Science of the Total Environment xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Science of the Total Environment

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

The oxidative stress in the liver of *Carassius auratus* exposed to acesulfame and its UV irradiance products

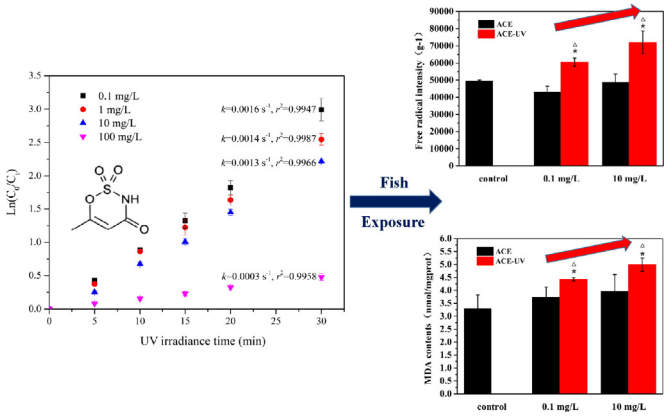
Yuhang Ren, Jinju Geng *, Fuchang Li, Hongqiang Ren, Lili Ding, Ke Xu

State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210023, People's Republic of China

HIGHLIGHTS

- The oxidative status in liver of *Carassius auratus* exposed to ACE had no distinct change.
- An increased oxidative stress of *Crassius auratus* was observed by exposed to ACE UV irradiance products.
- ACE after UV irradiance is more eco-toxicity than ACE itself by inducing the accumulation of OH.
- Eight UV irradiation products of ACE were identified.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 May 2016
Received in revised form 4 July 2016
Accepted 6 July 2016
Available online xxxx

Editor: Kevin V. Thomas

Keywords:
Artificial sweeteners
Eco-toxicity
UV irradiance
Hydroxyl radical

ABSTRACT

Acesulfame (ACE) is listed as an emerging contaminant due to its environmental persistence and wide occurrence in the environment. ACE can be degraded partially in the regular UV disinfection process but the eco-toxicity of its irradiation products remains unclear. This study focused on the possible oxidative status change in the liver of *Carassius auratus* exposed to ACE and its irradiation products. The UV degradation of ACE follows pseudo-first-order kinetics, and eight irradiation products were identified. Fish were exposed 7 days to 0.1 and 10 mg/L ACE (ACE group) and ACE after UV irradiance (ACE-UV group). The oxidative stress in fish liver exposed to ACE group had no distinct change. However, in the ACE-UV group, the quantity of •OH was induced by 17.96–55% and the MDA content increased by 16.28–68.28% compared to control. Time-effect exposure in the ACE-UV group showed that in the first 3 days the quantity of •OH reached its peak, causing severe inhibition of SOD and continuous inducement of GPx. GSH helped scavenge •OH and decreased below control after 3 days. An increased toxicity of ACE after UV irradiance was observed and its transfer after into aquatic environment needs to be recognized as an environmental risk.

1. Introduction

Artificial sweeteners (ASs) are used globally as no-sugar or low-calorie sugar substitutes in food, beverages, pharmaceuticals and personal

* Corresponding author.
E-mail address: jjgeng@nju.edu.cn (J. Geng).

http://dx.doi.org/10.1016/j.scitotenv.2016.07.047
0048-9697/

care products (Gan et al., 2013a). Most of these sweeteners remained unchanged through the human body after ingestion and ultimately enter the aquatic environment (Scheurer et al., 2009; Stolte et al., 2013). They have become a topic of concern in recent years as emerging contaminants. They have the highest known concentration among trace contaminants. They are persistent and present potential ecological risk (Lange et al., 2012).

Among the ASs, acesulfame (ACE) has caused greater concern because of its prevalent and wide occurrence in the environment (Lange et al., 2012). ACE has been detected mainly in western Europe and America with concentrations of up to 50 μg/L in wastewater treatment plant (WWTP) influents (Lange et al., 2012; Scheurer et al., 2009) and as high as 25 μg/L in surface water (Lange et al., 2012). ACE has also had the maximum median concentration of 14.3 μg/L in 90 WWTP effluents in Europe (Loos et al., 2013). In China, ACE concentration was determined to be in WWTP influent with up to 17 μg/L, and the surface water in Tianjin was reported to be 7.6 μg/L (Gan et al., 2013a).

WWTPs are considered to be the main 'hotspots' for the release of ACE into the environment. In general, ACE in WWTP can only be partly removed, which means residual amounts can reach surface water or groundwater. Previous studies have confirmed that ACE is extremely resistant to biodegradation and is generally resistant to hydrolysis (Gan et al., 2014). Coagulation, chlorination and sorption to active carbon were very limited in their ability to remove ACE, while ACE could be degraded in ozonation and some other advanced oxidation processes (AOPs) (Mailler et al., 2015; Scheurer et al., 2010; Soh et al., 2011). Several publications have documented that ACE could be degraded by UV radiation. For example, ACE could be degraded by 30% in UV oxidation process in a full-scale waterworks (Scheurer et al., 2014). A wide range of emerging contaminants including polycyclic aromatic hydrocarbons (PAHs), antibiotics and pharmaceuticals were found to be much more toxic than the parent compounds after UV irradiance (Jung et al., 2008; Klamerth et al., 2010; Sang et al., 2014). To evaluate the UV phototoxicity of ACE, Sang et al. (2014) confirmed a photo-enhancement toxicity factor of 575 for ACE under UV irradiance in the acute toxicity test using *Vibrio fischeri*. Our preliminary research also found that the inhibition rate of ACE to *Vibrio fischeri* was increased by 11.9% – 22.48% after 150 J/m^2 UV irradiance (unpublished data). A recent publication found that the transformation products of ACE under UVC would cause significant adverse effects during embryo development of zebrafish *Danio rerio* (Li et al., 2016). Limited research to date has been conducted to investigate the chronic ecotoxicity of ACE on aquatic organisms.

