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

*Corresponding author:

Jinju Geng

State Key Laboratory of Pollution Control and Resource Reuse

School of the Environment

Nanjing University

Nanjing

210023

People's Republic of China

Tel.: +86 25 89680360

Fax: +86 25 89680569.

E-mail: jjgeng@nju.edu.cn

Corresponding author. Tel: +86 25 89680360. E-mail address: jjgeng@nju.edu.cn

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 d 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 $\cdot\text{OH}$ 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 d the quantity of $\cdot\text{OH}$ reached its peak, causing severe inhibition of SOD and continuous inducement of GPx. GSH helped scavenge $\cdot\text{OH}$ and decreased below control after 3 d. 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.

Key words: Artificial sweeteners; Eco-toxicity; UV irradiance; Hydroxyl radical

1. Introduction

Artificial sweeteners (ASs) are used globally as no-sugar or low-calorie sugar substitutes in food, beverages, pharmaceuticals and personal 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, Klammerth 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/m² 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 M Ω ·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 (22W, 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 uW/cm², 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 $\pm$ 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 d 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 d). 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 ramped linearly to 90% over 7 min, after which it was held constant for 1 min. Then it came back to initial status and it was allowed to equilibrate for another 1 min.

The total organic carbon (TOC) concentration was analyzed by TOC 5000A analyzer (Shimadzu Corporation, Japan) after filtration through a 0.45 μm filter.

2.5 ROS analysis

ROS in the livers of *C. auratus* was extracted by PBN and detected by electron paramagnetic resonance (EPR). After rinsing with ice-cold physiological saltwater, the fish livers were immediately weighed, and a fraction (0.1 g) was removed and homogenized quickly in 1.0 mL of 50 mM PBN dissolved in DMSO using a

homogenizer in a centrifuge tube. Then 0.1 mL of supernatant was transferred to a capillary tube with a diameter of 0.9 mm, and frozen by liquid nitrogen for EPR analysis. The EPR spectra were recorded with Bruker EMX 10/12 X-band spectrometer (Bruker, Germany) at room temperature. The operation conditions were: center field, 3,470 G; scan range, 200 G; modulation frequency, 100 kHz; modulation amplitude, 0.5 G; receiver gain, 5×10^4 scans, five times; and microwave power, 20 mW.

2.6 Determination of the biochemical parameters

Fish liver samples were weighed and homogenized in normal saline with the ratio at 1/9 of weight/volume. The homogenate was centrifuged for 10 min at 4 °C at 2,500 rpm and the supernatant was used as the enzyme source. The total protein, malondialdehyde content (MDA) and reduced glutathione (GSH) were determined with a protein detection kit, an MDA detection kit and a GSH detection kit from Nanjing Jian Cheng Biology Company (China). Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activity were also determined with the corresponding detection kit from Nanjing Jian Cheng Biology Company.

2.7 Statistical analysis

Statistical analyses were performed using the SPSS statistical package. All data

were determined at least thrice and expressed as mean values $\pm$ standard deviation (SD). The significant difference between two experimental groups were compared by a one-way analysis of variance (ANOVA), and significance was identified by post hoc-LSD test at $p < 0.05$. A *significant difference was marked between exposure group and control group, Δ significant difference was marked between ACE group and ACE-UV group and $\diamond$ significant difference was marked between parameters in 0 day and other exposure time.

3. Results and discussion

3.1 UV degradation process of ACE

3.1.1 Effect of initial concentration on degradation of ACE

The UV degradation of ACE followed pseudo first-order kinetics in the test condition (Fig. 1). The degradation process exhibited similar change trends and the rate constant k ranged from $1.3 \times 10^{-3} \text{ s}^{-1}$ to $1.6 \times 10^{-3} \text{ s}^{-1}$ between 0.1 mg/L and 10 mg/L. At the higher concentration of 100 mg/L, the degradation was much slower ($k=0.3 \times 10^{-3} \text{ s}^{-1}$), and it may be explained by the limited photons emitted from the lamp (Scheurer et al. 2014). Five min UV irradiance time was chosen in the ACE-UV group as it was more close to the realistic condition. After 5 min UV irradiation, ACE could be degraded by $21.75 \pm 1.4\%$ in the 10 mg/L set and $34.77 \pm 0.55\%$ in the 0.1 mg/L set. TOC stayed unchanged during the UV irradiance process (data not shown),

and it was speculated that ACE translated into unknown UV irradiance products.

