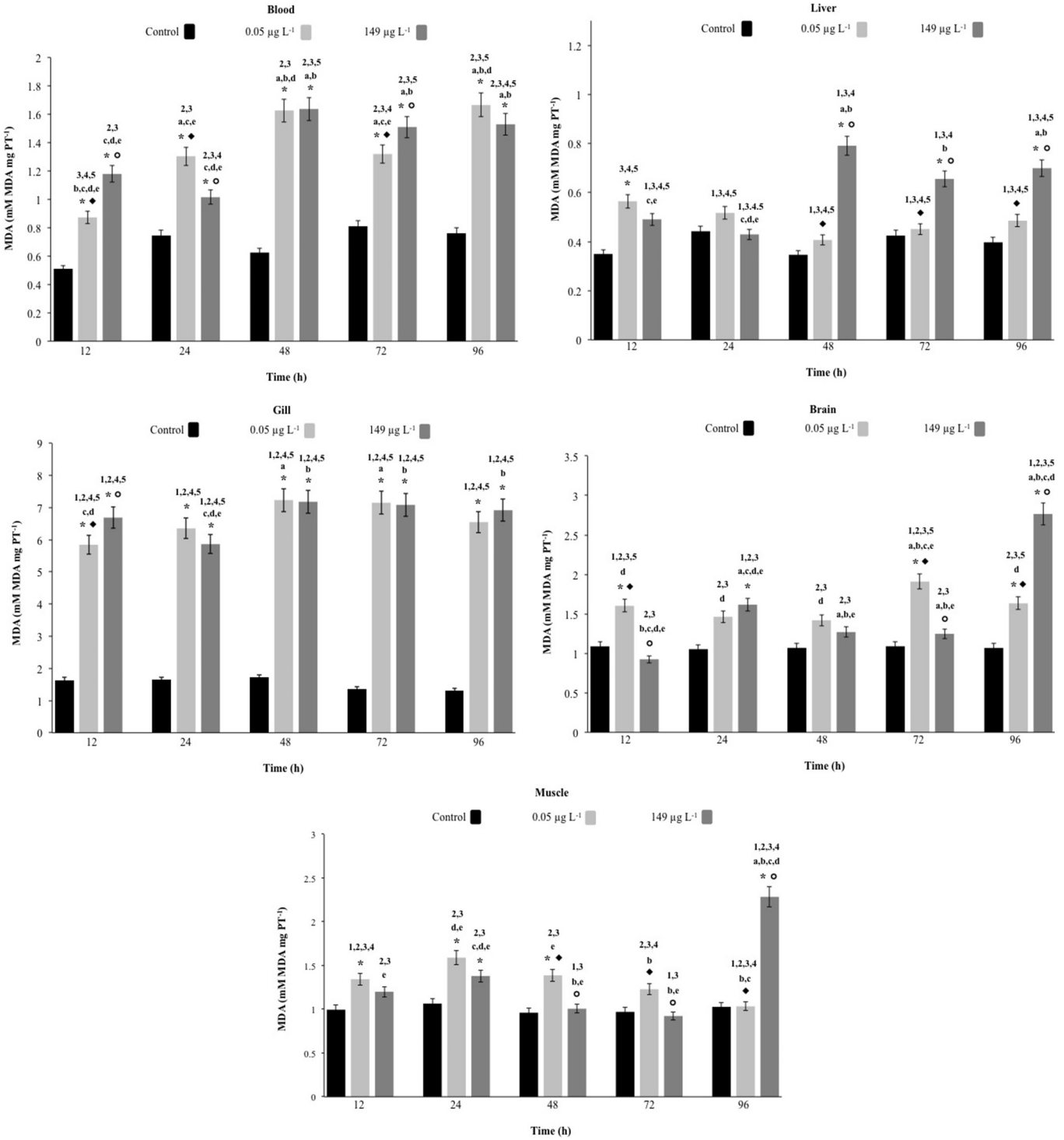

Fig. 2. LPX in blood, liver, gill, brain and muscle of *C. carpio* exposed for 12, 24, 48, 72 and 96 h to ACS concentrations of 0.05 μg L^{-1} and 149 μg L^{-1}. Values are the mean of three replicates ± SEM. MDA = malondialdehyde. Significantly different ($p < 0.05$) from: *control group; [a]12 h; [b]24 h; [c]48 h; [d]72 h; [e]96 h; ♦149 μg L^{-1}; º0.05 μg L^{-1}; [1]blood; [2]liver; [3]gill; [4]brain; [5]muscle. ANOVA and Bonferroni *post-hoc* test.

3. Results

3.1. Quantification of ACS in water and tissues of common carp (Cyprinus carpio)

Table 1 shows the concentration of ACS in the different matrices involved. The highest concentration in water is found at 12 h and diminishes as exposure time increases. On the other hand, in the organs and tissues evaluated, the concentration of ACS increases as exposure time increases. The organs with highest uptake of ACS were gill and blood; in muscle, a lesser uptake of the sweetener was observed.

3.2. HPC

Fig. 1 shows the results of HPC. For the *concentration of 0.05 μg L^{-1}*, significant increases with regard to the control group ($p < 0.05$) were observed at all exposure times in blood and brain. In liver, significant decreases were observed at 12 and 24 h, as well as a significant increase at

