Transformation pathways and acute toxicity variation of 4-hydroxyl benzophenone in chlorination disinfection process

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HIGHLIGHTS

- 7 transformation products in 4HB chlorination disinfection system were identified.
- Transformation pathways of 4HB during chlorination disinfection were proposed.
- Acute toxicity increased after chlorination due to the formation of 3,5-dichloro-4HB.
- The transformation mechanisms of 4HB can be repeated in ambient water matrices.

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ABSTRACT

Benzophenones compounds (BPs) are widely used as UV filters, and have been frequently found in multiple environmental matrices. The residual of BPs in water would cause potential threats on ecological safety and human health. Chlorination disinfection is necessary in water treatment process, in which many chemicals remained in water would react with disinfectant chlorine and form toxic by-products. By using ultra performance liquid phase chromatography quadrupole time of flight mass spectrometer (UPLC-QTOF-MS), nuclear magnetic resonance (NMR), the transformation of 4-hydroxyl benzophenone (4HB) with free available chlorine (FAC) was characterized. Eight major products were detected and seven of them were identified. Transformation pathways of 4HB under acid, neutral, and alkaline conditions were proposed respectively. The transformation mechanisms involved electrophilic chlorine substitution of 4HB, Baeyer-Villiger oxidation of ketones, hydrolysis of esters and oxidative breakage of benzene ring. The orthogonal experiments of pH and dosages of disinfectant chlorine were conducted. The results suggested that pH conditions determined the occurrence of reaction types, and the dosages of disinfectant chlorine affected the extent of reactions. Photobacterium assay demonstrated that acute toxicity had significant increase after chlorination disinfection of 4HB. It was proved that 3,5-dichloro-4HB, one of the major transformation products, was responsible for the increasing acute toxicity after chlorination. It is notable that, 4HB at low level in real ambient water matrices could be transformed during simulated chlorination disinfection practice. Especially, two major products 3-chloro-4HB and 3,5-dichloro-4HB were detected out, implying the potential ecological risk after chlorination disinfection of 4HB.

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1. Introduction

With the increasing concern about the effects of ultraviolet (UV) irradiation, more and more UV filters especially organic types with better sun protection factors, higher photo stability, and better water resistance have been produced and used throughout the last decades. Besides cosmetics such as lotions, shampoos, and lipsticks in personal care products, UV-filters are also widely used in

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insecticides, agricultural chemicals, pharmaceuticals and material preservatives, e.g. textiles and numerous other products (Christin et al., 2007; Roelandts et al., 1983; Stenback, 1977; Tomson et al., 1981). Benzophenones (BPs), one class of commonly used UV-filters, have wide absorption wavelength range at 200–400 nm. BPs are effectively used in preventing some skin diseases even skin cancer, for reducing the harms of UVA (320–400 nm) and UVB (290–320 nm) to human body completely (Moloney et al., 2002). In addition, BPs are also used as agricultural chemicals, flavor ingredients and UV stabilizers in plastic surface coatings for food packaging and in the manufacture of cosmetics and other personal care products due to their low cost (Suzuki et al., 2005; Zenker et al., 2008). The maximum authorized concentration of 2-hydroxy-4-methoxy-BP (BP-3) in sunscreens is 10% in EU, 6% in USA, 2,2'-dihydroxy-4-methoxy-BP (BP-8) is 3% in Korea (EEC, 1983; FDA, 1999; Jeon et al., 2006). 2-Hydroxy-4-methoxy-5-sulfonic acid BP (BP-4) is permitted for use in cosmetics at a maximum concentration of 10% in Japan and Australia (SJP, 1985; TGA, 2003). As a result, a large amount of residual BPs and their metabolites enters into the aqueous environment via washing off from skin and clothes, or discharging of sewage and swimming pool waters (Kim and Choi, 2014). The concentration of BP-4 ranged from 237 to 1481 ng L⁻¹ in wastewater from Galicia (NW Spain) (Rodil et al., 2008). 2,4-Dihydroxy-BP (BP-1) was detected with the mean concentration of 47 ng L⁻¹ in 25 rivers in Korea, and benzhydrol, a major metabolite of benzophenone was detected with the highest concentration of 204 ng L⁻¹ as well. Meanwhile, the concentration of BP-3 and BP-8 ranged from 730 to 3880 and 500–4170 ng kg⁻¹ in soil samples, respectively (Jeon et al., 2006). It has been proved that human bodies would absorb and accumulate BPs after skin administration. BP-3 was detected up to 445 ng g⁻¹ lipid in human breast milk samples (Hany and Nagel, 1995), up to 40 µg L⁻¹ in urine samples (Zwiener et al., 2007). BP-1, a metabolite of BP-3, has also been detected in urine after application of commercially available sunscreen products to the skin (Felix et al., 1998).

