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Toxicological hazard induced by sucralose to environmentally relevant concentrations in common carp (*Cyprinus carpio*)

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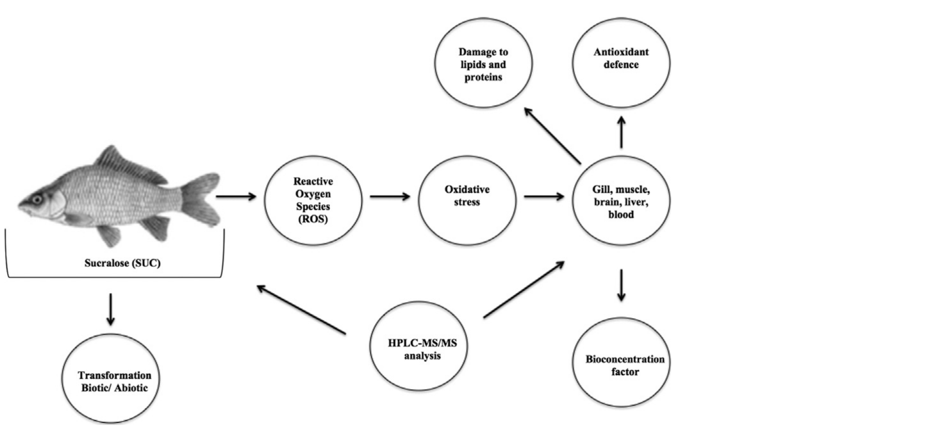
HIGHLIGHTS

- Sucralose was detected and quantified in different organs of *Cyprinus carpio*.
- Sucralose is not bio-accumulated in the organs and tissues of *Cyprinus carpio*.
- Sucralose induces the SOD and CAT activity in gills, muscle and brain of *Cyprinus carpio*.
- Sucralose induces damage to lipids and proteins in gills, muscle, brain and liver of *Cyprinus carpio*.

GRAPHICAL ABSTRACT



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ABSTRACT

Sucralose (SUC) is an artificial sweetener that is now widely used in North American and Europe; it has been detected in a wide variety of aquatic environments. It is considered safe for human consumption but its effects in the ecosystem have not yet been studied in depth, since limited ecotoxicological data are available in the peer-reviewed literature. This study aimed to evaluate potential SUC-induced toxicological hazard in the blood, brain, gill, liver and muscle of *Cyprinus carpio* using oxidative stress biomarkers. Carps were exposed to two different environmentally relevant concentrations (0.05 and 155 μg L^{-1}) for different exposure times (12, 24, 48, 72

Abbreviations: AChE, acetylcholinesterase; ACS, acesulfame; AGE, advanced glycation end product; ALI, alitame; ANOVA, analysis of variance; ASP, aspartame; BCF, bioaccumulation factor; CAT, catalase; CYC, cyclamate; DNPH, di-nitro phenyl hydrazine; ESI, electrospray ionization; HPLC-MS/MS, high pressure liquid chromatography tandem mass spectrometry; HPC, hydroperoxide content; LPX, lipid peroxidation; MDA, malondialdehyde; MEC, molar extinction coefficient; MRM, multiple reaction monitoring; NEO, neotame; NHDC, neohesperidin dihydrochalcone; ORAC, oxygen radical absorbing capacity; PCC, protein carbonyl content; RLS, restless leg syndrome; ROS, reactive oxygen species; SAC, saccharin; SOD, superoxide dismutase; SUC, sucralose; TBA, thiobarbituric acid; TCA, trichloroacetic acid.

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and 96 h). The following biomarkers were evaluated: lipid peroxidation (LPX), hydroperoxide content (HPC) and protein carbonyl content (PCC), as well as the activity of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT). SUC was determined by high pressure liquid chromatography tandem mass spectrometry techniques (HPLC)–MS/MS. Results show a statically significant increase in LPX, HPC, PCC ($P < 0.05$) especially in gill, brain and muscle, as well as significant changes in the activity of antioxidant enzymes in gill and muscle. Furthermore, the biomarkers employed in this study are useful in the assessment of the environmental impact of this agent on aquatic species.

1. Introduction

Artificial sweeteners are used worldwide as sugar substitutes in remarkable amounts in food, beverages, and also in drugs and sanitary products, such as mouthwashes. They provide no or negligible energy and thus are ingredients of dietary products (Kroger et al., 2006; Zygler et al., 2009). The most popular artificial sweeteners are aspartame (ASP), neotame (NEO), alitame (ALI), acesulfame (ACS), saccharin (SAC), cyclamate (CYC), sucralose (SUC), and neohesperidin dihydrochalcone (NHDC). Of the variety of artificial sweeteners being used, only ACS, CYC, SAC and SUC have been identified in wastewater effluents (Lange et al., 2012).

Artificial sweeteners are highly consumed, particularly in the U.S., with increasing trends in consumption, especially after the introduction of SUC in 1998. The global market for artificial sweeteners reaches $5.1 billion, of which the U.S. and Europe currently make up 65% (Bennett, 2008). Production volumes of artificial sweeteners vary between reports. The U.S. is currently the largest market for SUC, making use of more than 1500 tons per year, followed by Europe, with around 400 tons per year, as reported by a major Chinese company that recently entered into the SUC market. In the Asian Pacific market, the volume output in total of SAC, CYC, ACS, ASP, SUC, ALI and NEO, grew approximately 10% between 2009 and 2010, reaching approximately 109,000 tons per year (Kokotou et al., 2012). SUC constituted about 16% of the U.S. high intensity sweeteners market in 2009, and its growth is expected to be high, almost 5% annually, through the next few years (Haely, 2012).

