Water Research 117 (2017) 157–166

Contents lists available at ScienceDirect

Water Research

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

Transformation of acesulfame in chlorination: Kinetics study, identification of byproducts, and toxicity assessment

Adela Jing Li [a, d], Pengran Wu [a, c], Japhet Cheuk-Fung Law [a], Chi-Hang Chow [a], Cristina Postigo [e], Ying Guo [c, **], Kelvin Sze-Yin Leung [a, b, c, *]

[a] Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong Special Administrative Region
[b] HKBU Institute of Research and Continuing Education, Shenzhen Virtual University Park, Shenzhen, China
[c] School of Environment, Guangzhou Key Laboratory of Environmental Exposure and Health, Guangdong Key Laboratory of Environmental Pollution and Health, Jinan University, Guangzhou 510632, China
[d] Key Laboratory of Tropical Agro-environment, Ministry of Agriculture of China, South China Agricultural University, Guangzhou 510642, China
[e] Water and Soil Quality Research Group, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research – Spanish National Research Council (IDAEA-CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain

ARTICLE INFO

Article history:
Received 1 September 2016
Received in revised form
21 March 2017
Accepted 25 March 2017
Available online 28 March 2017

Keywords:
Acesulfame
Chlorination
Kinetics
Disinfection by-products
Environmental fate

ABSTRACT

Acesulfame (ACE) is one of the most commonly used artificial sweeteners. Because it is not metabolized in the human gut, it reaches the aquatic environment unchanged. In the present study, the reactivity of ACE in free chlorine-containing water was investigated for the first time. The degradation of ACE was found to follow pseudo-first-order kinetics. The first-order rate increased with decreasing pH from 9.4 to 4.8 with estimated half-lives from 693 min to 2 min. Structural elucidation of the detected transformation products (TPs) was performed by ultra-high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. Integration of MS/MS fragments, isotopic pattern and exact mass allowed the characterization of up to 5 different TPs in the ultrapure water extracts analyzed, including two proposed new chlorinated compounds reported for the first time. Unexpectedly, several known and regulated disinfection by-products (DBPs) were present in the ACE chlorinated solution. In addition, two of the six DBPs are proposed as N-DBPs. Time-course profiles of ACE and the identified by-products in tap water and wastewater samples were followed in order to simulate the actual disinfection process. Tap water did not significantly affect degradation, but wastewater did; it reacted with the ACE to produce several brominated-DBPs. A preliminary assessment of chlorinated mixtures by luminescence inhibition of *Vibrio fischeri* showed that these by-products were up to 1.8-fold more toxic than the parent compound. The generation of these DBPs, both regulated and not, representing enhanced toxicity, make chlorine disinfection a controversial treatment for ACE. Further efforts are urgently needed to both assess the consequences of current water treatment processes on ACE and to develop new processes that will safely treat ACE. Human health and the health of our aquatic ecosystems are at stake.

1. Introduction

Chlorine-based selective oxidation is a chemical process commonly used to remove pathogenic microorganisms in water reclamation plants, swimming pools as well as wastewater treatment systems. However, recent published studies have uncovered unintended, undesirable consequence of this process in that it produces disinfection by-products (DBPs) related to the reactions of chlorine with synthetic chemicals that are now commonly detected in aquatic environments (Sedlak and von Gunten, 2011; Postigo et al., 2016). Concerns over DBP exposure are increasing because of a potential link between intake of chlorinated water and negative effects for humans, e.g. increased risk of asthma, miscarriages, poor semen quality, birth defects and bladder/colorectal cancer (Postigo and Richardson, 2014; Richardson and Kimura, 2016).

Although there is a great deal of information regarding the products of the chlorination of pharmaceuticals (El Najjar et al., 2013; Negreira et al., 2015b, 2015c; Xiang et al., 2016), personal

* Corresponding author. Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong Special Administrative Region.
** Corresponding author.
E-mail addresses: yingguo2004@jnu.edu.cn (Y. Guo), s9362284@hkbu.edu.hk (K.S.-Y. Leung).

http://dx.doi.org/10.1016/j.watres.2017.03.053
0043-1354/

care products (de Oliveira e Sá et al., 2014; Abdallah et al., 2015; Trebše et al., 2016) and herbicides (Chusaksri et al., 2012; Tawk et al., 2015), very little is known about the fate of food additives in chlorinated waters (Gan et al., 2013; Subedi and Kannan, 2014). The artificial sweetener acesulfame (ACE) has become a hot topic because of its increasing consumption worldwide (now consumed in more than 100 countries), zero human metabolism, and consistent detection in finished drinking water (>1 μg L^{-1}, Buerge et al., 2009; Richardson and Kimura, 2016), marine seawater (up to 7.6 μg L^{-1}, Gan et al., 2013; Sang et al., 2014) and wastewater treatment plants (up to 2,500 μg L^{-1}, Loos et al., 2013). ACE is only partially removed in conventional municipal sewage systems and subsequently ends up in the bodies of water receiving these systems' effluent (Scheurer et al., 2010). The safety of ACE as a food additive has been evaluated (FDA, 2014). Assessing the environmental significance of ACE requires an extensive understanding of the processes affecting its transformation and fate. ACE may undergo different kinds of transformations like photolysis and ozonation (Scheurer et al., 2012; Gan et al., 2014; Li et al., 2016). Our earlier studies found that photo-induced transformation products (TPs) of ACE were more toxic by > 500 times to marine bacteria than the precursor (Sang et al., 2014), and they interrupted fish embryo development in tail detachment, and heart/hatching/survival rate (Li et al., 2016). Yet, there has been no satisfactory remedial solution for ACE mineralization so far.

When present in water, the reactivity of ACE towards chlorine is demanding because water disinfection process may influence ACE fate. Whether the attacks by the reactive chlorine species in the ACE chlorination process would form undesirable chlorinated by-products is unknown, just as whether chlorination can mineralize ACE residues in the aquatic environment is still unknown; both deserve comprehensive study. To date, only two research groups have done primary work on ACE chlorination. Their publications address these issues in a sentence, stating that ACE was not transformed or was degraded up to only 20% under certain experimental conditions (Scheurer et al., 2010; Soh et al., 2011). No kinetic studies of ACE chlorination or of any resultant TP or of TPs' environmental fate in real world applications have been published. Similarly, toxicity caused by ACE chlorinated derivatives has not been studied or reported, yet should also be kept under surveillance because these derivatives might pose a long-term threat to human and environmental health due to potential production of DBPs.

The present study was designed to assess ACE reactivity during chlorination in order to better understand its fate and potential threats to the environment. The report comprises three parts: (i) in the first part the chlorination kinetics of ACE are discussed, and rate constants are reported, determined as functions of pH; (ii) in the second part of the study, chlorination by-products were identified in pure and real waters; and (iii) the third part is a toxicity study of the TPs using marine bacteria *Vibrio fischeri*.

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 MΩ cm (Millipore, Billerica, MA, USA) and stored in the dark at 4 °C. A solution of sodium hypochlorite (≥5.2%) was purchased from Uni-Chem (China). The solution was stored at 4 °C and its exact concentration was accurately determined prior to chlorination experiments by the *N*,*N*-diethyl-*p*-phenylenediamine method (Clesceri et al., 1998). Sodium thiosulfate pentahydrate (extra pure) was from Junsei Chemical (Japan). Standard solutions of haloacetic acids (HAAs) and trihalomethanes (THMs) were obtained as mixed standard solutions (EPA 552.2 for HAAs and EPA 501/601 for THMs) (TraceCERT®) from Sigma-Aldrich (USA). For haloacetamides (HAcAms), individual standard for dichloroacetamide was obtained from Sigma-Aldrich (USA).

All solvents were HPLC or LC-MS grade, and all other chemicals were analytical reagent grade. Methanol and formic acid (98–100%) were acquired from Duksan (Korea) and International Laboratory (USA), respectively. Hydrochloric acid (HCl, 37%), sodium phosphate and sodium hydrogen phosphate were ordered from VWR Chemicals (Belgium). Methyl *tert*-butyl ether (MTBE) (>99.8%) was obtained from Sigma-Aldrich (USA).

Reagents for Microtox test were provided by Modern Water (Guildford, UK), entailing osmotic adjusting solution, diluent, reconstitution solution and acute reagent.