BPs are bioaccumulative in organisms due to their hydrophilicity, which would make adverse biological effects. For example, BPs exhibited both estrogenic and anti-androgenic properties in a variety of organisms, exerted autotrophic effect *in vivo* test, stimulated cell proliferation of human breast cancer cell line MCF-7 (Michigan Cancer Foundation7) and increased the secretion of tumor marker pS2 (an estrogen-induced protein) *in vitro* (Balmer et al., 2005; Ma et al., 2003; Schlumpf et al., 2001; Schreurs et al., 2005; Zenker et al., 2008). Moreover, some BPs such as benzophenone, BP-3 and BP-8, 2,3,4-trihydroxy-BP (BP-20) have been reported to have potential genotoxic effects (Fluck et al., 1976; French, 1992; Jeon et al., 2007; Mortelmans et al., 1986; Zeiger et al., 1987). Some BPs have also been observed obvious genotoxic effects at high milligram per liter level (Zhao et al., 2013). A current epidemiological survey suggested that male exposure to 2,2',4,4'-tetrahydroxy-BP (BP-2) or 4-hydroxyl benzophenone (4HB) may diminish couples' fecundity by about 30 percent, resulting in a longer time to pregnancy (Louis et al., 2014).

Disinfection is one of necessary processes for producing tap water, reclaimed water, and swimming pool water. Chlorine is still one of the most popular disinfectants in the world due to its high efficiency, convenience and low cost. However, except for killing pathogens, chlorine may react with the residual chemicals and natural organic matter in water, and generate some unintended toxic disinfection by-products (DBPs). More than 600 DBPs have been identified in drinking water, and many of them are mutagenic or carcinogenic (Richardson, 1998; Richardson et al., 2007). During

chlorination of BP compounds, halogenated aromatic DBPs might be formed. And recent studies have shown that halogenated aromatic DBPs were significantly more toxic than the regulated trihalomethanes and haloacetic acids (Yang and Zhang, 2013; Pan et al., 2016; Liu and Zhang, 2014). The formation mechanisms and potential ecological or health risk of DBPs should be paid more attention, which would provide scientific reference for optimizing disinfection operating parameters. The substitution and cleavage of two benzene rings of BP-3 and BP-4 in chlorination disinfection have been reported (Negreira et al., 2008, 2012; Xiao et al., 2013). Bromoform was found to be generated from BP-3 during the chlorination of seawater swimming pools (Manasfi et al., 2015). However, the toxicity changes after chlorination disinfection of BPs, identification and formation pathways of toxic products have not been disclosed systematically. It is well known that lots of chemical reactions occur in chlorination disinfection system, and the components in chlorinated solution are quite complex. Furthermore, the target chemicals selected in the previous literature usually had complex molecular structures, which increased the difficulty in identifying products structures and analyzing transformation mechanisms. If a model chemical with simple structure and similar reactivity is selected, it will be quite easier to disclose common transformation characteristics of pollutants comprehensively. Therefore, the present paper focused on the chlorination disinfection of 4HB, identified the main transformation products, and proposed the plausible transformation pathways under acid, neutral and alkaline conditions, respectively. Moreover, the acute toxicity variation of 4HB after chlorination treatment under different conditions was monitored to screen transformation products with high toxicity. The results of this study would throw more light on the transformation mechanism of BPs in water disinfection treatment, and provide scientific basis to the risk evaluation of this kind of compounds.