Although from the beginning of their use there have been controversies over their risk as potential carcinogens (Weihrauch and Diehl, 2004), these sweetener compounds are generally considered to be safe for use in foodstuffs (Cohen et al., 2008; Kroger et al., 2006; Ahmed and Thomas, 1992). Moreover, due to these compounds are metabolically inert in the human body it has been believed that are also inert in the environment. However in recent years the concern is shifting from health concerns to ecosystem concerns (Sang et al., 2014).

Excretion after human consumption is undoubtedly a major source of artificial sweeteners in the environment, but it is surely not the only one (Kokotou et al., 2012). From households and industries, all artificial sweeteners enter into wastewater treatment plants, where in most cases passes without any change through these processes, as a result they eventually reside in the receiving environmental water bodies (Houtman, 2010). In addition, direct discharges from industry, households, animal farming and agriculture burden surface waters with artificial sweeteners (Houtman, 2010).

SUC (also known as Splenda) is a relatively new artificial sweetener that is now widely used in North American and Europe. SUC is produced by the chlorination of sucrose, which leads to a stabile compound that is poorly absorbed in the mammalian gastro-intestinal (GI) tract. The majority of orally ingested sucralose is excreted as unchanged parent compound, with <1% of the original oral dose excreted as two glucuronide adduct metabolites (Sims et al., 2000). It may seem like an odd compound to include as an emerging contaminant, but it is now being found in environmental waters and it is extremely persistent (half-life up to several years) (Richardson, 2010).

Sucralose has been detected in a wide variety of aquatic environments. A Swedish study reported concentrations of SUC in treated effluent to be ≤11 μg L^{-1}, while surface water concentrations were ≤3.6 μg L^{-1} (Brorstrom-Lunden et al., 2008). Other studies have measured sucralose in effluents in surface waters at concentrations ≤2.5 μg L^{-1} (Ferrer and Thurman, 2010; Neset et al., 2010; Loos et al., 2009; Scheurer et al., 2009). One hundred and twenty samples were collected from rivers in 27 European countries, and sucralose was found up to 1 μg L^{-1}, predominantly in samples from the United Kingdom, Belgium, The Netherlands, France, Switzerland, Spain, Italy, Norway, and Sweden, with only minor levels (<100 ng L^{-1}) detected in samples from Germany and Eastern Europe, suggesting a lower use of sucralose in those countries (Richardson and Ternes, 2011).

SUC is considered safe for human consumption (the acceptable daily intake for SUC was set at 5 mg kg^{-1} of body weight per day) (Grotz and Munro, 2009; Brusick et al., 2010; Viberg and Fredriksson, 2011), but its effects in the ecosystem have not yet been studied in depth, since limited ecotoxicological data are available in the scientific literature. Hjorth et al. (2010) evaluated egg production, hatching rate, food intake and mortality of two species of copepods, *Calanus glacialis* and *Calanus finmarchicus* exposed to six different concentrations (0–50 mg L^{-1}) of SUC. The results showed that both species responded weakly to SUC, but with *C. glacialis* being possibly slightly more sensitive than *C. finmarchicus*. Huggett and Stoddard (2011) assessed the effects of SUC on the survival, growth and reproduction of *Daphnia magna* and *Americamysis bahia* (mysid shrimp). They concluded that the concentrations of SUC detected in the environment are well below those required to elicit chronic effects in freshwater or marine water bodies. On the other hand, recently, a study on crustaceans showed for the first time that physiology and locomotive behaviour could be affected by exposure to SUC (0.0001–5 mg L^{-1}). The behavioural response of *Daphnia magna* manifested as altered swimming height and increased swimming speed, whereas in gammarids the time to reach food and shelter was prolonged. These authors suggest that exposure to sucralose may induce neurological and oxidative mechanisms with potentially important consequences for *D. magna* behaviour and physiology (Eriksson-Wiklund et al., 2014). Research on the ecotoxicology of SUC is expected to increase in next years, since both short and long-term effects resulting from exposure to low levels of this compound is largely unknown.

Biomarkers are measurable internal indicators of changes in organisms at the molecular or cellular level, which can offer great potential to understand the environmentally mediated disease, and to improve the process of risk assessment (Valavanidis and Vlachogianni, 2010). Oxidative stress, is considered as one of the major mechanisms of action of toxicants, and is among the most frequently used biomarkers since it is able to evaluate general damage to biomolecules such as lipids, proteins and DNA (Barata et al., 2005). Oxidative damage to lipids, proteins and DNA and adverse effects on enzymatic antioxidant defence mechanisms in aerobic organisms has been used in recent years as biomarkers for monitoring environmental pollution (Valavanidis et al., 2006). The most important oxidative stress biomarkers used in toxicological studies of aquatic systems are lipid peroxidation (LPX), hydroperoxide content