2.2. Chlorination experiments

Kinetic experiments were performed in a conical flask (25 mL) wrapped in aluminum foil in a temperature controlled (23 °C) water bath with constant shaking at 100 rpm (SW 22, Julabo, Germany). Under pseudo-first-order conditions, the initial ACE concentration was 20 mg L^{-1} with the molar ratio of free chlorine at 1:25. To cover the typical aquatic pH range of real waters, which is of 6–9, a pH range of 4.8–9.4 was studied. The pH of the tested aqueous solution was adjusted using phosphate buffer (100 mM). At constant time intervals, 2 mL of solution were withdrawn and mixed with an excess of $Na_2S_2O_3$ (100 μM) to quench the residual chlorine. Chlorine/pH variation was within 20% and 0.1, respectively. Samples filtered by 0.2 μm nylon membrane were analyzed using HPLC-DAD to determine the remaining ACE concentration. Each kinetic test was repeated twice.

Search for TPs was performed on 2, 20 and 400 mg L^{-1} of ACE in the presence of different chlorine molar ratios of 1:2 and 1:20. The transformation was observed after addition of NaOCl to unbuffered/unquenched system with an initial pH of 7.0. Samples for TPs identification were not subjected to any pre-concentration. Control (without chlorine) and blank (with chlorine but without ACE) treatments were run in parallel.

Finally, samples of tap water (pH 8.1) and wastewater (pH 8.3) were adjusted to pH 7.0 by HCl, spiked with 20 mg L^{-1} of ACE with chlorine molar ratio at 1:20. The samples were analyzed by UHPLC-MS at different reaction times in order to compare the results with the ones obtained in ultrapure water.

2.3. Analytical methods

During kinetic experiments, the concentration of ACE was determined by HPLC (Agilent 1200 series) (Agilent Technologies, Santa Clara, CA, USA) connected to a UV detector. Separation was performed using an XSelect CSH C18 column (150 × 2.1 mm, 5 μm, Waters, USA) and using a flow rate of 0.5 mL/min. The mobile phase was a 20 mM ammonium acetate buffer/methanol mixture (50/1 *v*/*v*). Injection volume was set at 10 μL. The UV detection was performed at 227 nm for ACE.

Chlorination TPs of ACE were analyzed by UHPLC (Agilent 1290 series) coupled with QTOF-MS (Agilent 6540 series) (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was performed on a Luna 3μ CN column (150 × 2.0 mm, 3 μm, Phenomenex, USA) in the isocratic elution mode. Water and methanol both containing 0.1% formic acid (*v*/*v*) were used as mobile phases A and B, respectively. The mobile phase composition was set at 95% A and 5% B for 5 min. The flow rate was set to 0.25 mL/min and the injected volume was 2 μL. Q-TOF mode was

applied with ion source of Dual AJS electrospray interface (ESI) with mass correction at reference mass of *m/z* 112.9855 at negative mode while *m/z* 149.0233 at positive mode. High resolution mass spectra (*m/z* 40–500) were obtained at a rate of 2 spectra per second. TPs were screened in both positive and negative electrospray ionization modes, though the negative mode showed higher responses of TPs. Therefore, data derived from negative mode were applied for subsequent analyzes. The dry gas of nitrogen flowed at 8 L/min under 325 °C while sheath gas of nitrogen was at 8 L/min with 350 °C, nebulizer was at 20 psig, fragmentor voltage was at 120 V, skimmer voltage was at 65 V, octapole RFPeak voltage was at 750 V, Vcap was to 4,000 V and the nozzle voltage was set to 500 V. The instrument was operated at 4 GHz mode.

Instrumental control, data acquisition and evaluation were performed with the software of MassHunter (Agilent Technologies). By removing background signals and grouping isotopic patterns, MassHunter generated a list of chemically discrete molecular features from the spectral data. The detected compounds were putatively identified by producing candidate formulae using a mass accuracy limit of 7 ppm. Thereafter, molecular ions were fragmented in order to obtain fragmentation patterns of ACE TPs at two collision energy of 5 V and 10 V.

Trihalomethanes (THMs), haloacetic acids (HAAs) and haloacetamides (HAcAms) were identified by GC-MS and LC-QTRAP-MS with mass spectra matching and reference standards matching. Detailed instrumental conditions are shown in the Supplementary S1 and S2.

2.4. Microtox test

The toxicity of ACE TPs was assessed by using acute toxicity screening in chlorinated samples. 1 L of collected samples were lyophilized with a laboratory freeze dryer (IlShin®, FD5512, Netherlands) which yielded approximate 1 g of dried powder. The freeze-dried powder of the chlorinated mixture was accurately weighed and reconstituted with 10 mL ultrapure water for subsequent toxicity testing.

The toxicity tests for *V. fischeri* were performed according to the standard method BS EN ISO 11348-3 (2008). Luminescence measurements were carried out by using a Microtox Model 500 analyzer (Modern Water, Guildford, UK) with a thermo block maintained at 15 °C. The toxicant-induced inhibition of luminescence (%) was calculated upon decay after 15 min of exposure to ACE TPs and a control without ACE TPs. Solutions were adjusted to pH 7.5 ± 0.2 with HCl or NaOH before tests, as suggested by the standard method. Parallel tests were conducted in phenol as a reference toxicant for positive control with an EC50 criterion set in a typical 13–26 mg L^{-1} range for quality control (BS EN ISO 11348-3, 2008).

3. Results and discussion

3.1. Chlorination kinetics

In the control without chlorine, no obvious loss of ACE was observed, proving that ACE decay from non-chlorine mediated reaction was negligible (data not shown). The kinetics of initial chlorination of ACE was studied under experimental conditions developed for pseudo-first-order kinetics in excess of chlorine as represented by equation (1).

$$-\frac{d[ACE]}{dt} = k_{obs}[ACE] \quad (1)$$

Where k_{obs} is the first-order rate constant (min^{-1}) and [ACE] is the concentration of ACE (mg L^{-1}). After integration between t = 0 and t,

$$\ln\frac{[ACE]_t}{[ACE]_0} = -k_{obs}t \quad (2)$$

Fig. 1 shows the representation of $\ln([ACE]_t/[ACE]_0)$ versus *time* at different pHs for ACE. The results indicated that the rate of ACE

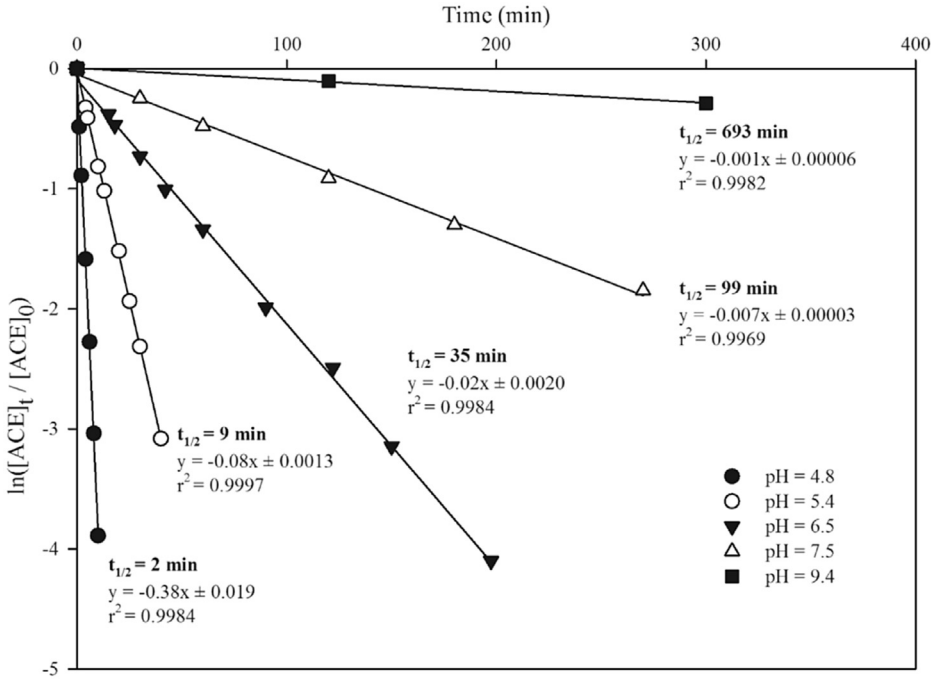

Fig. 1. Pseudo-first-order kinetic plot of ACE chlorination at pH of 4.8, 5.4, 6.5, 7.5, $[ACE]_0$ = 20 mg L^{-1}, $[ACE]_0$: $[HOCl]_0$ at 1:25 while pH of 9.4, $[ACE]_0$ = 20 mg L^{-1}, $[ACE]_0$: $[HOCl]_0$ at 1:340.

chlorination could be well linearized using a pseudo-first-order kinetic law with $r^2 > 0.99$ for all reactions. The order of the reaction was 1 with respect to ACE, and the observed first-order rate constants k_{obs} were given as the slope of the curve according to equation (2). Degradation rates were speeded up by decreasing the sample pH, and so estimated half-lives ($t_{1/2}$) varied from 693 min at pH 9.4 to only 2 min at pH 4.8 although chlorine input at pH 9.4 was over ten-fold (Fig. 1 and Fig. S1). This pH-dependent behavior suggests that the reaction of ACE with chlorine is faster and more efficient at lower pH values due to the greater oxidizing strength of HOCl compared to dissociated OCl^-. This phenomenon has been previously reported for trichloroethylene, benzoic acid, etoposide and ibuprofen (Wang et al., 2012; Fang et al., 2014; Negreira et al., 2015a; Xiang et al., 2016).