Fig. 1 UV photodegradation of ACE in ultrapure water at different initial concentrations

3.1.2 Identification of UV irradiance products

Upon UV irradiance of ACE in this study, eight UV irradiance products were identified based on ion peaks observed in a UPLC-MS full scan analysis (Fig. 2). Previous studies reported hydroxyl-acesulfame ($\text{C}_4\text{H}_6\text{NO}_5\text{S}^-$) ($m/z=180$) as a main transformation product and aminosulfonic acid ($m/z=96$) as a minor product in the UV irradiation of ACE (Scheurer et al. 2014). With the fragment ions of product 180 detected at $m/z=96$ (NH_2SO_3), 80 (SO_3) and 122, and fragment ions of product 136 found at $m/z=78$ and 84 (Fig. S1), it is in agreement with Gan et al. (2014) that

product 180 might be formed by the addition of water at the C=C bond and subsequently yield product 136. While this pathway is different with that described by Sang et al. (2014) and Li et al. (2016). The mechanism of regular detected product 194, 196 and 212 was described elsewhere (Gan et al. 2014). The unknown product ion of $m/z=154$ and 230 was also found in the UV irradiance of ACE in a recent report (Li et al. 2016). Same fragment ion of product 154 with $m/z=96$ and 136 (Fig. S1) were also found comparing to Li but none fragment ion of $m/z=230$ was found in our research (Li et al. 2016). Even more complex 12 transformation products were confirmed under prolonged UV irradiance, and the rapid forming products of m/z 180, 194, 154 and 212 through the UV process was also present in our study (Sang et al. 2014). Complex irradiation products may induce an uncertain environmental risk potential so it is meaningful to evaluate the risk led by these photocatalytic transformation products.

Fig. 2 Changes of UV irradiance products with irradiation time ($C_0=10$ mg/L)

Isopropanol, sodium azide and sorbic acid were used as hydroxyl radical ($\cdot\text{OH}$) scavenger, $^1\text{O}_2$ scavenger and triplet excited state inhibitor, respectively (concentration with 1 mg/L and 5 mg/L). As can be seen in Fig. 3, the addition of 1 mg/L sorbic acid (same with 5 mg/L sorbic acid) completely suppressed the UV photolysis of ACE suggesting that the formation of a triplet excited state played a major role in the degradation process. Gan et al. (2014) also found that the addition of sorbic acid would suppress the degradation of ACE under simulated sunlight (Gan et al. 2014). The addition of 1 mg/L and 5 mg/L sodium azide slightly inhibited the UV photolysis of ACE, indicating that dissolved oxygen participated in the photolysis and $^1\text{O}_2$ involved in the mechanism (Wang et al. 2015). No significant changes in the

photolysis of ACE occurred when 1 mg/L and 5 mg/L isopropanol was added, which excluded the influence of hydroxyl radical.

Fig. 3 UV degradation of ACE (0.1 mg/L) with the addition of NaN₃, isopropanol and sorbic acid

3.2 Effect of UVC on the toxicity of ACE

3.2.1 Free radical production and MDA content

The EPR spectrum of fish hepatic free radicals trapped by PBN in the 10 mg/L ACE-UV group is shown in Fig. 4. The EPR spectrums of control group and ACE group are similar to that of ACE-UV group; thus, data are not shown. After analyzing the EPR spectrum, the hyperfine splitting constants for the PBN-radical adduct ($g=2.0084$, $a^N=13.88$, $a^H=2.34$) were similar to the characteristics of PBN/ $\cdot\text{OCH}_3$ and PBN/ $\cdot\text{CH}_3$. It has been reported that the reaction of DMSO with $\cdot\text{OH}$ produces $\cdot\text{CH}_3$,

after which $\cdot\text{CH}_3$ was oxidized to $\cdot\text{OCH}_3$ under aerobic conditions (Takeshita et al. 2004). Thus the free radical generated in the ACE group and ACE-UV group was confirmed as $\cdot\text{OH}$.

Fig. 4 EPR spectrum of free radicals in liver of *C. auratus* in ACE-UV group

The quantity of $\cdot\text{OH}$ generation of *C. auratus* exposed to 0.1 and 10 mg/L ACE and ACE-UV group for 7 days could be calculated by the EPR spectrum as well. Fig. 5a confirmed that there was no significant difference of $\cdot\text{OH}$ generation in the ACE group compared to the control experiment, suggesting that there was no accumulation of $\cdot\text{OH}$ when exposed to ACE itself. ACE proved to have no distinct affection on the oxidative status of *C. auratus* under the testing condition. A minimally toxic of ACE was also proven in zebrafish and golden orfe since ACE is a legally allowed food

additive (Sang et al. 2014). Up to 1000 mg/L ACE also posed no hazard to green algae *Scenedesmus vacuolatus* (24 h), water fleas *Daphnia magna* (48 h) and duckweed *Lemna minor* (7 d) (Stolte et al. 2013). This results suggests that the aquatic environmental level of ACE does not increase the risk to organisms.

