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Photocatalytic transformation of acesulfame: Transformation products identification and embryotoxicity study

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ABSTRACT

Artificial sweeteners have been recognized as emerging contaminants due to their wide application, environmental persistence and ubiquitous occurrence. Among them, acesulfame has attracted much attention. After being discharged into the environment, acesulfame undergoes photolysis naturally. However, acesulfame photodegradation behavior and identity of its transformation products, critical to understanding acesulfame's environmental impact, have not been thoroughly investigated. The present study aimed to fill this knowledge gap by a laboratory simulation study in examining acesulfame transformation products and pathways under UV-C photolysis in the presence of TiO_2. Photodegradation products of acesulfame were isolated and analyzed using the LC-IM-QTOF-MS coupled with LC Ion Trap MS in the MS^n mode. Our results show six new transformation products that have not been previously identified. The molecular structures and transformation pathways were proposed. Further embryotoxicity tests showed that acesulfame transformation products at the low g L^{-1} level produced significant adverse effects in tail detachment, heart rate, hatching rate and survival rate during fish embryo development. The identification of additional transformation products with proposed transformation pathways of acesulfame, the increased toxicity of acesulfame after photolysis, and the fact that the accumulation of acesulfame transformation products is increasingly likely make acesulfame contamination even more important. Water resource control agencies need to consider legislation regarding acesulfame and other artificial sweeteners, while further studies are carried out, in order to protect the safety of this most vital resource.

1. Introduction

Artificial sweeteners (ASs) have been consumed in considerable quantities as sugar substitutes in low-calorie beverages, foods and personal care products since the 1950s (Buerge et al., 2011; Berset and Ochsenbein, 2012). They are a class of emerging environmental contaminants with growing scientific concern due to their potential undesirable impacts on ecosystems and human health (Richardson and Ternes, 2014; Sang et al., 2014; Tran et al., 2014). Among the ASs, acesulfame (ACE) has drawn much attention due to its worldwide consumption, persistence when released, and subsequent ubiquitous occurrence in the natural environment (Nödler et al., 2013; Tran et al., 2013). Available data indicates that the global consumption of ACE increased from 2.5 to 4.0 metric tons from 2001 to 2005 due to its greater sweetening power and cheaper price compared to other ASs (Bahndorf and Kienle, 2004; ISO, 2008; Subedi and Kannan, 2014). The ingredient is applied in over 4000 foods and beverages in around 90 countries (Gisel, 2009).

Due to human excretion mostly as the parent compound and

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high persistence in waste water even after treatment (Gan et al., 2013), levels of ACE were among the highest measured for trace pollutants in receiving waters of surface water, groundwater and wastewater influent and effluent (Table S1) and even tap water (Buerge et al., 2009; Mawhinney et al., 2011) with up to 100% detection frequency. Once released into nature, ACE may undergo different kinds of transformations. Stadler et al. (2012) suggest that estimating ACE removal rates by monitoring only the original compound will give distorted figures because it fails to take into consideration transformation products (TPs). This viewpoint is supported by findings of several other independent studies (Gan et al., 2014; Sang et al., 2014; Scheurer et al., 2012, 2014). Research has subsequently shown that ACE can be degraded under simulated solar/UV irradiation or ozone treatment into at least ten TPs. The transformation ACE undergoes in the natural environment is now considered to be quite complex.

Identification of the TPs structures would be extremely valuable in understanding, including predicting, how they might behave in the environment. Our previous study showed that many photo by-products were produced from ACE under prolonged UV irradiation, and the transformation process yielded by-products more persistent than the original ACE (Sang et al., 2014).

According to several toxicological tests, ACE appears to be nontoxic to humans within regulated concentrations (Shankar et al., 2013; Gardner, 2014), and poses a low hazard to green algae *Scenedesmus vacuolatus*, duckweed *Lemna minor* and water fleas *Daphnia magna* (Stolte et al., 2013). Nevertheless, the ubiquitous occurrence of ACE raises safety concern and call for further research, particularly in the aspect of ACE TPs (Lange et al., 2012; Toth et al., 2012; Stolte et al., 2013; Richardson and Ternes, 2014). The only earlier relevant work by us indicated that photo-induced TPs of ACE are >500 times more toxic than the mother compound in the marine bacterium *Vibrio fischeri* (Sang et al., 2014). The current study, therefore, will further evaluate the risk led by those TPs.

The primary objectives of this study were to search for and identify the molecular structure of any new photo-induced ACE TP; and to evaluate the embryotoxicity of the TPs in mixture by embryo toxicity testing (FET) using the zebrafish *Danio rerio*.