Actually, the reaction of chlorine with most organic compounds has been shown to be a second-order reaction, first-order with respect to each reactant (González-Mariño et al., 2015; Tawk et al., 2015). The second-order rate constants k_{app} were determined at several pH values according to equation (3) (Soufan et al., 2013; Tawk et al., 2015).

$$k_{obs} = k_{app}[\text{chlorine}]_0^n \quad (3)$$

$$\log(k_{obs}) = \log(k_{app}) + n\log([\text{chlorine}]_0) \quad (4)$$

By plotting $\log(k_{obs})$ versus $\log([chlorine]_0)$ (Equation (4)), the reaction order "n" in $[\text{chlorine}]_0$ is the slope of the regression line. Fig. 2 shows the pH dependence of reaction order rate constant "n" in $[\text{chlorine}]_0$. At pH 6.5 and 7.5, the reaction order in $[\text{chlorine}]_0$ is around 2, indicating two-order of reactive chlorine species between OCl^- and HOCl. At pH 9.4, the value is close to 1, which can be explained by the lower reactivity of OCl^- compared to HOCl (Deborde and von Gunten, 2008; Negreira et al., 2015c; Xiang et al., 2016). Consistent with previous work, these results demonstrate that the role of Cl_2O is significant at neutral pH (Sivey et al., 2010; Chusaksri et al., 2012; Sivey and Roberts, 2012; Cai et al., 2013).

The pseudo-first-order ACE chlorinated reaction was applied in the following TPs identification study with a varied molar excess of free chlorine at neutral pH, reflecting the application of chlorine typically used in water treatment plants.

3.2. Identification of TPs

Generation of TPs was evaluated by chlorinating ACE at three different concentrations (i.e. 2, 20 and 400 mg L^{-1}) with two molar ratios of chlorine at 1:2 and 1:20, respectively. Samples were analyzed by UHPLC-QTOF-MS in scan mode and screened for potential TPs by using MassHunter software. Molecular formulae for the detected TPs were inferred from the accurate mass measurements, the observed isotopic patterns, and MS/MS fragments. A high confidence in the TPs identification attributed to high mass accuracy measurements of the fragment ions.

A total of 5 TPs were identified for ACE chlorination, matching the proposed molecular formulas within 4 ppm of mass measurement error (Table 1). Based on the characteristic chlorine isotopic pattern, one of them was concordant with mono-chlorinated derivatives, two presented the isotopic cluster distinctive of dichlorinated species and one TP corresponded to trichlorinated compounds. MS/MS fragmentation of TP-130, TP-164, TP-206 and TP-240 mainly yielded the characteristic fragment ion (*m/z* 79.9575) corresponding to the sulfur trioxide moiety, indicating that for these TPs the transformation reactions did not affect this side chain (Fig. 3). At the initial spike of 400 mg L^{-1} ACE and chlorine equivalent of 1:2, all the 5 TPs were detected. Analysis of the reaction mixture of aquatic chlorination at low levels of ACE, 20 and 2 mg L^{-1}, allowed detecting only a few major products (Table S1). The TPs detected at the medium concentration of ACE (i.e. 20 mg L^{-1}; Column c and d of Supplementary Table S1) are those that showed with a higher intensity at the highest ACE concentration (400 mg L^{-1}; Fig. S2). Taking the molar ratio of 1:20 ACE: chlorine as an example, reaction of ACE 400 mg L^{-1} showed that TP-130 had the highest intensity, followed by dichlorinated TP-164 and then TP-96 (Fig. S2b). Under ACE 20 mg L^{-1}, the intensities

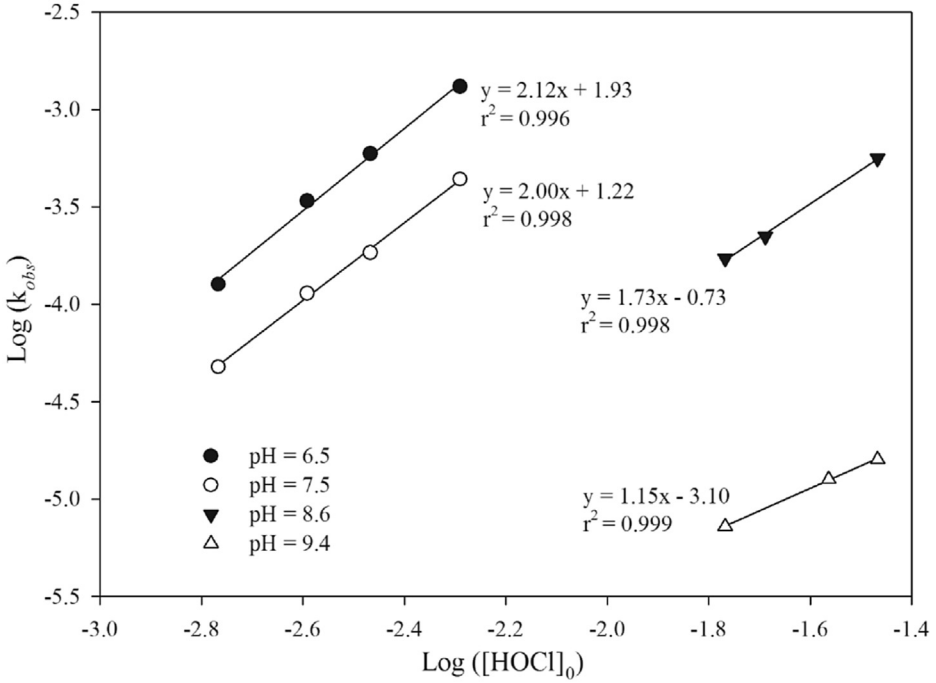

Fig. 2. Reaction order plots in chlorine (as log (k_{obs}) versus log $([HOCl]_0)$) at pH 6.5, 7.5, 8.6, and 9.4. The slopes indicate the reaction order in chlorine.

Table 1
Exact mass information of acesulfame chlorination TPs and DBPs by using UHPLC-QTOF-MS.[e]

Compound	Chemical Formula $[M-H]^-$	Retention time (min)	Experimental mass (relative abundance) $[M-H]^-$	Exact mass $[M-H]^-$	Mass error Δm (ppm)	DBE[d]
ACE	$[C_4H_4NO_4S]^-$	2.898	161.9865	161.9867	−1.23	3
TP-96	$[H_2NO_3S]^-$	1.892	95.9763	95.9761	2.08	0
TP-130	$[ClHNO_3S]^-$	1.879	129.9370 (100), 131.9340 (34)	129.9371	−0.77	0
TP-164	$[Cl_2NO_3S]^-$	1.962	163.8980 (100), 165.8950 (70)	163.8981	−0.61	0
TP-206	$[C_2H_2Cl_2NO_4S]^-$	2.046	205.9087 (100), 207.9057 (66)	205.9087	0.00	1
TP-240	$[C_2HCl_3NO_4S]^-$	2.689	239.8697 (98), 241.8668 (100)	239.8697	0.00	1
Chlorite [a]	$[ClO_2]^-$	1.997	66.9593 (100), 68.9565 (32)	66.9592	1.49	0
Chlorate [b]	$[ClO_3]^-$	2.016	82.9541 (100), 84.9511 (35)	82.9541	0.00	0
Dichloroacetic acid [a]	$[C_2HCl_2O_2]^-$	2.284	126.9359 (100), 128.9330 (60)	126.9359	0.00	1
Dichloroacetamide [c]	$[C_2H_2Cl_2NO]^-$	2.245	125.9522 (100), 127.9476 (71)	125.9519	2.38	1
Trichloroacetamide [c]	$[C_2HCl_3NO]^-$	3.280	159.9128 (100), 161.9096 (90)	159.9129	−0.63	1