2. Methods and materials

2.1. Reagents and instruments

4HB (>97.5% purity) was purchased from Sigma-Aldrich (St Louis, MO, USA) which was used directly without further purification. Salts including HOAc (acetic acid), NaOAc (sodium acetate), Na₂CO₃ (sodium carbonate), Na₂HPO₄·12H₂O and NaH₂PO₄·2H₂O for preparing buffer (pH = 4.6, 7.5 and 11.0 with the concentration of 0.02 M), KH₂PO₄, NaCl and Na₂SO₃ for toxicity test medium and quenching solutions, were purchased from Sigma-Aldrich (St Louis, MO, USA). Yeast extract, tryptone and glycerol were obtained from BD (Franklin Lakes, NJ, USA). Methanol and acetone for HPLC analysis were obtained from Fisher Sci (Fair Lawn, NJ, USA). Dimethylsulfoxide (DMSO) was purchased from Amresco (Solon, OH, USA). The NaClO (8%) aqueous solution was purchased from Wako Co (Tokyo, Japan). The test bacteria *Photobacterium phosphoreum* T3 Straus was provided as freeze-dried powder by the Institute of Soil Science, Chinese Academy Sciences, Nanjing, China. The ultrapure water used in solution preparation and dilution was produced by a Milli-Q purification system (Millipore, Billerica, MA, USA). An Ultimate3000 UPLC (Dionex, USA) and QTOF-MS (microTOF QII, Bruker, Germany) were used to separate and identify the main transformation products of 4HB after chlorination in different buffer system.

2.2. Chlorination experiments

Considering the relative distribution and properties of free

available chlorine (FAC) species under different pH conditions (Fig. S1), the chlorination experiments were conducted at three pH values 4.6 (100% as HOCl), 7.5 (50% as HOCl and 50% as ClO^-) and 11.0 (100% as ClO^-) for exploring the chlorination mechanisms of 4HB within a relatively wide pH range. To maintain the pH value within 0.1 unit of variance during the reaction period, 0.02 M acetate buffer (pH = 4.6), phosphate buffer (pH = 7.5), and carbonate buffer (pH = 11.0) were used.

The chlorination reaction was conducted in a 1000 mL borosilicate glass conical flask, which was wrapped with aluminum foil and placed in a water bath with a magnetic stirring apparatus to maintain the reaction temperature at $25 \pm 0.5^\circ\text{C}$. To avoid missing the detection of the trace chlorination products and make a more comprehensive research, the initial concentration of 4HB was set as 2 mM in 500 mL of buffer solutions (0.02 M), respectively. The co-solvent, methanol concentration in reaction system was less than 1% (Schwarzenbach et al., 2003). The chlorination reaction was initiated by adding certain amount of FAC. The initial doses of FAC were set as 0, 1, 3, 5, 10 and 15 molar equivalent to 4HB, respectively. Five milliliters of samples were subsequently collected at certain time intervals from the reaction system and a slightly excessive sodium sulfite (1.2 molar equivalent of sulfite to FAC) was added immediately to quench the reaction.

2.3. Sample preparation

The samples were freeze-dried by a lyophilizer (FD-1D-50, Beijing, China), and 4 mL of mixed solvents (50% methanol and 50% acetone) was added to extract organic components. The extract was filtered through a $0.22\ \mu\text{m}$ glass fiber membrane to remove undissolvable inorganic salts. One milliliter of the filtrate was taken to detect the residual 4HB and transformation products by UPLC-QTOF-MS. Two milliliters of filtrate was taken to be dried with a gentle flow of ultrapure nitrogen gas, and the solid residue was re-dissolved in 400 μL DMSO as stock solution. For the photobacterium acute toxicity test, the stock solution was diluted with 3% NaCl aqueous solution. The content of DMSO in the exposure solution for the bioassay was less than 1% (v/v) at which had been proved to be no significant influence on photobacterium bioassay (Grzegorz et al., 1997).

2.4. Acute toxicity test

The pretreated sample was diluted into a series of solutions with gradient concentrations, and added into a 96-well microplate. NaCl solution (3%) was set as negative control, and Hg^{2+} (prepared with HgCl_2) was set as positive control to obtain the calibration curve. The volume of sample or Hg^{2+} in each well was 180 μL . Then 20 μL of pre-cultured *P. Phosphoreum* bacteria suspension was added into each well and mixed sufficiently. After a 15 min of shake and exposure period at $22 \pm 1^\circ\text{C}$, the bioluminescence intensity in each well was measured by Synergy™ 2 Multi-Mode Microplate Reader (Biotek, USA). Toxicity was expressed as the luminescence intensity inhibition ratio (Ir), which could be described as follows:

$$\text{Ir} = \frac{L_{\text{NC}} - L_{\text{sample}}}{L_{\text{NC}}} \times 100\%$$