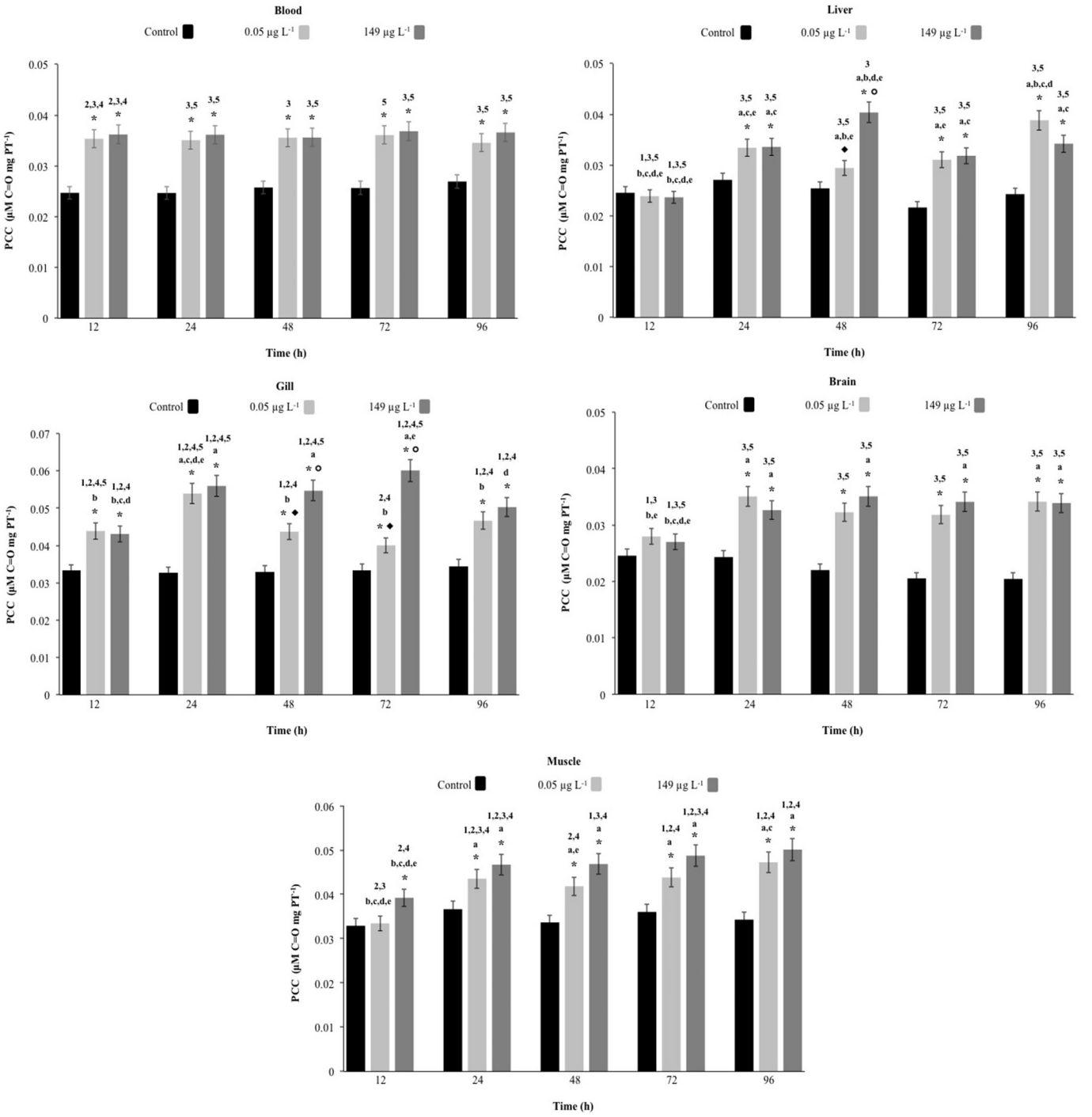

Fig. 3. PCC in blood, liver, gill, brain and muscle of *C. carpio* exposed for 12, 24, 48, 72 and 96 h to ACS concentrations of 0.05 μg L^{-1} and 149 μg L^{-1}. Values are the replicates ± SEM. C=O = reactive carbonyls. Significantly different ($p < 0.05$) from: *control group; [a]12 h; [b]24 h; [c]48 h; [d]72 h; [e]96 h; ♦149 μg L^{-1}; [g]0.05 μg L^{-1}; [1]blood; [2]liver; [3]gill; [4]brain; [5]muscle. ANOVA and Bonferroni *post-hoc* test.

72 h. In gills, significant increases with regard to the control group at 12, 24 and 48 h, were observed. Finally, in muscle, no significant differences were observed with regard to the control group. In the case of the *concentration of 149 μg L^{-1}*, significant increases were observed at 12, 48, 72 and 96 h of exposure for the liver. Likewise, significant increases with regard to the control group ($p < 0.05$) at all exposure times in blood, gills, brain and muscle, were observed. Also, significant differences were observed between the different organs and concentrations (in liver and muscle at all exposure time; in gill after 48 h; in brain only at 72 h). The percentages of increase and decrease are shown in Table 2.

3.3. LPX

The level of lipoperoxidation (LPX) is shown in Fig. 2. For the lowest *concentration (0.05 μg L^{-1})*, significant increases were observed with regard to the control group ($p < 0.05$) at the five exposure times for blood and gill; in liver, a significant increase was also observed at 12 h; in brain, a significant increase with regard to the control group was seen at 12, 72 and 96 h and in muscle, at 12, 24 and 48 h. As for the *concentration of 149 μg L^{-1}*, significant increases were obtained with regard to the control group ($p < 0.05$), in blood at 12, 48, 72 and 96 h; in brain and muscle