Although ACE was proved safe for human use by the US Food and Drug Administration (FDA), the public protection agencies in the European Union and China, it was reported that ACE may cause DNA damage in the bone marrow cells of mice (Bandyopadhyay et al., 2008) and interfere with photosynthesis in plants (Subedi and Kannan, 2014). Increased reactive oxygen species (ROS) production in zebrafish embryo and a higher embryo death rate was found when the fish were exposed to low g/L dosage ACE (Kim et al., 2015). Some other ASs would cause developmental toxicity to *Oryzias latipes* (Lee and Wang, 2015). Thus a comprehensive evaluation of the toxicity of ACE is needed. UV radiation is currently used as a disinfection method in WWTPs. This study assesses oxidative stress in the liver of *Carassius auratus* (*C. auratus*, a commonly used test organism in China) exposed to ACE and its UV irradiance products. The purpose of this research is: (1) to compare the toxicity of ACE and its UV irradiation products using *C. auratus* as testing organism and (2) to describe some oxidative stress parameters and antioxidant defense factors in vivo.

2. Materials and methods

2.1. Reagents

Acesulfame potassium, N-tert-Butyl-α-phenylnitrone (PBN), dimethyl sulfoxide (DMSO), ammonium acetate and the ion pair reagent TRIS were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol and HPLC-grade acetonitrile were supplied by Merck (Darmstadt, Germany). Isopropanol, sodium azide, sorbic acid and other chemicals were analytically graded and obtained from Nanjing Chemical Reagent Factory, China. Milli-Q water, with a resistivity of at least 18.2 MΩ cm, was produced from a Millipore purification system (Billerica, CA, USA).

2.2. UV irradiation of ACE

The UV irradiation of ACE was conducted in a photoreaction operation reactor (XPA-7, Nanjing Xujiang Motor Factory, China). A low pressure mercury light (22 W, 254 nm) was placed in the center of the reactor with a quartz cover and light was supplied by an electronic ballast. The UV irradiation intensity was 520 μW/cm^2, measured by a UV radiation meter (purchased from Photoelectric Instrument Factory of Beijing Normal University, China).The irradiated sample was placed in a 50 mL quartz tube filled with ACE solution and stirred by an electromagnetic stirrer. To determine the degradation kinetics of ACE, ACE solution with different concentrations (0.1, 1, 10 and 100 mg/L) was irradiated, and samples were taken at specific time intervals within 30 min. Finally, 0.1 mg/L and 10 mg/L ACE solution was irradiated for 5 min in preparation for the toxicity exposure experiment.

2.3. Fish breeding and exposure treatment

C. auratus with a mean body length of 10.00 ± 0.1 cm, was obtained from Wetland park aquatic breeding base (Nanjing, China) with a mean body length of 10.00 ± 0.10 cm. All fish were acclimatized to aerated tap water over 3 days before the experiment and the total mortality of fish was below 1%. Fish were randomly selected into different group ($n = 6$) and kept in 25 L glass aquaria. Fish were fed with commercial feed once a day.

During 7 days of dose-effect experiments, fish were exposed to 0 (control), 0.1 and 10 mg/L of ACE (ACE group) and ACE solution after 5 min of UV irradiation (ACE-UV group) for 7 days, respectively. The 0.1 mg/L ACE was set as a concentration relatively close to that in the aquatic environment (Lange et al., 2012). In addition, 10 mg/L concentration of ACE was set as a higher concentration. Another time-effect toxicity experiment was conducted with fish exposed to 0.1 mg/L ACE-UV group over different periods (1, 3, 5, 7 and 9 days). Half of the water was replaced by the same designated solution during the whole exposure experiment and metabolites were removed daily. At the end of the exposure period, fish were sacrificed to obtain fresh livers. Exact 0.1 g treated fish liver was used for the determination of ROS and the rest of the livers were homogenized by homogenizer at 4 °C for the determination of other biochemical parameters.

2.4. Analytical methods

ACE was quantified by LC-MS (Waters Xevo TQ-S UPLC-MS system, USA) equipped with an electrospray ionization (ESI) interface using the method modified from previous studies (Arbelaez et al., 2015; Gan et al., 2013b). Chromatographic separation was performed on an Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm) at 30 °C in the gradient elution mode. The mobile phase was composed of water (A) and acetonitrile (B), both containing 5 mM ammonium acetate and 1 mM TRIS. Gradient elution was carried out at a flow rate of 0.1 mL/min, and the mobile phase gradient was ramped linearly from 10% to 70% B in 3 min, returned back to 10% within 2 min and the system was allowed to equilibrate for 1 min before the next injection. To distinguish the irradiation products of ACE under UV irradiance, the experiment was carried out at an initial concentration of 10 mg/L. Full scan spectra (m/z 10–400) were obtained with ESI in negative ion mode. Deionized water and methanol were applied as mobile phase A and B, respectively. An 8 min gradient elution started with 5% mobile phase B and then

Please cite this article as: Ren, Y., et al., The oxidative stress in the liver of *Carassius auratus* exposed to acesulfame and its UV irradiance products, Sci Total Environ (2016), http://dx.doi.org/10.1016/j.scitotenv.2016.07.047