Where, L_{NC} represents the luminescence intensity of negative control, L_{sample} represents the luminescence intensity of sample. For each toxicity test, two dose-response curves could be obtained, one was of positive reference substance Hg^{2+} , and the other was of tested sample. Based on the two dose-response curves, the half maximal effective concentrations (EC50) were calculated for positive reference substance Hg^{2+} and samples, respectively (Ye et al.,

2011). In order to visually reflect the toxicity of samples before and after disinfection of 4HB under various conditions, the toxicity results of samples were converted into equivalent concentration of positive reference substance Hg^{2+} . Specifically, after acquiring the dose-response curves of Hg^{2+} (X-axis is mass of Hg^{2+} , mol) and sample (X-axis is volume of sample, L), EC50 values for Hg^{2+} and samples were calculated, respectively. The ratio of $\text{EC50}_{\text{Hg}^{2+}}^+$ to $\text{EC50}_{\text{Sample}}^+$, defined as equivalent concentration of Hg^{2+} (M), indirectly reflects the toxicity of one sample. The control trials of three buffer solutions proved that buffer salts had no effect on the toxicity test of the *P. Phosphoreum* bacteria.

2.5. Liquid chromatography-mass spectrometry

The residual of 4HB and possible transformation products were separated by an ultra performance liquid chromatography (Ultimate 3000, Dionex, USA) system with Agilent SB-C18 column ($4.6 \times 150\ \text{mm}$, $5\ \mu\text{m}$), and analyzed by quadrupole-time of flight mass spectrometer (microTOF QII, Bruker, Germany). The mass spectrometer has high resolution (resolution > 16,500 FWHM (full width at half maximum) and $\Delta M/M < 2\ \text{ppm}$), which can provide accurate molecular mass for identifying the possible structure of the transformation products. Injection volume was 1 μL and flow rate was 0.4 mL/min, gradient elution was mixture of 0.1% (v/v) formic acid aqueous solution and methanol. The gradient elution method is described in Table S1. The MS analyses were conducted using negative mode electrospray ionization (ESI^-) over a mass scan range of 50–1000 m/z . The mass spectrometer fragmentation voltage was set at 175 eV. Temperature of spray chamber and dry gas flow rate were set at 250°C and 6 L/min, respectively.

2.6. Synthesis of 3,5-dichloro-4HB

Firstly, 0.4 g 4HB and 0.2 mL NaClO (8%) solution were added into a 250 mL round bottom flask containing 100 mL of 0.02 M carbonate buffer solution (pH 10). After stirring 2 h, 0.24 mL of sulfite solution (1 M) was added to quench the reaction. The reaction solution was filtered through $0.22\ \mu\text{m}$ glass fiber membrane, and the solid was collected. The solid was re-dissolved in 0.5 mL methanol and the solution was evenly applied on a line 1.5 cm from the bottom edge of a $20 \times 20\ \text{cm}$ silica gel plate (TLC Silica gel 60 F254, Merck, Germany) by using capillary. After evaporating off the solvent methanol, the silica gel plate was placed into a transparent separation chamber containing mixed solvents (petroleum ether: ethyl acetate = 2:1, v/v) with a depth of less than 1 cm. The chamber was closed with a glass cover. The solvents carried the products up the plate (elute and separate the products). The plate was removed from the chamber before the solvents front almost reached the top of the stationary phase. After drying, scraped off the silica gel adsorbed the target product (3,5-dichloro-4HB), then extracted it into methanol and filtrated. The filtrate was dried with gentle N_2 flow, and stocked for further analysis.

2.7. Nuclear magnetic resonance (NMR)

In order to confirm the molecular structure of some key transformation products in chlorination disinfection system, the ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker ARX 400 spectrometer for solutions in deuterated- CHCl_3 , and the chemical shifts were given in ppm downfield from an internal tetramethylsilane standard. Before the nuclear magnetic resonance experiment, thin layer chromatography was used to acquire the high purity product.