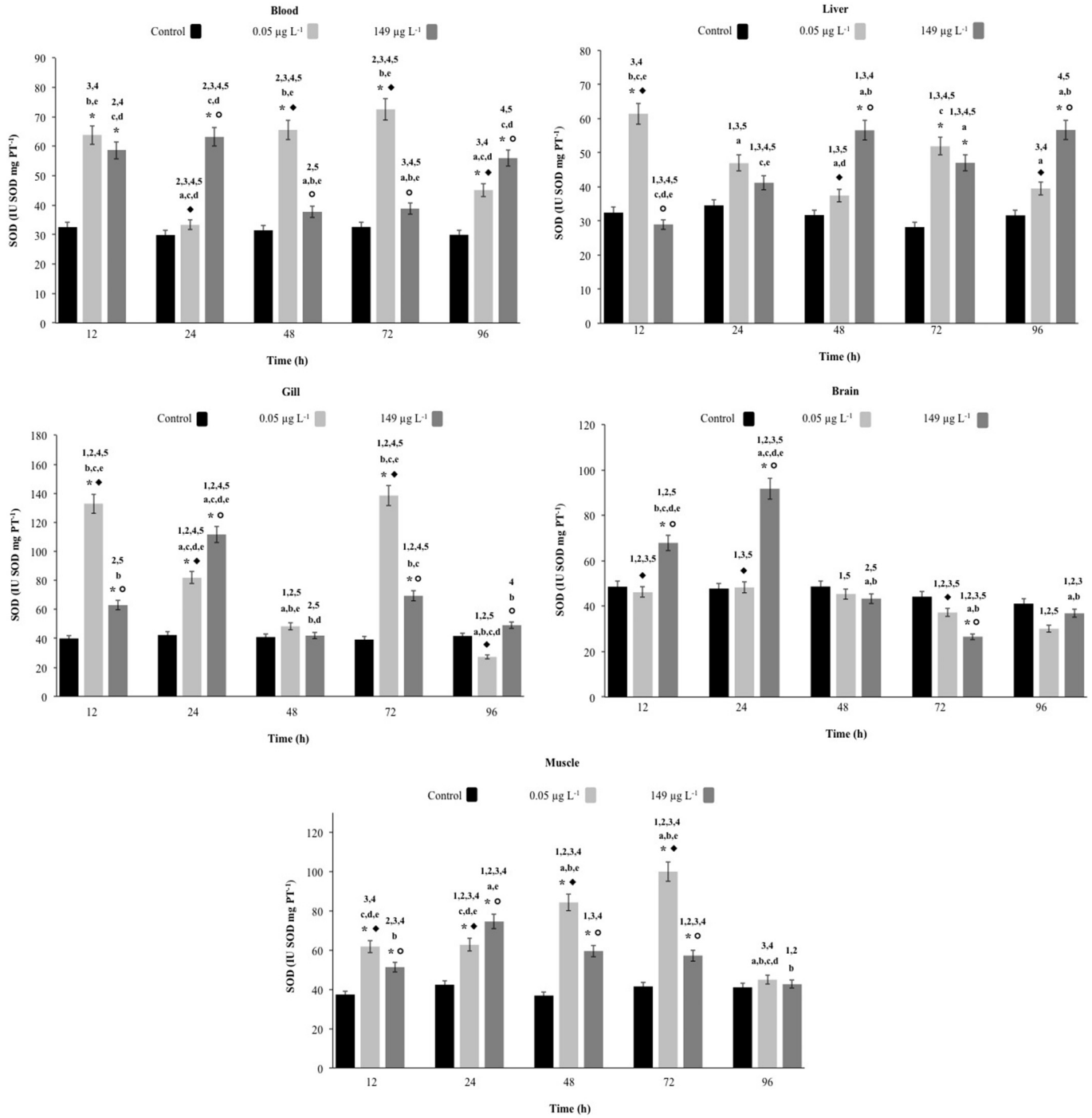

Fig. 4. SOD activity in blood, liver, gill, brain and muscle of *C. carpio* exposed for 12, 24, 48, 72 and 96 h to ACS concentrations of 0.05 μg L^{-1} and 149 μg L^{-1}. Values are the mean of three replicates ± SEM. IU = international units. Significantly different ($p < 0.05$) from: *control group; [a]12 h; [b]24 h; [c]48 h; [d]72 h; [e]96 h. ♦149 μg L^{-1}; º0.05 μg L^{-1}; [1]blood; [2]liver; [3]gill; [4]brain; [5]muscle. ANOVA and Bonferroni *post-hoc* test.

at 24 and 96 h; in gill, significant increases were also observed at all exposure times. In addition, significant differences were observed between the different organs and concentrations (in blood at 12, 24 and 72 h; in liver and muscle after 48 h; in gill only at 12 h; in brain at 12, 72 and 96 h).

3.4. PCC

PCC results are shown in Fig. 3. As for the *concentration of 0.05 μg L^{-1}*, a significant increase is observed with regard to the control group ($p < 0.05$) in blood and gill at the 5 exposure times; in liver, an increase is observed at 24, 72 and 96 h. Finally, in brain and muscle, significant increases are seen at 24, 48, 72 and 96 h of exposure. For the *concentration of 149 μg L^{-1}*, significant increases with regard to the control group ($p < 0.05$) were observed in blood, gill and muscle at all exposure times. At 24, 48, 72 and 96 h, significant increases were found in liver and brain. Moreover, significant differences were observed between the different organs and concentrations (in liver only at 48 h; in gill at 48 and 72 h).

3.5. SOD

Fig. 4 shows the results of SOD activity. For the *concentration of 0.05 μg L^{-1}* significant increases were observed with regard to the control