Compared to the ACE group, the quantity of $\cdot\text{OH}$ could be induced by $22.29\pm 8.77\%$ in the 0.1 mg/L ACE-UV group and $28.11\pm 16.93\%$ in the 10 mg/L ACE-UV group, indicating that the degree of oxidant stress in the ACE-UV group was higher than that in the ACE group. It could be speculated that the complex UV irradiation products of ACE should be responsible for the accumulation of $\cdot\text{OH}$. It was reported that UV enhanced phototoxicity of PAHs was dependent on the absorption properties of the compound and formation of damaging singlet oxygen (Huovinen et al. 2001). While the maximum absorbance of ACE was ~ 225 nm and tails over 290 nm (Gan et al. 2014), UVC light would potentially induce the toxicity of ACE. Such photo-enhanced toxicity was also found in sulfonamide, and the EC_{50} of sulfonamides to *Daphnia magna* were reported to increase by several fold after UVB irradiance; which was associated with the production of ROS (Selvaag et al. 1997). It was hard to distinguish which irradiation product of ACE was the main contributor to the enhancement of toxicity since it was more of a combined effect

Fig. 5 Changes of (a) $\cdot\text{OH}$ signal intensity and (b) MDA contents in fish liver exposed to the ACE group and the ACE-UV group for 7 days

Lipid peroxidation (LPO) is the most frequent cellular damage caused by ROS, and MDA is the main terminal production in the reaction between ROS and lipids (Li et al. 2015). Fig. 5b shows that compared with the control group, MDA contents in the ACE group increased slightly but no significant difference could be observed ($p > 0.05$). MDA contents increased significantly in the ACE-UV group ($p < 0.05$), ranging from 3.29 nmol/mgprot to the level of 4.43 nmol/mgprot (0.1 mg/L) and 4.99 nmol/mgprot (10 mg/L). MDA contents was in strong positive accordance with $\cdot\text{OH}$ quantity ($r = 0.889$, $n = 5$, $p = 0.05$), suggesting LPO in fish liver was induced by $\cdot\text{OH}$ accumulation in ACE-UV group. Previous studies have confirmed that phenanthrene, pentachlorophenol, pyrene and 2-Chlorophenol could significantly induce $\cdot\text{OH}$ in fish liver and resulted in LPO (Luo et al. 2008, Yin et al. 2007). The degree of LPO in fish liver was also enhanced when exposed to PCBs, PAHs and various metals (Livingstone 2001). The increased MDA contents indicated that LPO in fish liver was

promoted in the ACE-UV group thus an oxidative damage occurred.

3.2.2 Changes of the total protein, SOD, and CAT activity

Chemoreceptor studies have shown that herbivorous and omnivorous species are sensitive to carbohydrates (Corotto and O'brien 2002), and the oxidative stress may be associated with ACE exposure stimulating feeding and increasing calorie intake (Eriksson Wiklund et al. 2014). This supposed association was expressed in the increase of the total protein. However no significant difference in fish liver of the total protein (Fig. 6a) was found exposed to the ACE group and ACE-UV group for seven days ($p > 0.05$). No changes in the total protein excluded the ACE/oxidative stress rationalization and confirmed that $\cdot\text{OH}$ accumulation was due to the induction by ACE irradiation products.

A slight inhibition (2.39~5.32%) of CAT activity (Fig. 6b) occurred but no significant difference was found with the control (except for the 0.1 mg/L ACE-UV group, $p < 0.05$). As for the SOD activity (Fig. 6c), a significant inhibition by 8.5~29.53% compared to the control was found in all exposure groups and more severe inhibition occurred in the ACE-UV group. The SOD system is the first line of defense against oxygen toxicity. CAT and GPx are also important antioxidant enzymes (Li et al. 2015). SOD and CAT activity in fish liver (*Pimephales promelas*) was also inhibited by some pharmaceutical and personal care products (Jasinska et al. 2015). The inhibition could be explained in two ways. ACE and its irradiation products could

possibly not be metabolized by fish and the conjugation with SOD would inactivate the enzymes. Since a significant accumulation of ROS occurred in the ACE-UV group (Fig. 5a), it exceeded the ability of antioxidant enzymes to scavenge ROS and other cellular defense systems (such as GSH) were activated to remove ROS (Luo et al. 2006). Compared with CAT activity, SOD enzymes were a more sensitive biomarker showing the oxidative stress exposed to ACE in this research.

More severe accumulation of ROS and oxidative damage occurred in the 10 mg/L ACE-UV group but more research is still needed to confirm the dose-effect toxicity. The following time-effect relationship toxicity experiment was conducted in the 0.1 mg/L ACE-UV group, which is relatively close to the aquatic environment.