3. Results and discussion

3.1. Identification of transformation products

According to the distribution of chlorine species and the influence of chlorine dosages, the orthogonal experiments were respectively conducted with different FAC dosages (0-, 1-, 3-, 5-, 10- and 15-equivalents to 4HB) under three pH conditions (pH 4.6, 7.5, 11.0) to characterize the transformation behaviors and acute toxicity changes. It was found that the transformation behaviors of 4HB were strongly dependent on the operating conditions (e.g., pH, FAC dose). Fig. S2 shows the chromatographic peaks of major products of 4HB after chlorination with 5-equiv FAC under three pH conditions, respectively.

From the base peak chromatogram, 8 major products were found. Among them, 7 products were identified. Fig. S3 shows the mass spectrum information, isotopic peaks of the products and the identified structural formula. The high resolution mass spectrometer (resolution > 16,500 FWHM and $\Delta M/M < 2$ ppm) used in this study could provide accurate molecular mass, true isotopic pattern, and high sensitivity, which are beneficial to structure identification of products. Take monochlorinated product P2 for example, from the mass spectrum as shown in Fig. S3, the exact molecular weight is m/z 231.0246/233.0216, the intensity ratio of isotopic peaks is about 3:1. It can be determined that one hydrogen atom was substituted by one chlorine atom on one benzene ring. Meanwhile, considering the orientation effect of hydroxyl group, it is easily to determine the structure of P2. With the similar method, the molecular structures of products P5 and P6 were identified. Since product P5 is a key intermediate during the chlorination transformation of 4HB, it was synthesized, and its NMR spectrum was obtained to verify its absolute structure. Fig. S4 shows the ^1H NMR and ^{13}C NMR spectra, which provides solid evidence to determine the structure of P5. The assignment of chemical shifts for 4HB and P5 are listed in Text S1. Product P4 has one more oxygen atom than that in P5, and the possible reason is the oxidation of ketone group into ester group by FAC. This reaction is named Baeyer-Villiger oxidation, which has been reported in our previous study (Xiao et al., 2013).

Except for the chlorinated and ester compounds, P3' and P4' were detected in chlorination system as well. According to the MS¹ information, the structure of parent compound 4HB and the components in buffer solutions, we conjectured that they were acetylation derivatives of intermediate phenolics by CH_3COOH in the buffer solution. In order to affirm this suppose, the acetate buffer system (HOAc-NaOAc , pH 4.6) was replaced by phosphate buffer system ($\text{Na}_2\text{HPO}_4\text{--KH}_2\text{PO}_4$, pH 4.6), and the chlorination experiment was performed again. Products P3' and P4' were not detected in the phosphate buffer system, implying P3' and P4' were acetylation derivatives from P3 and P4, respectively. Fig. S5 shows the base peak chromatogram of products in two different buffer systems with $[\text{4HB}]_0:[\text{FAC}]_0 = 1:5$.

Because of the instability of P1 under the ionization conditions of ESI, MS² spectrum was almost same as MS¹. The molecular structure of P1 was identified according to the information of molecular ion and fragment ions shown in Fig. S6. With the collision of insert gas, the molecular ions would lose some pieces and generate sequence fragment ions. From Fig. S6, it can be seen that two HCl molecules, a CO_2 molecule, and $-\text{CO}$, $-\text{C}_2\text{H}_2$ fragments were sequentially broken down from the molecular ion. Product P1' was detected out in acetate buffer system but not in phosphate buffer system. Based on the identification of P3' and P4', we speculated that P1' might be derivate product of P1 in acetate buffer

system, but the specific structure of P1' can not be identified yet.

3.2. Transformation pathways of 4HB in chlorination process

The proposed transformation pathways of 4HB chlorination system is shown in Fig. 1, the compounds in the dashed boxes were undetected intermediates under the current instrumental conditions. From the transformation pathways, it can be found that the reaction in alkaline system was the simplest and chlorination occurred only. In neutral system, chlorination, Baeyer-Villiger oxidation and cleavage of benzene ring were occurred. Except for the reactions occurred in neutral system, hydrolysis reaction was found in acid system. Moreover, occurrence of P1', P3' and P4' were the proof of esterification between intermediate products and buffer salts. In fact, the species distribution of FAC determines the reaction types in different pH systems. At pH 11.0, the major species of FAC is ClO^- , as electrophilic reagent, chlorination occurred between ClO^- and 4HB. At pH 7.5, HClO and ClO^- were coexisted, HClO can oxidize carbonyl to ester, and the strong oxidability of HClO may break the benzene ring as well. At pH 4.6, HClO is the major species, except of all the reaction types contained in neutral system, hydrolysis could occur either. The hydrolysis of esters easily occurred in both acid and alkaline systems.