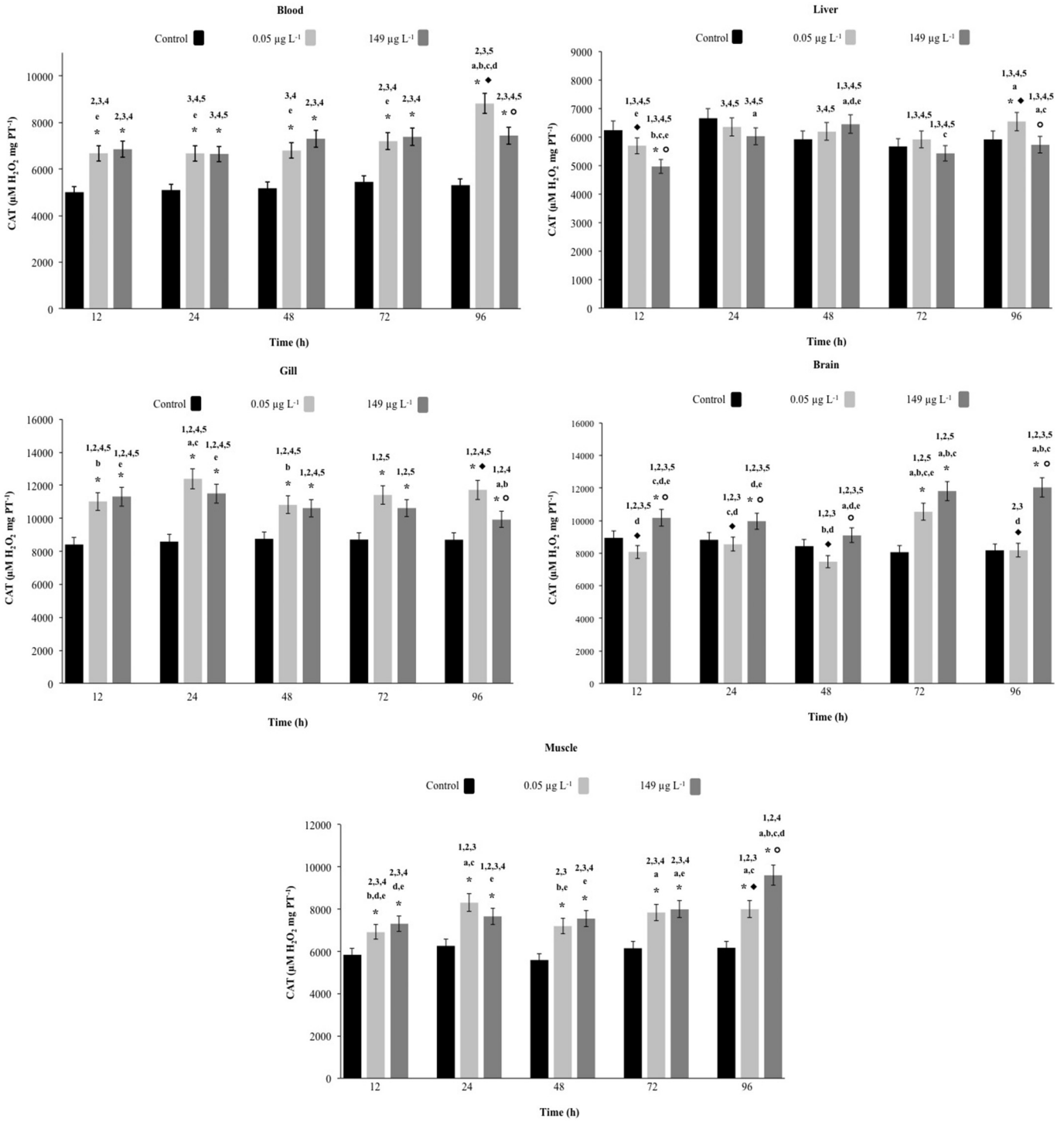

Fig. 5. CAT activity in blood, liver, gill, brain and muscle of *C. carpio* exposed for 12, 24, 48, 72 and 96 h to ACS concentrations of 0.05 μg L^{-1} and 149 μg L^{-1}. Values are the mean of three replicates ± SEM. Significantly different ($p < 0.05$) from: *control group; [a]12 h; [b]24 h; [c]48 h; [d]72 h; [e]96 h; ♦149 μg L^{-1}; º0.05 μg L^{-1}; [1]blood; [2]liver; [3]gill; [4]brain; [5]muscle. ANOVA and Bonferroni *post-hoc* test.

group ($p < 0.05$) in blood at 12, 48, 72 and 96 h; in liver, at 12 and 72 h; in gill, at 12, 24 and 72 h; in muscle, at 12, 24, 48 and 72 h. However, in the brain, no significant differences were observed at any of the exposure times. As for the *concentration of 149 μg L^{-1}*, significant increases with regard to the control group were observed ($p < 0.05$) in blood at 12, 24 and 96 h; in liver, at 48, 72 and 96 h; in gill, at 12, 24 and 72 h; in muscle, at 12, 24, 48 and 72 h; in brain, at 12 and 24 h. However, a significant decrease was also observed in the brain at 72 h of exposure. Also, significant differences were observed between the different organs and concentrations (in blood after 24 h of exposure; in liver at 12, 48 and 96 h; in gill at 12, 24, 72 and 96 h; in brain at 12, 24 and 72 h; in muscle from 12 to 72 h).

3.6. CAT

The results of CAT antioxidant activity are shown in Fig. 5. Significant increases with regard to the control group were observed ($p < 0.05$) at the *concentration of 0.05 μg L^{-1}* at all exposure times for blood, gill and

muscle. In liver, a significant increase was observed at 96 h of exposure and in brain at 72 h. As for the *concentration of 149 μg L^{-1}*, significant increases with regard to the control group ($p < 0.05$) were observed at all exposure times in blood, gill and muscle, while in the brain an increase was observed at 12, 24, 72 and 96 h. Finally, in liver, a significant decrease was observed at 12 h. Furthermore, significant differences were observed between the different organs and concentrations (in blood, gill and muscle at 96 h of exposure; in liver at 12 and 96 h; in brain at 12, 24, 48 and 96 h).

3.7. Pearson correlation

Table 3 shows the correlations found between the oxidative stress biomarkers, exposure times and ACS concentration in the different organs. The values in bold letter indicate a higher degree of correlation between the variables analyzed. All the biomarkers used show a correlation at both exposure concentrations.