[a] These chemicals are on the list of US EPA National Primary Drinking Water Regulations.
[b] The chemical is on the list of US EPA Drinking Water Contaminant Candidate List 4 – Draft.
[c] The chemicals are nitrogenous-DBPs which have not been regulated.
[d] DBE: double bond equivalent.
[e] Instrumental details for the analysis of each acesulfame chlorinated by-products are given in Table S2 of Supplementary. The disinfection byproduct, chloroform, was detected by GC-MS.

of TP-130, TP-164 and TP-96 were all decreasing (Fig. S2d). Under ACE 2 mg L^{-1}, TP-130 and TP-164 were detected at a much lower intensity while TP-96 was not observed at all (Fig. S2f). Thus, these results suggest that the relative intensities of all observed TPs depend on the original concentration of ACE. Besides, high chlorine equivalents likely caused further transformation of TPs, signifying in shrinking abundance of TP-206 and TP-240, and increasing abundance of TP-130 and TP-164 (Fig. S2). Such chlorine-dependent production of TPs has been delivered to UV filters, pharmaceutical and herbicides during chlorine disinfection of water (Armbruster et al., 2015; Manasfi et al., 2015; Tawk et al., 2015). Thus, the time profile of ACE chlorination deserves further attention in order to clarify TPs fate in the environment.

To elucidate the structure of the chlorinated TPs, MS/MS spectra of the parent compound and its four chlorinated transformation products are detailed in Fig. 3. With the data of exact mass, isotopic pattern and MS/MS fragmentation, structural elucidation of the chlorinated TPs followed the confirmation criteria of a proposed level system (Schymanski et al., 2014). According to zero unsaturation and fragments determined at *m/z* 79.9575 $[SO_3]^-$, 81.9531 $[ClHNS]^-$ and 93.9599 $[NSO_3]^-$ (Fig. 3), TP-130 with *m/z* 129.9370 ($[ClHNO_3S]^-$, Δm = −0.32) is proposed to have an open ring structure. Observation of the corresponding $[M+2-H]^-$ ion with abundance of about 1/3 of the $[M-H]^-$ ion indicated one chlorine in the structure. Subsequent chlorination of TP-130, TP-164 presented the characteristic chlorine isotopic pattern of dichlorinated species, with molecular formula ($[Cl_2NO_3S]^-$, Δm = 0.07). MS/MS fragmentation yielded a fragment ion at *m/z* 128.9287 ($[ClNO_3S]^-$) revealing that a substitution reaction occurred in the nitrogen moiety (Fig. 3). TP-130 and TP-164 have a closer structural relationship, as demonstrated by three identical fragments ($[SO_3]^-$, $[ClHNS]^-$ and $[NSO_3]^-$) and close elution times (1.879 and 1.962 min). According to SciFinder, TP-130 and TP-164 could probably be *N*-mono- and *N*,*N*-dichlorosulfamic acids, respectively. Sulfamic acid (TP-96, $[H_2NO_3S]^-$, Δm = 2.08, Table 1) can react rapidly with HOCl to form *N*-chlorosulfamic acid (Kolar et al., 1983). This verifies the proposed pathway (Fig. 4). In practice, the *N*-monochlorinated and *N*,*N*-dichlorinated sulfamates have been patented for use in control of microorganisms in processing waters and have been used as preservatives to prevent spoilage of stored paper (US 3328294 and 6471974). The empirical formula proposed for TP-206 ($[C_2H_2Cl_2NO_4S]^-$, Δm = −2.66) has two atoms of carbon/hydrogen less, two atoms of chlorine more and two unsaturated atoms less than the precursor compound (Table 1). Its structure was inferred on fragment detections at *m/z* 79.9576 $[SO_3]^-$, 82.9465 $[CHCl_2]^-$, 95.9763 $[H_2NO_3S]^-$, 96.9606 $[HSO_4]^-$, 105.9609 $[CNO_3S]^-$, 125.9528 $[C_2H_2Cl_2NO]^-$ and 169.9325 $[C_2HClNO_4S]^-$ (Fig. 3) which showed the dichlorinated species' isotopic pattern. It is hypothesized that nucleophilic attack of the nitrogen was to the carbonyl carbon, accompanied by demethylation; the reactive chlorine species then induced chlorine substitution to form a closed five-member heterocyclic ring. The structure of TP-240 is estimated to be an open ring, depending on fragment ions of *m/z* 79.9575 $[SO_3]^-$, 96.9596 $[HSO_4]^-$, 121.9546 $[CHNO_4S]^-$ and 159.9132 $[C_2HCl_3NO]^-$ (Fig. 3) together with one degree of unsaturation and the isotopic pattern of trichlorinated species (Table 1). The transformation pathway from ACE to TP-206 or TP-240 could not be elucidated as no intermediate was detected during this process. Hypothetically, we may say that ACE chlorination is initiated by oxidation, demethylation and reactive chlorine species incurred chlorination, and sustained through hydrolysis, chlorination and ring cleavage to form more stable products (Fig. 4). With all the above information, probable structures of transformation products of ACE chlorination were proposed with identification confidence of 'level 2b' according to the proposed level system (Schymanski et al., 2014).

Besides the halogenated transformation products, six known DBPs were also formed unpredictably during ACE chlorination (Table 1 and Table S2). These known DBPs were identified and confirmed their structures by LC-QTOF-MS, LC-QTRAP-MS and/or GC-MS with reference standards or library spectrum match (Table S2 and Fig. S3). The production of these DBPs deserves special attention due to their known and potential toxicity. Among them, three are on the list of US EPA National Primary Drinking Water Regulations, inclusive of chlorite, chloroform and dichloroacetic acid. The formation of chloroform from chlorination has also been reported to β-triketones herbicides of tembotrione and sulcotrione (Tawk et al., 2015) and UV filters of oxybenzone, dioxybenzone and avobenzone (Duirk et al., 2013; Trebše et al., 2016). Chlorate is on the draft of the US EPA Drinking Water Contaminant Candidate List 4. Disturbingly, two N-DBPs of dichloroacetamide and trichloroacetamide were found in the ACE chlorinated solution. This might be rationalized as an electrophilic substitution of amine with HOCl (Negreira et al., 2015c). Even though these two N-DBPs are not on any regulated compound list, there is an urgent need to enlarge the scientific knowledge about transformation of ACE as precursor under chlorination. N-DBP is of particular concern due to its carcinogenic properties, which are far greater than halogenated DBPs (Shah and Mitch, 2012; Sgroi et al., 2015).