2. Experimental

2.1. Chemicals and reagents

Chemical standard for the artificial sweetener acesulfame potassium was purchased from Sigma–Aldrich (≥99.0%, HPLC, Germany). The ACE stock solution of 400 mg L^{-1} was prepared with Milli-Q water of 18.2 ΩM cm (Millipore, Billerica, MA, USA) and stored in the dark at 4 °C. Titanium (IV) oxide of 21 nm particle size was supplied by Sigma–Aldrich (TiO_2, ≥99.5%, trace metals basis). For chromatographic analyses in HPLC, Milli-Q water and HPLC grade methanol (Tedia, OH, USA) were used to prepare the mobile phases.

Reagents for embryotoxicity bioassay were prepared for reconstituted moderately hard water (MHW) using the U.S. Environmental Protection Agency recipe: 75.9 mg L^{-1} calcium sulfate dihydrate ($CaSO_4 \cdot 2H_2O$, ≥99.0%, ISO9001:2000, China), 123.2 mg L^{-1} magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$, ≥99.0%, A.R., China), 96 mg L^{-1} sodium bicarbonate ($NaHCO_3$, 99%, Sigma–Aldrich, China), 4 mg L^{-1} potassium chloride (KCl, ≥99.5%, A.R., China). For all embryotoxicity testing, 2 times of MHW was used as test medium (Bone et al., 2012).

2.2. Analysis of acesulfame transformation products

2.2.1. TiO_2-assisted photolysis experiment

Initially, we conducted a comparative study of ACE under UV-C illumination for 19 h with and without TiO_2. The ACE (at a concentration of 400 mg L) showed a 63.94% (n = 3) degradation without TiO_2 while with a 99.99% (n = 3) in the presence of a catalyst. TiO_2-assisted photodegradation was therefore adopted. Experimentally, 10 mg of ACE was dissolved in 25 mL of Milli-Q water. Freshly prepared TiO_2 was then added to the solution at ACE/TiO_2 mass ratio of 1:20. After the reaction under UV-C light (Sankyo G8T5, Japan; 1630 μW/cm^2) for 19 h at room temperature, the solution was filtered through 0.2 μm nylon membrane, and freeze-dried for use.

2.2.2. Analytical methods

Both ACE and its transformation products at 100 mg L^{-1} were analyzed by the Ion Mobility Q-TOF MS (Agilent 6560 IM Q-TOF) coupled with LC system (Agilent 1290 Infinity, USA) (LC-IM-QTOF-MS). Chromatographic separation was performed on a Phenomenex Luna 3μ CN column (150 × 2.0 mm, 3 μm) in the gradient elution model. The injection volume was 1 μL. The mobile phase was composed of water and methanol, both containing 0.1% formic acid (98–100%, Merck, Suprapur®). The flow rate was 0.2 mL/min. The gradient program started with 95% water for 5 min, followed by ramping to 50% water within 5 min, and then kept at this condition for 1 min, and then returned to the initial setup in 1 min. Q-TOF mode was applied with ion source of Dual AJS electrospray interface (ESI) with mass correction at reference masses of *m/z* 119.0363 and 966.0070. High resolution mass spectra (*m/z* 40–1700) were obtained at a rate of 2 spectra per second with electrospray ionization in the negative ion mode. Both positive and negative modes were initially applied to analyze samples, but only data from negative ion mode offered adequate information with allowance in detecting of potential TPs. Therefore, data derived from negative mode runs were focused for subsequent analyses. The dry gas of nitrogen flowed at 5 L/min under 200 °C while sheath gas of nitrogen was at 12 L/min with 325 °C, Vcap was set to 5000 V and the nozzle voltage to 500 V.

Specific mass of target transformation products was further fragmented by using the Ion Trap (Bruker amaZon speed) in the MS^n mode coupled with HPLC (Thermal Scientific Accela). Chromatographic separation was performed under the same conditions for Q-TOF except with injection volume of 10 μL and *m/z* of 50–300. The fragmentation mass took place at energy of 2.78 V. Nitrogen was taken as dry gas with flow rate at 8.2 L/min and 350 °C. The nebulizer pressure was 15.0 psi.

2.3. Embryotoxicity testing

2.3.1. Animal care and breeding

Adult zebrafish *D. rerio* were cultured in the Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou. In the laboratory, breeding stocks of males and females were maintained in separate glass aquaria filled with oxygenated tap water maintained at 26 ± 1 °C and kept on a 14 h:10 h light:dark cycle. Fish were fed with *Artemia franciscana nauplii*. Before breeding the zebrafish in a ratio of 3:2 females: males, were kept in a tank with a board separating females from males, overnight. In the morning, when the light came on, the board was removed and the fish were allowed to breed. Embryos were collected after 30 min.

2.3.2. Toxicity exposure

The embryotoxicity tests of ACE and the preparation of ACE