4. Discussion

Recently, artificial sweeteners have been identified in the environment as emerging contaminants (Lange et al., 2012) and the environmental levels strictly depend on their use in different countries and their degradability. ACS is not eliminated in wastewater treatment plants (WWTPs) and is very persistent in surface waters and even in groundwater due to the fact that it is a highly-hydrophilic compound and is stable at room temperature (Buerge et al., 2011). For this reason, it is considered an ideal chemical marker of domestic wastewaters in the environment. Concentrations of ACS in waste and surface waters have been monitored in multiple studies and show relatively high concentrations (Buerge et al., 2009). The results suggest that ACS is ingested by *Cyprinus carpio*, being detected in decreasing order, in blood, gill, liver, brain and muscle. While the organism does not metabolize this sweetener, ACS is an amphipathic molecule that does not necessarily require specific transport mechanisms in order to penetrate or spread across the phospholipid bilayer of the membrane (Schiffman, 2012; Szakacs et al., 2008). Because of the aforementioned and due to its high stability and resistance to hydrolysis (Lange et al., 2012), this sweetener cans bioconcentrate itself in different organs and tissues. Diverse studies which report the presence of sweeteners in fish, such as the study carried out by Saucedo-Vence et al. (2017), who report the presence of SUC in blood, gill, liver and muscle of *Cyprinus carpio*. Lillicrap et al. (2011) also reported an accumulation of the same sweetener in *Pseudokirchneriella subcapitata*, *Daphnia magna* y *Danio rerio*. Despite that the US Food and Drug Administration (FDA) and the public protection agencies in the European Union and China have demonstrated that ACS is safe for human consumption, diverse studies have demonstrated that this sweetener can generate toxicity (Subedi and Kannan, 2014; Bandyopadhyay et al., 2008). ACS contains, within its structure, the chemical methylene chloride, a known carcinogen. Also, one of its decomposition subproducts within the organism is acetoacetamide, which is toxic at high doses (Findikli and Türkoglu, 2014). Oxidative stress (OS) is a mechanism in which an imbalance exists between the production of reactive oxygen species (ROS) and antioxidant systems, altering cell metabolism and its regulation, thereby damaging cell components (Lushchak, 2011). The reaction between ROS and lipids is considered one of the most frequent mechanisms of cellular damage and is known as lipid peroxidation, a process of autopropagation in which the peroxyl radical is formed when an ROS such as the hydroxyl radical (OH•) has sufficient reactivity to abstract a hydrogen atom from an intact lipid (Halliwell and Gutteridge, 1999). Hydroperoxides are the main products of lipid peroxidation, which afterwards degrade into products of low molecular weight, including MDA (Niki et al., 2005). In this study, an increase in the content of hydroperoxides and MDA is observed (Figs. 1 and 2). This could be explained by the fact that ACS can suffer a photolytic transformation, generating subproducts such as hydroxylated acesulfame, isoacesulfame and amidosulfonic acid (Scheurer et al., 2014).

Hydroxylation is one route of common degradation in photolysis (Salgado et al., 2013). This type of reaction requires oxygen as a substrate, for which the stage of hydroxylation is a process dependent on oxygen (Brunelle et al., 2005). During this process, ROS can be created, which could have generated the damage to lipids, as observed in this study. In addition to the aforementioned, methylene

Table 3
Pearson's correlation between ACS concentrations and biomarkers of oxidative stress in blood, liver, gill, brain and muscle of *C. carpio*.

Organs	Exposure time (h)	Oxidative stress biomarkers									
		HPC		LPX		PCC		SOD		CAT	
		0.05 μg L^{-1}	149 μg L^{-1}	0.05 μg L^{-1}	149 μg L^{-1}	0.05 μg L^{-1}	149 μg L^{-1}	0.05 μg L^{-1}	149 μg L^{-1}	0.05 μg L^{-1}	149 μg L^{-1}
Blood	12	0.116	−0.437	**−0.803**	0.012	**0.570**	**1.000**	−0.047	**−0.907**	**0.907**	**−0.650**
	24	0.029	−0.317	−0.076	**−0.647**	**0.975**	−0.452	**0.504**	−0.496	0.218	**0.895**
	48	**−0.700**	0.417	−0.087	0.240	−0.200	**0.509**	**−0.845**	**−0.866**	0.225	**0.660**
	72	**0.859**	**−0.773**	**−0.932**	0.117	0.399	0.496	**−0.999**	−0.496	**0.957**	**0.994**
	96	−0.404	**0.695**	**0.585**	**−0.883**	**−0.858**	0.223	**0.706**	−0.062	0.271	0.410
Liver	12	−0.437	**0.980**	**−0.851**	**0.954**	0.117	**0.983**	0.084	**0.999**	0.125	**0.996**
	24	−0.444	−0.421	**−0.514**	0.213	0.415	**−0.943**	**0.687**	**0.760**	**0.818**	**−0.899**
	48	**−0.999**	−0.016	**−0.999**	**−0.626**	**−0.910**	**−0.754**	−0.314	**0.742**	**−0.993**	−0.030
	72	**0.963**	**0.995**	−0.094	**−0.735**	**0.999**	**0.517**	**0.616**	**0.750**	**0.850**	**0.954**
	96	**0.854**	0.338	**−0.906**	**0.738**	**0.890**	**0.558**	**−0.676**	**0.696**	**0.703**	**0.924**
Gill	12	0.439	−0.251	**−0.644**	0.110	**0.527**	**0.954**	**0.795**	0.015	**0.999**	−0.156
	24	−0.200	**−0.529**	**0.988**	**−0.991**	**0.986**	−0.334	**0.985**	**0.747**	**0.975**	**0.980**
	48	**0.802**	−0.201	**0.573**	**−0.999**	**0.999**	**−0.987**	0.234	**0.761**	**0.862**	**−0.539**
	72	**−0.664**	**0.966**	**0.987**	**−0.693**	0.042	−0.387	0.104	**−0.985**	**0.742**	**0.588**
	96	**0.910**	−0.051	**0.914**	**0.676**	**−0.789**	0.370	**−0.526**	**0.798**	**0.973**	**0.774**
Brain	12	–	−0.046	–	−0.272	–	−0.121	–	−0.306	–	−0.029
	24	–	**−0.508**	–	**0.872**	–	**−0.997**	–	**−0.812**	–	**−0.928**
	48	–	**−0.898**	–	**−0.832**	–	**0.871**	–	**−0.899**	–	**0.832**
	72	**−0.968**	**0.605**	**−0.843**	0.319	**−0.976**	0.422	**0.686**	0.477	**−0.992**	**0.907**
	96	−0.126	−0.358	**−0.807**	**0.746**	**0.960**	**−0.817**	**−0.628**	**−0.793**	**0.558**	**−0.968**
Muscle	12	–	0.234	–	**−0.643**	–	0.283	–	**−0.763**	–	−0.279
	24	–	**0.615**	–	0.211	–	0.304	–	**0.607**	–	**0.594**
	48	–	0.460	–	**−0.670**	–	**0.843**	–	−0.174	–	0.059
	72	**−0.754**	−0.104	**−0.848**	**−0.893**	−0.100	**−0.925**	−0.240	**−0.998**	−0.042	**−0.945**
	96	**−0.992**	**0.696**	**−0.823**	**−0.827**	**0.982**	0.038	**0.830**	**0.788**	**0.697**	0.458

