Science of the Total Environment 647 (2019) 772–784

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

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

Claudia Cruz-Rojas [a], Nely SanJuan-Reyes [a,c], María Paulina Aideé Gracia Fuentes-Benites [b], Octavio Dublan-García [a], Marcela Galar-Martínez [c], Hariz Islas-Flores [a], Leobardo Manuel Gómez-Oliván [a,*]

[a] *Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad Autónoma del Estado de México. Paseo Colón intersección Paseo Tollocan s/n, Col. Residencial Colón, 50120 Toluca, Estado de México, México*

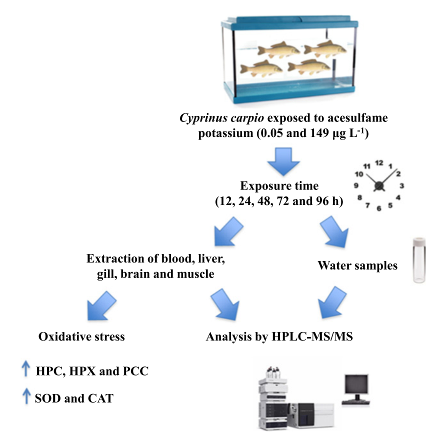
[b] *Laboratorio de Química Orgánica, Facultad de Química, Universidad Autónoma del Estado de México. Paseo Colón intersección Paseo Tollocan s/n, Col. Residencial Colón, 50120 Toluca, Estado de México, Mexico*

[c] *Laboratorio de Toxicología Acuática, Departamento de Farmacia, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Unidad Profesional Adolfo López Mateos, Av. Wilfrido Massieu Esq. Cda. Miguel Stampa s/n, Delegación Gustavo A. Madero, C.P.07738 CDMX, Mexico.*

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



ARTICLE INFO

Article history:
Received 1 June 2018
Received in revised form 2 August 2018
Accepted 3 August 2018
Available online 04 August 2018

Editor: D. Barcelo

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.

* Corresponding author at: Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad Autónoma del Estado de México, Paseo Colón intersección Paseo Tollocan s/n, Col. Residencial Colón, Toluca CP 50120, Estado de México, Mexico.
E-mail address: lmgomezo@uaemex.mx (L.M. Gómez-Oliván).

https://doi.org/10.1016/j.scitotenv.2018.08.034

Keywords:
Sweetener
Damage
Antioxidant enzymes
HPLC-MS/MS

(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