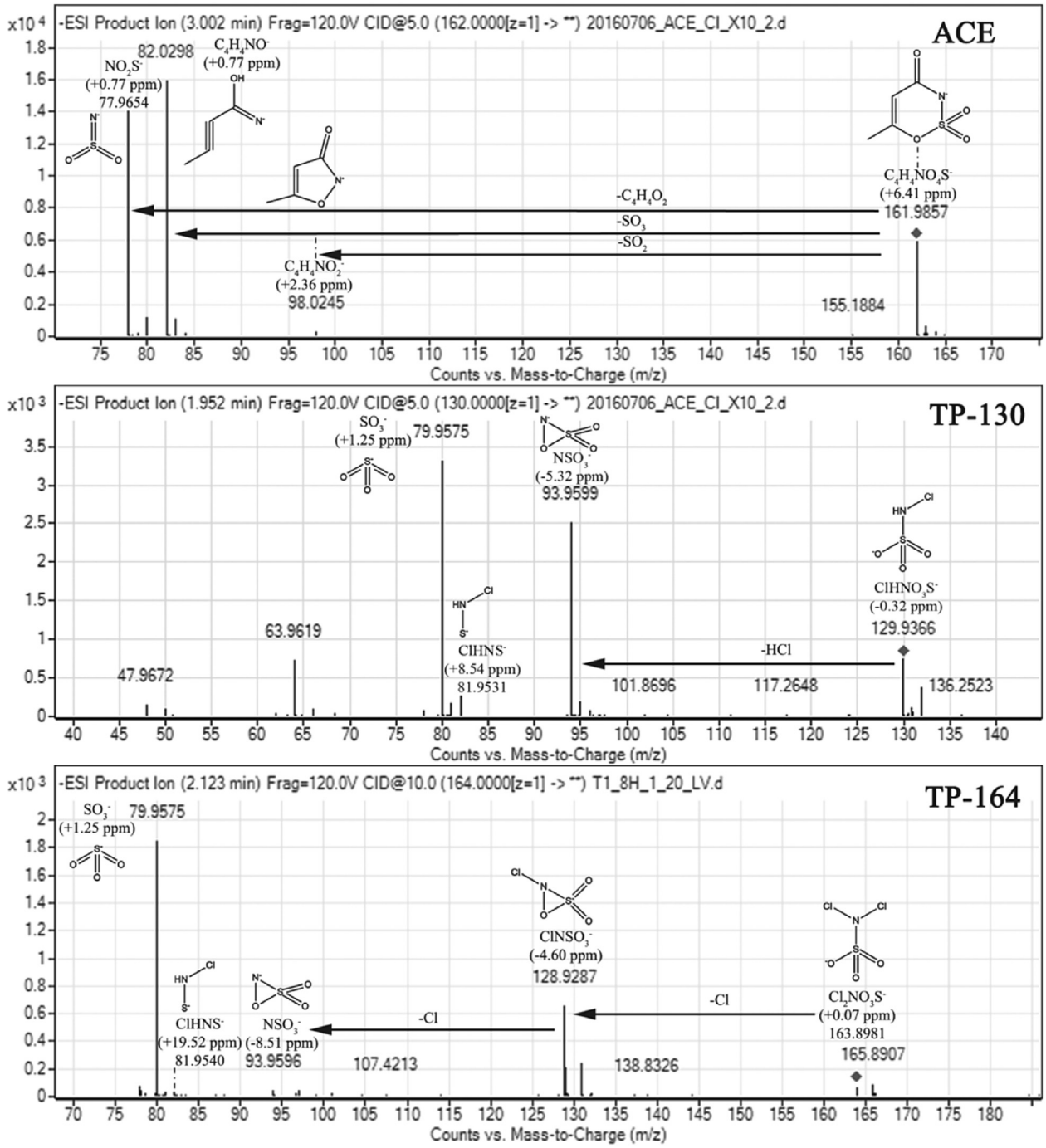

Fig. 3. Accurate MS/MS spectra and fragmentation routes for acesulfame and its four transformation products (TP-130, TP-164, TP-206, TP-240); mass deviations from theoretical *m/z* values are indicated in brackets.

3.3. Reactivity of ACE in real waters

Since ACE may react with free chlorine during water treatment and potabilization, in order to know how it will affect the environment, we must investigate it under conditions that simulate actual water treatment. To this end, tap water and wastewater samples collected from water supply and Sha Tin Sewage Treatment Works (Supplementary Fig. S4) of Hong Kong were spiked with ACE at 20 mg L^{-1} and free chlorine at a 1:20 molar ratio. Ultrapure water experiment was run in parallel so as to compare results. However, due to the complexity of the wastewater matrix, neither the abundance of ACE nor its TPs could be tracked using the above experimental design. A pilot study determined that a solution of ACE at 400 mg L^{-1} with a molar ratio of free chlorine at 1:2.5 produced detectable signals. The initial pH was adjusted to neutral. The water samples were not buffered in order to demonstrate environmental relevance of the reactivity. Aliquots of ultrapure water and tap water were taken at specified times up to 6 h while wastewater was up to 1 h.

In both ultrapure and tap water, removal of ACE was almost complete (>93%) after 6 h, whereas in the wastewater sample it reached a maximum yield of 68% after 10 min, with no further ACE

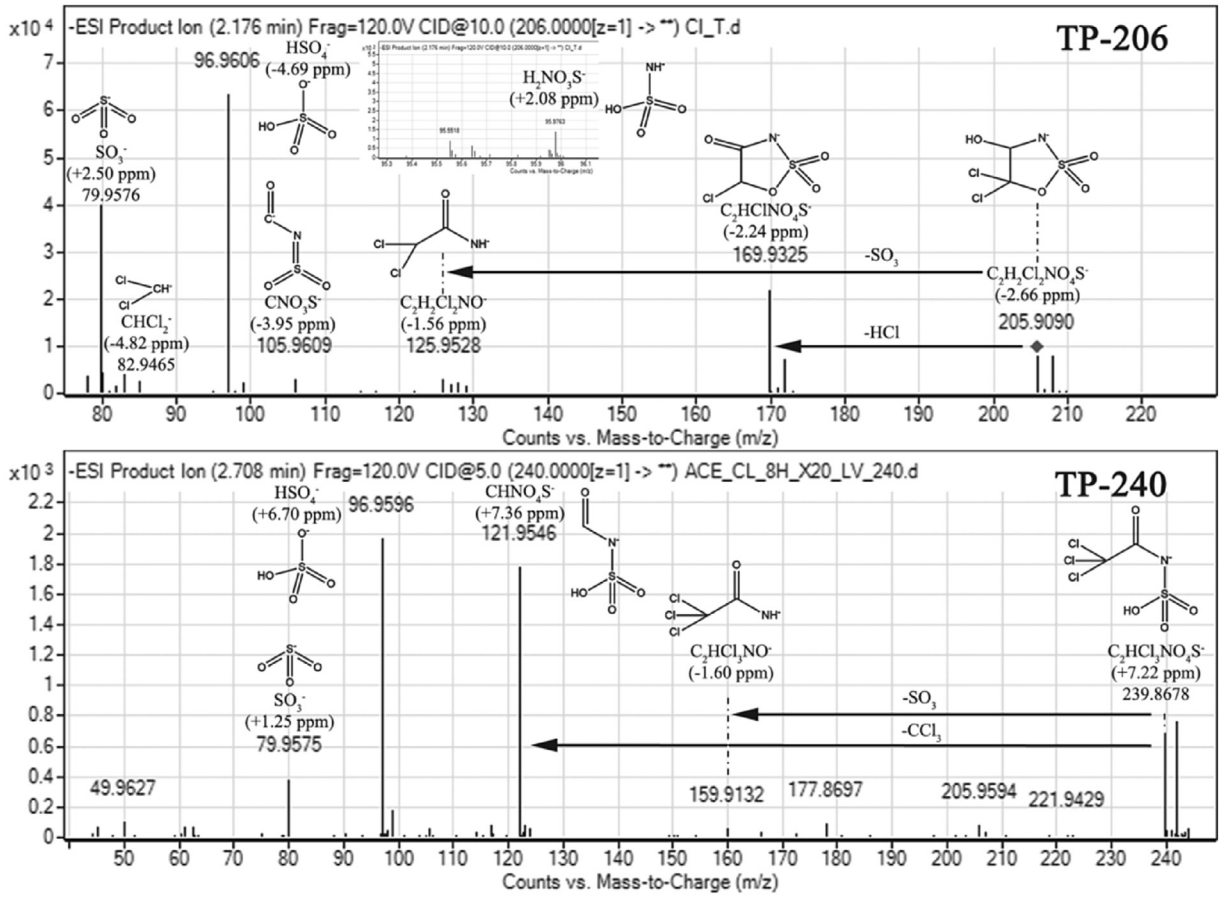

Fig. 3. (*continued*)