Correlation coefficients > 0.5 are significant (shown in bold).

chloride is metabolized by cytochrome P-450 (CYP2E1) (Schlosser et al., 2015; EPA, 2011), also contributing to the generation of ROS in this study. CYP is present in the smooth endoplasmic reticulum of cells in different organs such as the liver, gut, kidney, gills, brain, heart and gonads (Sarasquete and Segner, 2000). Different P450 gene families have been characterized in fish, such as CYP1, CYP2, CYP3, CYP4, CYP11, CYP17 and CYP19 (Stegeman and Livingstone, 1998). In addition, acetoacetamide has a toxic effect on its own. The gills carry out the oxidative metabolism of many toxic agents, thus promoting ROS production responsible for damage to lipids in the present study (Islas-Flores et al., 2013). Also, the brain has a high lipid content that makes it susceptible to oxidative damage. Our results are consistent with those of others, such as Wiklund et al., 2014, who concluded that SUC induces lipid peroxidation in *Daphnia magna* after its exposure.

Lipids along with proteins are the major organic constituents of fish, and they play major roles as sources of metabolic energy for growth and movement (Tocher, 2003). The muscle contains the pro-oxidative heme proteins myoglobin or hemoglobin, which have been showed to be able to be good initiators of protein oxidation. The modifications caused by ROS in muscle proteins could be implicated in the loss of their functionality and therefore, in the loss of the quality of meat (Lund et al., 2011). Blood is susceptible of oxidative damage since, in addition to fulfilling diverse functions such as the transport of xenobiotics throughout the body, it also transports proteins and other biomolecules to all tissues (San Juan-Reyes et al., 2013).

Free amino acids and amino acid residues in proteins are highly susceptible to oxidation by one or more ROS (Stadtman and Levine, 2003). The abstraction of a hydrogen atom by an ROS leads to the generation of a protein carbon-centered radical (P•) which is consecutively converted into a peroxyl radical (POO•) in the presence of oxygen, and an alkyl peroxide (POOH) by abstraction of a hydrogen atom from another molecule (Lund et al., 2011). In this study, the damage to proteins is evidenced by an increase in PCC (Fig. 3). The formation of carbonyl compounds has been employed as a protein oxidation marker in vitro as well as in vivo (Davies, 2005). Damage to the proteins include sulfhydryl group oxidation, reduction of disulfides, the fragmentation of peptides, the modification of prosthetic groups and the nitration of proteins. All these modifications lead to the loss of protein function (Cabiscol et al., 2000). The generation of ROS by ACS has been reported by Kim et al. (2015), who reported a major production of ROS in zebra fish embryo and a higher embryo mortality rate when the fish were exposed to a low dose of ACS (mg mL^{-1}). While the mechanism by which ACS generates toxicity is not yet clear, studies have demonstrated that this sweetener generates DNA damage (Findikli and Türkoglu, 2014; Bandyopadhyay et al., 2008; Mukherjee and Chakrabarti, 1997) and a mechanism that induces damage to this macromolecule in aquatic organisms through OS (San Juan-Reyes et al., 2015; Valavanidis et al., 2006).

The antioxidant defenses may be induced by environmental contaminants in peroxidant conditions, as in the case of SOD and CAT enzymes. In this study, an increase in both enzymes is observed (Figs. 4 and 5), this as a consequence of ROS action. The SOD enzyme participates in the conversion of the superoxide anion (O_2•) into hydrogen peroxide (H_2O_2). Posteriorly, the CAT enzyme converts H_2O_2 into water and oxygen (San Juan-Reyes et al., 2013). However, some decreases in enzyme activity are also observed, which could be explained due to the fact that antioxidant levels initially increase in order to offset oxidative stress, but prolonged exposure leads to their depletion (Bebianno et al., 2005). Diverse authors have reported an increase in antioxidant enzymes SOD and CAT in response to different environmental contaminants (Cortes-Diaz et al., 2017; Saucedo-Vence et al., 2017; San Juan-Reyes et al., 2013; Oviedo-Gómez et al., 2010). Finally, all the biomarkers used in this study showed a correlation at both exposure concentrations (Table 3), for which damage induced by ACS is countered by the defense systems of *Cyprinus carpio*.

5. Conclusions

ACS induces oxidative stress in common carp (*Cyprinus carpio*) at the two concentrations of environmental relevance (0.05 and 149 μg L^{-1}), since significant increases were observed in HPC, degree of LPX and PCC in all the organs studied, as well as significant changes in the activity of SOD and CAT antioxidant enzymes. However, more studies are required in order to carry out an evaluation of risk of artificial and natural sweeteners, in particular of ACS, and implement more strict regulations in order to protect the environment.

Acknowledgments

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Conflict of interest

The authors declare they have no actual or potential competing financial interests.