(a)

(b)

(c)

Fig.6 Changes of the (a) total protein contents, (b) SOD activity and (c) CAT activity in fish liver exposed to the ACE group and the ACE-UV group for 7 days

3.3 Time-effect of oxidative stress induced by 0.1 mg/L ACE irradiation products

Fig. 7 shows the time-effect changes of some biochemical parameters representing the oxidative status. As can be seen in Fig. 7a, $\cdot\text{OH}$ generation increased significantly in the first 3 days and then showed a fluctuation. An increase by 21.49~52.6% was induced in all exposure groups and the maximum accumulation of $\cdot\text{OH}$ occurred on the 3rd day. An increase of MDA content during the whole exposure period was found with the maximum increase of 100.63% and also occurred on the 3rd day, exhibiting similar change trends with $\cdot\text{OH}$ intensity (Fig. 7b). No significant changes in the total protein was observed (data not shown), which was similar to the dose-effect observations. Fig. 7c showed that the SOD activity was significantly inhibited in 1 and 3 d by 61.69% and 64.57% respectively. After 3 d, SOD activity rebounded to a lower level of control group. CAT activity showed a slight inhibition except for 5 d (Fig. 7d).

Together, enzymatic and nonenzymatic components such as GSH played an important role in antioxidant defense system. GPx was an efficient enzyme protecting the cell membranes from LPO by scavenging ROS at the expense of GSH (Moreno et al. 2005). GSH showed a little increase in the first 3 d and then decreased significantly

by 22.55~30.38% until 9 d (Fig.7f). The GPx enzyme catalyzes the reduction reaction of GSH towards hydrogen peroxide with specificity to protect the structure and function of the cell. GPx was induced significantly by up to 58.28% in the first 3 d and gradually decreased after 3 d (Fig.7e).

(a)

(b)

(c)

(d)

(e)

(f)

Fig. 7 Changes of (a) $\cdot\text{OH}$ intensity, (b) MDA content, (c) SOD activity, (d) CAT activity, (e) GSH-PX activity and (f) GSH content in fish liver exposed to ACE-UV group during 9-d exposure time

The changes of $\cdot\text{OH}$ and MDA in fish liver were similar to that when *C. auratus* was exposed to pentachlorophenol (Luo et al. 2009) and glyphosate (Fan et al. 2013). MDA contents had a moderate positive correlation with $\cdot\text{OH}$ during their 9-day exposure ($r = 0.707$, $p < 0.05$), proving a ROS-triggered LPO in fish. MDA content was significantly increased through the whole exposure period suggesting that irradiation products of ACE could cause severe oxidative damage in fish livers (Fig. 7b). As ROS was rapidly accumulated in the first 3d when fish were exposed to the irradiation products of ACE, SOD activity was seriously inhibited in the first 3d while GPx was significantly induced, with moderate positive correlation with $\cdot\text{OH}$ ($r=0.536$, $p<0.05$). Severe inhibition was also found in *C. auratus* when exposed to herbicide as atrazine (Husak et al. 2014) and pharmaceutical as diclofenac associated with excess ROS (Saucedo-Vence et al. 2015). It could be speculated that another cellular defense system was promoted to clear the excess ROS, and the sharp decrease in GSH level after 3 d confirmed the hypothesis (Fig. 7f). GSH was a scavenger of $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$ and it could also scavenge ROS in a reaction catalyzed by GPx. The restoration of SOD activity indicated that a non-enzymatic defense system protected SOD from $\cdot\text{OH}$ -mediated damage (Luo et al. 2006). GSH content also sensitively decreased

when *C. auratus* was exposed to other man-made xenobiotics (Yin et al. 2014, Zhang et al. 2005). Loss of GSH implied a weaker ability of organisms to resist oxidation.

Considering the severe LPO in fish liver caused by the irradiation products of ACE, ACE could be harmful to *C. auratus* after UV irradiance. Previous studies showed that activated carbon filters may remove the ozonation products of ACE (Scheurer et al. 2012); moreover, such a combination of carbon filters with UV or AOPs might be efficient to control the eco-toxicity of ACE degradation products. More comprehensive and complex assessment of hazards is necessary to fully evaluate the eco-toxicity effects of ACE from advanced wastewater treatment effluent. The results of this study are an important contribution to a more realistic understanding of the eco-toxicity of ACE after it enters into the aquatic environment suffering from light irradiation.

4. Conclusions

ACE could translate into complex irradiation products after UV irradiance, and they are confirmed more toxic than the parent compound. Exposure to the ACE-UV group caused oxidative stress in liver of *C. auratus*. Rapid accumulation of $\cdot\text{OH}$ and severe LPO could be observed, suggesting that fish suffered from oxidative damage. Simultaneously, GSH was significantly reduced; thus, fish were weakened in their defense from oxidation stress. The results of this research indicated that the

environmental risk of ACE was underestimated in that it had not taken into account the influence of its intermediate metabolic products. The study of ACE should be given more attention and a comprehensive assessment of its environmental risk is still urgently needed.

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Supplementary Material

Detailed information on statistically significant correlations analysis is described in the Supplementary Material section. This information is available online alongside the electronic version of this article.

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Fish
Exposure