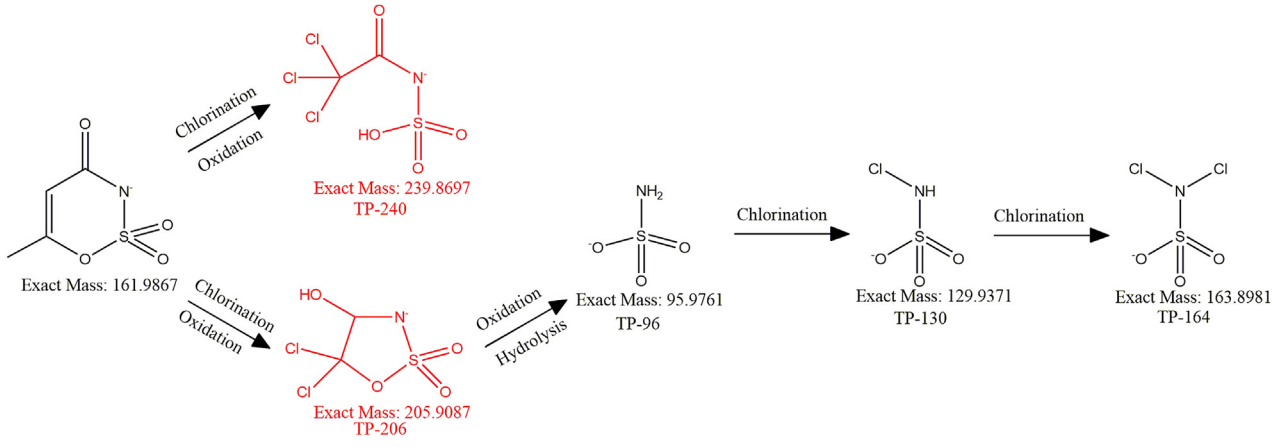

Fig. 4. Proposed transformation pathway for acesulfame with free chlorine.

loss being observed afterwards (Fig. 5). This is likely due to the competition between the organic matter and ACE for chlorine, as has been illustrated in recent studies (González-Mariño et al., 2015; Negreira et al., 2015b, 2015c; Xiang et al., 2016). Most of the chlorine is expected to be consumed by substances in wastewater other than ACE, preventing its complete degradation.

In general terms, good agreement was gained in the results with ultrapure and real waters (Fig. 5). The four TPs previously identified in ultrapure water were also detected in the tap water and wastewater chlorination experiments. Those six known DBPs were not all detected in real waters possibly because their levels were below the detection limit of the mass spectrometric system, thus they were not included in the current discussion. The response of each TP was expressed as $(TP\ Area)_t/(ACE\ Area)_0$ versus *time*. This approach is only for the purpose of discussing variation in TP abundance, its results do not indicate the concentration of TPs in the solution. TP formation showed a very similar profile in ultrapure and tap water (Fig. 5a and b; Fig. S5a) as follows: the relative abundance of TP-130 and TP-164 increased monotonically with increasing reaction time; the responses of TP-206 and TP-240 showed a fast increase

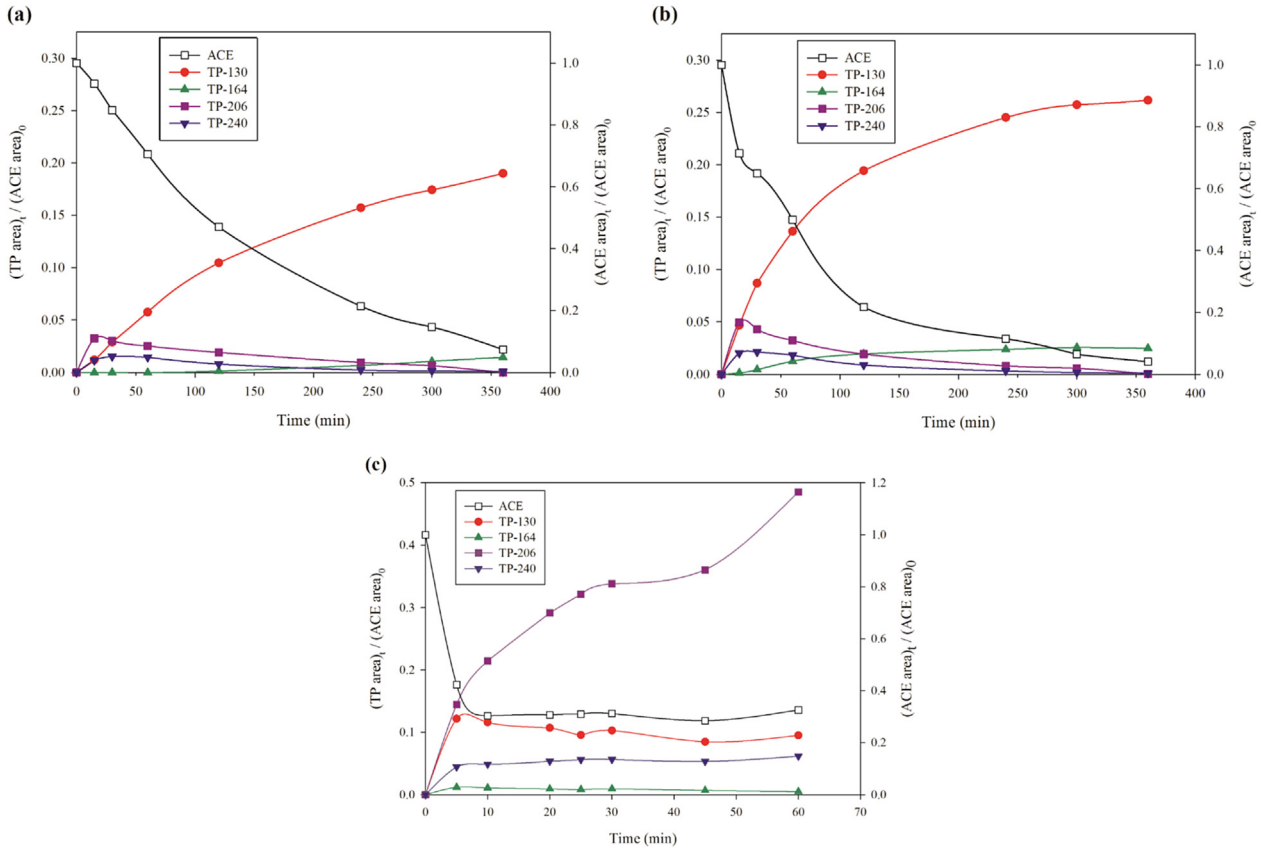

Fig. 5. Evolution profiles of acesulfame transformation products after aquatic chlorination with $[ACE]_0$ 20 mg L^{-1}, $[ACE]_0$: $[HOCl]_0$ at 1:2 in (a) ultrapure water, (b) tap water, and $[ACE]_0$ 400 mg L^{-1}, $[ACE]_0$: $[HOCl]_0$ at 1:2.5 in (c) wastewater.

reaching maximal abundance after 15 min, and then decayed slightly to near zero after 6 h as a consequence of their transformation into TP-130 and TP-164 under the condition of excess of free chlorine. Since Hong Kong uses seawater as flushing water, the wastewater is thus mainly composed of seawater and would normally contain a high level of bromide (20–32 mg L^{-1}) (Yang et al., 2015). Reaction of active chlorine to wastewater triggered further formation of brominated-DBPs not produced in freshwater (Fig. S6). Some of these Br-DBPs are on the regulation list (Table S3). The potential formation of Br-DBPs should be a theme of further study due to their higher activity compared with chlorinated analogues in terms of mutagenicity, cytotoxicity, genotoxicity and developmental toxicity (Richardson et al., 2007; Yang and Zhang, 2013; Liu and Zhang, 2014; Manasfi et al., 2015). Furthermore, the time-course profile in wastewater showed a different picture (Fig. 5c). Due to the limited free chlorine supply, transformation from TP-206 and TP-240 to TP-130 and TP-164, respectively, was not obvious. Alternatively, the signal of TP-206 rose steadily with time and showed relatively high abundance. The responses of TP-130, TP-164 and TP-240 increased up to 5 min and remained mostly constant from then on. Abundance of TP-164 was quite low.

The time-course results are of great importance since these TPs are actually produced under typical real water disinfection conditions. The information in ACE chlorination in real waters provides an overview of its fate in the aquatic environment, which can be used for further monitoring studies.

3.4. Toxicity evaluation of the DBPs

The original ACE compound has been proven marginally toxic from Microtox screening with EC50 > 70,000 mg L^{-1}, water flea acute immobilization with No Observed Effect Concentration (NOEC) at 1,000 mg L^{-1}, fish embryo assay with NOEC at 10,000 mg L^{-1} (Stolte et al., 2013; Sang et al., 2014; Li et al., 2016). Actually, degradations of ACE involve more complex TPs. Our previous works have found 16 TPs derived from ACE photolysis, inducing significantly elevated toxicity in both marine bacteria and zebrafish embryos (Sang et al., 2014; Li et al., 2016). Due to the production of chlorine-containing intermediates/products as well as known DBPs, ACE could still not be efficiently mineralized during chlorination disinfection. Alternatively, evaluation of the risk caused by the chlorination residues is of great importance in defining its environmental safety in the long run.