References

Arbeláez, P., Borrull, F., Pocurull, E., Marcé, R.M., 2015. Determination of high-intensity sweeteners in river water and wastewater by solid-phase extraction and liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1393 (1), 106–114.
Bahndorf, D., Kienle, U., 2004. World market of sugar and sweeteners. https://www.uni-hohenheim.de/fileadmin/einrichtungen/stevia/downloads/World_Market_Sugar.pdf.
Bandyopadhyay, A., Ghoshal, S., Mukherjee, A., 2008. Genotoxicity testing of low-calorie sweeteners: aspartame, acesulfame-K, and saccharin. Drug Chem. Toxicol. 31 (4), 447–457.
Bassoli, A., Merlini, L., 2003. Sweeteners. In: Caballero, B. (Ed.), Encyclopedia of Food Sciences and Nutrition, 2nd ed. Academic Press, Oxford, pp. 5688–5695.
Bebianno, M.J., Serafim, A., Camus, L., Cosson, R.P., Fiala-Medoni, A., 2005. Antioxidant systems and lipid peroxidation in *Bathymodiolus azoricus* from Mid-Atlantic Ridge hydrothermal vent fields. Aquat. Toxicol. 75 (4), 354–373.
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 (1), 248–254.
Brunelle, J.K., Bell, E.L., Quesada, N.M., Vercauteren, K., Tiranti, V., Zeviani, M., Scarpulla, R.C., Chandel, N.S., 2005. Oxygen sensing requires mitochondrial ROS but not oxidative phosphorylation. Cell Metab. 1 (6), 409–414.
Büege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation. Methods Enzymol. 52 (1), 302–310.
Buerge, I.J., Buser, H.R., Kahle, M., Muller, M.D., Poiger, T., 2009. Ubiquitous occurrence of the artificial sweetener acesulfame in the aquatic environment: an ideal chemical marker of domestic wastewater in groundwater. Environ. Sci. Technol. 43 (12), 4381–4385.
Buerge, I.J., Buser, H.R., Poiger, T., 2011. The artificial sweetener acesulfame as marker of domestic wastewater in groundwater. IAHS AISH Publ. 342 (1), 103–106.
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.
Cabiscol, E., Tamarit, J., Ros, J., 2000. Oxidative stress in bacteria and protein damage by reactive oxygen species. Int. Microbiol. 3 (1), 3–8.
Celanese, 2014. Annual report pursuant to section 13 or 15(d) of the securities exchange act of 1934. http://corpdocs.gmianalyst.com/annual/ar_2014_105299.pdf.
Cortes-Diaz, M.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 (1), 811–823.
Davies, M.J., 2005. The oxidative environment and protein damage. BBA-Proteins Proteomics 1703 (2), 93–109.
EPA, 2011. Toxicological review of dichloromethane (methylene chloride). https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0070tr.pdf.
Findikli, Z., Türkoglu, S., 2014. Determination of the effects of some artificial sweeteners on human peripheral lymphocytes using the comet assay. J. Toxicol. Env. Health Sci. 6 (8), 147–153.
Halliwell, B., Gutteridge, J.M.C., 1999. Free Radicals in Medicine and Biology. Oxford University Press, Clarendon, Oxford.
Huang, D.J., Zhang, Y.M., Song, G., Long, J., Liu, J.H., Ji, W.H., 2007. Contaminants-induced oxidative damage on the carp *Cyprinus carpio* collected from the upper Yellow River. Environ. Monit. Assess. 128 (1–3), 483–488.
Huidobro, A., Pastor, A., Tejada, M., 2000. Quality index method developed for raw gilthead seabream (*Sparus aurata*). J. Food Sci. 65 (7), 1202–1205.
Islas-Flores, H., 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 (1), 32–38.