To quantify chlorine-related toxicity of ACE, chlorinated samples were quenched and collected at defined intervals corresponding to different levels of ACE degradation (%). Two controls, one with no ACE input and the other with no chlorine additive, were run in parallel and aimed to verify toxicity origin. The initial findings of the current Microtox test displayed a dynamic variation in the toxicity of ACE chlorinated by-products (Fig. 6). At the beginning, i.e. the zero ACE chlorination point, the advanced toxicity relative to the two controls should derive from buffer and quench agents. Tawk et al. (2015) has found that phosphate buffer inhibits bacteria luminescence. The lowest toxicity occurred at around 50% ACE chlorination while the highest toxicity was at 75% ACE disappearance at a measureable magnification factor of 1.8 with respect to zero ACE degradation. As we know, excess chlorine facilitates a shift from TP-206 and TP-240 to TP-130 and TP-164 (Fig. 5a), respectively, which might contribute to toxicity variations accordingly. In such cases, chlorination disinfection may be still unable to remove

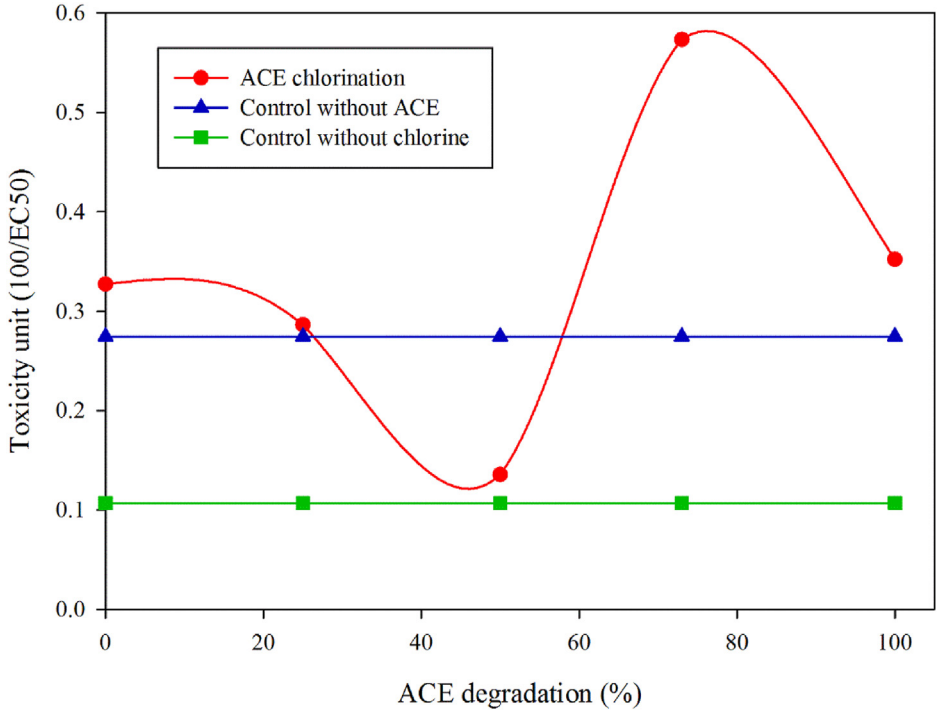

Fig. 6. Toxicity patterns during acesulfame chlorination with $[ACE]_0$ 20 mg L^{-1}, $[ACE]_0$: $[HOCl]_0$ at 1:20 in ultrapure water.

ACE residues from the aquatic environment due to not only its production of TPs, regulated DBPs and even N-DBPs but also the enhanced accompanying toxicity. Results of the current toxicity work provided only preliminary data to evaluate potential impact of ACE chlorination. A comprehensive assessment is demanded for studies with multiple model organisms/organisms from different tropic levels.

4. Conclusions

- In excess of chlorine, ACE undergoes pseudo-first-order removal reactions. The first-order rates were enhanced by decreasing the sample pH with estimated ACE half-lives from 693 min to 2 min. Chlorine species were also affected by pH and consequently impacted ACE reactivity in the solution.
- Significant formation of 5 TPs and 6 Cl-DBPs was recognized in the ultrapure water. Two chlorinated TPs were firstly identified in the current study. Unpredictably, ACE was found to be precursor of several regulated known DBPs and two N-DBPs. This raises new concerns about the fate of degradation by-products in ACE disinfection and underscores the necessity to pursue more precise data.
- With regard to ACE chlorination, toxicity as revealed by the Microtox assay showed a dynamic trend, which might be the result of further transformation of by-products during chlorination. Relative to the start of ACE chlorination, the maximal toxic effect (1.8-fold) to marine bacteria occurred during the period with 75% ACE degradation.
- Time-course profiles in chlorinated tap water and wastewater presented TPs identical to those found in ultrapure water. The results are of great importance since information on the transformation pathway of ACE provides an overview of its fate in the aquatic environment. Notably, a batch of Br-DBPs was detected during ACE chlorination in wastewater, which is reported to be more toxic than Cl-DBPs.
- To summarize, chlorine disinfection is not an optimal process for ACE removal because of its production of TPs, regulated DBPs and/or N-DBPs as well as its greater toxicity compared to the original chemical. Continuous study and technological development along these lines will be needed in order to improve ACE residue treatment such that it achieves decontamination and mineralization.

Acknowledgements

We thank the Hong Kong Research Grants Council (HKBU 12302915), and the Science, Technology and Innovation Commission of Shenzhen (JCYJ20150630164505506) for the financial support. Kelvin S.–Y. Leung also thanks the grants from the inter-institutional Collaborative Research Scheme (RC-ICRS/14-15/02) and Faculty of Science (FRG2/15-16/069), Partner State Key Laboratory of Environmental and Biological Analysis (SKLP-1415-P006, SKLP-1617-P03), Hong Kong Baptist University. J.C.-F. Law and C.-H. Chow gratefully acknowledge their receipt of postgraduate studentships from the University Grants Committee.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2017.03.053.

References

Abdallah, P., Deborde, M., Berne, F.D., Leitner, N.K.V., 2015. Kinetics of chlorination of benzophenone-3 in the presence of bromide and ammonia. Environ. Sci. Technol. 49, 14359–14367.

Armbruster, D., Happel, O., Scheurer, M., Harms, K., Schmidt, T.C., Brauch, H.-J., 2015. Emerging nitrogenous disinfection byproducts: transformation of the antidiabetic drug metformin during chlorine disinfection of water. Water Res. 79, 104–118.

BS EN ISO 11348-3, 2008. Water Quality – Determination of the Inhibitory Effect of Water Samples on the Light Emission of *Vibrio fischeri* (Luminescent Bacteria Test) – Part 3: Method Using Freeze-dried Bacteria (ISO 11348-3:2007). British

Standards.
Buerge, I.J., Buser, H.-R., Kahle, M., Müller, M.D., Poiger, T., 2009. Ubiquitous occurrence of the artificial sweetener acesulfame in the aquatic environment: an ideal chemical marker of domestic wastewater in groundwater. Environ. Sci. Technol. 43, 4381–4385.
Cai, M.-Q., Feng, L., Jiang, J., Qi, F., Zhang, L.-Q., 2013. Reaction kinetics and transformation of antipyrine chlorination with free chlorine. Water Res. 47, 2830–2842.
Chusaksri, S., Sutthivaiyakit, S., Sedlak, D.L., Sutthivaiyakit, P., 2012. Reactions of phenylurea compounds with aqueous chlorine: implications for herbicide transformation during drinking water disinfection. J. Hazard. Mater. 209–210, 484–491.
Clesceri, L.S., Greenberg, A.E., Eaton, A.D., 1998. Standard Methods for the Examination of Water and Wastewater, twentieth ed. American Water Works Association, Maryland, pp. 461–465.
Deborde, M., von Gunten, U., 2008. Reactions of chlorine with inorganic and organic compounds during water treatment-kinetics and mechanisms: a critical review. Water Res. 42, 13–51.
de Oliveira e Sá, M.M., Miranda, M.S., Esteves da Silva, J.C.G., 2014. Study of the transformation of two salicylates used in personal care products in chlorinated water. Water Res. 65, 32–39.
Duirk, S.E., Bridenstine, D.R., Leslie, D.C., 2013. Reaction of benzophenone UV filters in the presence of aqueous chlorine: kinetics and chloroform formation. Water Res. 47, 579–587.
El Najjar, N.H., Deborde, M., Journel, R., Leitner, N.K.V., 2013. Aqueous chlorination of levofloxacin: kinetic and mechanistic study, transformation product identification and toxicity. Water Res. 47, 121–129.
Fang, J., Fu, Y., Shang, C., 2014. The roles of reactive species in micropollutant degradation in the UV/free chlorine system. Environ. Sci. Technol. 48, 1859–1868.
FDA (U.S. Food and Drug Administration), 2014. How Sweet it Is: All about Sugar Substitutes, U. S. http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm397711.htm.
Gan, Z., Sun, H., Feng, B., Wang, R., Zhang, Y., 2013. Occurrence of seven artificial sweeteners in the aquatic environment and precipitation of Tianjin, China. Water Res. 47, 4928–4937.
Gan, Z., Sun, H., Wang, R., Hu, H., Zhang, P., Ren, X., 2014. Transformation of acesulfame in water under natural sunlight: joint effect of photolysis and biodegradation. Water Res. 64, 113–122.
González-Mariño, I., Quintana, J.B., Rodríguez, I., Cores, M., Cela, R., 2015. Transformation of methadone and its main human metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), during water chlorination. Water Res. 68, 759–770.
Kolar, J.J., Lindgren, B.O., Pettersson, B., 1983. Chemical reactions in chlorine dioxide stages of pulp bleaching. Wood Sci. Technol. 17, 117–128.
Li, A.J., Schmitz, O.J., Stephan, S., Lenzen, C., Yue, P.Y.-K., Li, K., Li, H., Leung, K.S.-Y., 2016. Photocatalytic transformation of acesulfame: transformation products identification and embryotoxicity study. Water Res. 89, 68–75.
Liu, J., Zhang, X., 2014. Comparative toxicity of new halophenolic DBPs in chlorinated saline wastewater effluents against a marine alga: halophenolic DBPs are generally more toxic than haloaliphatic ones. Water Res. 65, 64–72.
Loos, R., Carvalho, R., António, D.C., Comero, S., Locoro, G., Tavazzi, S., Paracchini, B., Ghiani, M., Lettieri, T., Blaha, L., Jarosova, B., Voorspoels, S., Servaes, K., Haglund, P., Fick, J., Lindberg, R.H., Schwesig, D., Gawlik, B.M., 2013. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. Water Res. 47, 6475–6487.
Manasfi, T., Storck, V., Ravier, S., Demelas, C., Coulomb, B., Boudenne, J.-L., 2015. Degradation products of benzophenone-3 in chlorinated seawater swimming pools. Environ. Sci. Technol. 49, 9308–9316.
Negreira, N., de Alda, M.L., Barceló, D., 2015a. Degradation of the cytostatic etoposide in chlorinated water by liquid chromatography coupled to quadrupole-Orbitrap mass spectrometry: identification and quantification of by-products in real water samples. Sci. Total Environ. 506–507, 36–45.
Negreira, N., Regueiro, J., de Alda, M.L., Barceló, D., 2015b. Degradation of the anticancer drug erlotinib during water chlorination: non-targeted approach for the identification of transformation products. Water Res. 85, 103–113.
Negreira, N., Regueiro, J., de Alda, M.L., Barceló, D., 2015c. Transformation of tamoxifen and its major metabolites during water chlorination: identification and *in silico* toxicity assessment of their disinfection byproducts. Water Res. 85, 199–207.
Postigo, C., Cojocariu, C.I., Richardson, S.D., Silcock, P.J., Barceló, D., 2016. Characterization of iodinated disinfection by-products in chlorinated and chloraminated waters using Orbitrap based gas chromatography-mass spectrometry. Anal. Bioanal. Chem. 408, 3401–3411.
Postigo, C., Richardson, S.D., 2014. Transformation of pharmaceuticals during oxidation/disinfection processes in drinking water treatment. J. Hazard. Mater. 279, 461–475.
Richardson, S.D., Kimura, S.Y., 2016. Water analysis: emerging contaminants and current issues. Anal. Chem. 88, 546–582.
Richardson, S.D., Plewa, M.J., Wagner, E.D., Schoeny, R., DeMarini, D.M., 2007. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. Mutat. Res. 636, 178–242.
Sang, Z., Jiang, Y., Tsoi, Y.-K., Leung, K.S.-Y., 2014. Evaluating the environmental impact of artificial sweeteners: a study of their distributions, photodegradation and toxicities. Water Res. 52, 260–274.
Scheurer, M., Godejohann, M., Wick, A., Happel, O., Ternes, T.A., Brauch, H.-J., Ruck, W.K.L., Lange, F.T., 2012. Structural elucidation of main ozonation products of the artificial sweeteners cyclamate and acesulfame. Environ. Sci. Pollut. Res. 19, 1107–1118.
Scheurer, M., Storck, F.R., Brauch, H.-J., Lange, F.T., 2010. Performance of conventional multi-barrier drinking water treatment plants for the removal of four artificial sweetener. Water Res. 44, 3573–3584.
Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying small molecules via high resolution mass spectrometry: communicating confidence. Environ. Sci. Technol. 48, 2097–2098.
Sedlak, D.L., von Gunten, U., 2011. The chlorine dilemma. Science 331, 42–43.
Sgroi, M., Roccaro, P., Oelker, G.L., Snyder, S.A., 2015. N-nitrosodimethylamine (NDMA) formation at an indirect potable reuse facility. Water Res. 70, 174–183.
Shah, A.D., Mitch, W.A., 2012. Halonitroalkanes, halonitriles, haloamides, and N-nitrosamines: a critical review of nitrogenous disinfection byproduct formation pathways. Environ. Sci. Technol. 46, 119–131.
Sivey, J.D., Mccullough, C.E., Roberts, A.L., 2010. Chlorine monoxide (Cl_2O) and molecular chlorine (Cl_2) as active chlorinating agents in reaction of dimethenamid with aqueous free chlorine. Environ. Sci. Technol. 44, 3357–3362.
Sivey, J.D., Roberts, A.L., 2012. Assessing the reactivity of free chlorine constituents Cl_2, Cl_2O, and HOCl toward aromatic ethers. Environ. Sci. Technol. 46, 2141–2147.
Soh, L., Connors, K.A., Brooks, B.W., Zimmerman, J., 2011. Fate of sucralose through environmental and water treatment processes and impact on plant indicator species. Environ. Sci. Technol. 45, 1363–1369.
Soufan, M., Deborde, M., Delmont, A., Legube, B., 2013. Aqueous chlorination of carbamazepine: kinetic study and transformation product identification. Water Res. 47, 5076–5087.
Stolte, S., Steudte, S., Schebb, N.H., Willenberg, I., Stepnowski, P., 2013. Ecotoxicity of artificial sweeteners and stevioside. Environ. Int. 60, 123–127.
Subedi, B., Kannan, K., 2014. Fate of artificial sweeteners in wastewater treatment plants in NewYork State, U.S.A. Environ. Sci. Technol. 48, 13668–13674.
Tawk, A., Deborde, M., Labanowski, J., Gallard, H., 2015. Chlorination of the β-triketone herbicides tembotrione and sulcotrione: kinetic and mechanistic study, transformation products identification and toxicity. Water Res. 76, 132–142.
Trebše, P., Polyakova, O.V., Baranova, M., Kralj, M.B., Dolenc, D., Sarakha, M., Kutin, A., Lebedev, A.T., 2016. Transformation of avobenzone in conditions of aquatic chlorination and UV-irradiation. Water Res. 101, 95–102.
Wang, D., Bolton, J.R., Hofmann, R., 2012. Medium pressure UV combined with chlorine advanced oxidation for trichloroethylene destruction in a model water. Water Res. 46, 4677–4686.
Xiang, Y., Fang, J., Shang, C., 2016. Kinetics and pathways of ibuprofen degradation by the UV/chlorine advanced oxidation process. Water Res. 90, 301–308.
Yang, M., Zhang, X., 2013. Comparative developmental toxicity of new aromatic halogenated DBPs in a chlorinated saline sewage effluent to the marine polychaete *platynereis dumerilii*. Environ. Sci. Technol. 47, 10868–10876.
Yang, M., Liu, J., Zhang, X., Richardson, S.D., 2015. Comparative toxicity of chlorinated saline and freshwater wastewater effluents to marine organisms. Environ. Sci. Technol. 49, 14475–14483.