Jiang, Z.Y., Hunt, J.V., Wolff, S.P., 1992. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal. Biochem. 202 (2), 384–389.
Kelly, K.A., Havrilla, C.M., Brady, T.C., Abramo, K.H., Levin, E.D., 1998. Oxidative stress in toxicology: established mammalian and emerging piscine model systems. Environ. Health Perspect. 106 (7), 375–384.
Kim, J.Y., Park, K.H., Kim, J., Choi, I., Cho, K.H., 2015. Modified high-density lipoproteins by artificial sweetener, aspartame, and saccharin, showed loss of anti-atherosclerotic activity and toxicity in zebrafish. Cardiovasc. Toxicol. 15 (1), 79–89.
Kokotou, M.G., Thomaidis, N.S., 2013. Determination of eight artificial sweeteners in wastewater by hydrophilic interaction liquid chromatography-tandem mass spectrometry. Anal. Methods 5 (16), 3825–3833.
Kokotou, M.G., Asimakopoulos, A.G., Thomaidis, N.S., 2012. Artificial sweeteners as emerging pollutants in the environment: analytical methodologies and environmental impact. Anal. Methods 4 (10), 3057–3070.
Lange, F.T., Scheurer, M., Brauch, H.J., 2012. Artificial sweeteners—a recently recognized class of emerging environmental contaminants: a review. Anal. Bioanal. Chem. 403 (9), 2503–2518.
Leatherhead Food Research Association, 2014. Future Directions for the Global Functional Foods Market. Unknown Publisher.
Levine, R.L., Williams, J.A., Stadtman, E.R., Shacter, E., 1994. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol. 233 (1), 346–357.
Lillicrap, A., Langford, K., Tollefsen, K.E., 2011. Bioconcentration of the intense sweetener sucralose in a multitrophic battery of aquatic organisms. Environ. Toxicol. Chem. 30 (3), 673–681.
Lund, M.N., Heinonen, M., Baron, C.P., Estévez, M., 2011. Protein oxidation in muscle foods: a review. Mol. Nutr. Food Res. 55 (1), 83–95.
Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol. 101 (1), 13–30.
Magnani, L., Gaydou, M., Jean, C.H., 2000. Spectrophotometric measurement of antioxidant properties of flavones and flavonols against superoxide anion. Anal. Chim. Acta 411 (1–2), 209–216.
Marcon, J.L., Filho, W.D., 1999. Antioxidant processes of the wild tambaqui, *Colossoma macropomum* (Osteichthyes, Serrasalmidae) from the Amazon. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 123 (3), 257–263.
Marklund, S., Marklund, G., 1974. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47 (3), 469–474.
Mukherjee, A., Chakrabarti, J., 1997. *In vivo* cytogenetic studies on mice exposed to acesulfame-K—a non-nutritive sweetener. Food Chem. Toxicol. 35 (12), 1177–1179.
Müller, C.E., Gerecke, A.C., Alder, A.C., Scheringer, M., Hungerbühler, K., 2011. Identification of perfluoroalkyl acid sources in Swiss surface waters with the help of the artificial sweetener acesulfame. Environ. Pollut. 159 (5), 1419–1426.
Nelson, J.S., 1994. Fishes of the World. John Wiley & Sons, New York, USA.
Niki, E., Yoshida, Y., Saito, Y., Noguchi, N., 2005. Lipid peroxidation: mechanisms, inhibition, and biological effects. Biochem. Biophys. Res. Commun. 338 (1), 668–676.
Oviedo-Gómez, D.G.C., Galar-Martínez, M., García-Medina, S., Razo-Estrada, C., Gómez-Oliván, L.M., 2010. Diclofenac-enriched artificial sediment induces oxidative stress in *Hyalella azteca*. Environ. Toxicol. Pharmacol. 29 (1), 39–43.
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.
Perkola, N., Sainio, P., 2014. Quantification of four artificial sweeteners in Finnish surface waters with isotope-dilution mass spectrometry. Environ. Pollut. 184 (1), 391–396.
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.
Salgado, R., Pereira, V.J., Carvalho, G., Soeiro, R., Gaffney, V., Almeida, C., Cardoso, V.V., Ferreira, E., Benoliel, M.J., Ternes, T.A., Oehmen, A., Reis, M.A.M., Noronha, J.P., 2013. Photodegradation kinetics and transformation products of ketoprofen, diclofenac and atenolol in pure water and treated wastewater. J. Hazard. Mater. 244 (1), 516–527.
San Juan-Reyes, N., Gómez-Oliván, L.M., Galar-Martínez, M., Vieyra-Reyes, P., García-Medina, S., Islas-Flores, H., Neri-Cruz, N., 2013. Effluent from an NSAID-manufacturing plant in Mexico induces oxidative stress on *Cyprinus carpio*. Water Air Soil Pollut. 224 (9), 1–14.
San Juan-Reyes, N., Gómez-Oliván, L.M., Galar-Martínez, M., García-Medina, S., Islas-Flores, H., González-González, E.D., Cardoso-Vera, J.D., Jiménez-Vargas, J.M., 2015. NSAID-manufacturing plant effluent induces geno-and cytotoxicity in common carp (*Cyprinus carpio*). Sci. Total Environ. 530 (1), 1–10.
Sang, Z., Jiang, Y., Tsoi, Y.K., Leung, K.S.Y., 2014. Evaluating the environmental impact of artificial sweeteners: a study of their distributions, photodegradation and toxicities. Water Res. 52 (1), 260–274.
Sarasquete, C., Segner, H., 2000. Cytochrome P4501A (CYP1A) in teleostean fishes. A review of immunohisto- chemical studies. Sci. Total Environ. 247 (2), 313–332.
Saucedo-Vence, K., Elizalde-Velázquez, A., Dublán-García, O., Galar-Martínez, M., Islas-Flores, H., San Juan-Reyes, N., García-Medina, S., Hernández-Navarro, M.D., Gómez-Oliván, L.M., 2017. Toxicological hazard induced by sucralose to environmentally relevant concentrations in common carp (*Cyprinus carpio*). Sci. Total Environ. 575 (1), 347–357.
Scheurer, M., Schmutz, B., Happel, O., Brauch, H.J., Wülser, R., Storck, F.R., 2014. Transformation of the artificial sweetener acesulfame by UV light. Sci. Total Environ. 481 (1), 425–432.
Schiffman, S.S., 2012. Rationale for further medical and health research on high-potency sweeteners. Chem. Senses 37 (8), 671–679.
Schlosser, P.M., Bale, A.S., Gibbons, C.F., Wilkins, A., Cooper, G.S., 2015. Human health effects of dichloromethane: key findings and scientific issues. Environ. Health Perspect. 123 (2), 114–119.
Stadtman, E.R., Levine, R.L., 2003. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino Acids 25 (3–4), 207–218.
Stegeman, J.J., Livingstone, D.R., 1998. Forms and functions of cytochrome P450. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 121 (1), 1–3.
Subedi, B., Kannan, K., 2014. Fate of artificial sweeteners in wastewater treatment plants in New York State, USA. Environ. Sci. Technol. 48 (23), 13668–13674.
Szakacs, G., Varadi, A., Özvegy-Laczka, C., Sarkadi, B., 2008. The role of ABC transporters in drug absorption, distribution, metabolism, excretion and toxicity (ADME–Tox). Drug Discov. Today 13 (9–10), 379–393.
Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11 (2), 107–184.
Valavanidis, A., Vlahogianni, T., Dassenakis, M., Scoullos, M., 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol. Environ. Saf. 64 (2), 178–189.
Wiklund, A.K.E., Adolfsson-Erici, M., Liewenborg, B., Gorokhova, E., 2014. Sucralose induces biochemical responses in *Daphnia magna*. PLoS One 9 (4), e92771.
Zygler, A., Wasik, A., Namieśnik, J., 2009. Analytical methodologies for determination of artificial sweeteners in foodstuffs. TrAC Trends Anal. Chem. 28 (9), 1082–1102.