3.3. Influence of pH values on the transformation of 4HB

pH values determine the species distribution of FAC and the target compound. The time-dependent removal behaviors of 4HB ($[\text{4HB}]_0 = 2$ mM) and the formation of the intermediates P2 and P5 in the FAC chlorination system ($[\text{FAC}]_0 = 6$ mM) are shown in Fig. 2. It can be revealed that the reaction reached equilibrium swiftly in three pH systems. The 4HB had the highest transformation rate in alkaline system and lowest in acid system, the formation of chlorinated products P2 and P5 were the most in alkaline system and the least in acid system. These phenomenas were most likely attributed to the different species of FAC and 4HB under various pH conditions. In alkaline system (pH 11.0), ClO^- is the single species of FAC, and hydroxy group in 4HB ($-\text{OH}$) is converted into phenolate anion ($-\text{O}^-$), the phenolate anion easily occurs chlorination substitution with ClO^- and accumulates intermediates P2 and P5. In acid system (pH 4.6), HClO is the single species with high redox potential, which rapidly reacts with 4HB to form chlorinated intermediates P2 and P5. Subsequently, P2 and P5 are oxidized to form ester products and some other products rapidly. As a result, the intermediates P2 and P5 were exhausted and 4HB was remained. The formation of other products also could be understood in the similar way.

3.4. Influence of FAC dosages on the formation of products

In order to investigate the effects of the FAC dosages on the transformation of 4HB, different FAC doses were set to react with 4HB under three pH conditions ($[\text{FAC}]_0:[\text{4HB}]_0 = 1, 3, 5, 10$ and 15). The formation and relative contents of major transformation products are shown in Fig. 3. The higher FAC dosage is, the more transformation of 4HB will be. Some intermediates sequentially increased to maximum concentrations and then decreased. Take P2 for example, it reached a maximum concentration at 3-equiv of FAC and subsequently decreased due to its further transformation with high dosages of FAC. In addition, from Fig. 3, it can be concluded that, with the increasing FAC dosages, P1', P1 and P5 increased continuously and became the major products at pH 4.6, 7.5 and 11.0, respectively.

Fig. 1. The proposed transformation pathways of 4HB in chlorination system ($[4HB]_0$: 2 mM, $[FAC]_0$: 20 mM, reaction time: 1 h) at three pH values (pH 4.6, pH 7.5, pH 11.0).

Fig. 2. The time-dependent concentration changes of 4HB, P2 and P5 in the chlorination disinfection systems ($[4HB]_0$ = 2 mM, $[FAC]_0$ = 6 mM) at three pH values ((a) pH 4.6; (b) pH 7.5; (c) pH 11.0).

Fig. 3. Formation and relative contents of main products of 4HB (2 mM) chlorination with different doses of FAC (0-, 1-, 3-, 5-, 10- and 15-equiv) under three pH systems ((a) pH 4.6; (b) pH 7.5 (c) pH 11.0). The relative content for each product was calculated through standardizing peak area. Specifically, for each product, totally 18 peak areas could be obtained in this experiment (conducted at six of FAC doses (0-, 1-, 3-, 5-, 10-, and 15-equiv.) and three of pH conditions (pH 4.6, 7.5 and 11), respectively). Among the 18 peak areas, the highest peak area was defined as 100% of relative content, and the other peak areas were standardized referencing the highest one. The red color indicates high concentration, and the light blue color indicates low concentration. The relative content from 0 to 100% was divided into 10 ranks at 10% intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.5. Acute toxicity variation

After chlorination treatment of 4HB under different operating conditions, the acute toxicity of the reaction mixtures were preliminarily screened using *Photobacterium* bioassay. The acute toxicity changes of 4HB before and after chlorination treatment (1 h) under various dosages of FAC (1-, 3-, 5-, 10- and 15-equiv to 4HB) and pH (4.6, 7.5 and 11.0) are shown in Fig. 4. It can be observed that the toxicity decreased slightly when the FAC dosage increased from 0 to 3-equiv, followed by a sudden increase at the FAC dosage of 5-equiv, then decreased with the FAC increasing in acid system. And the toxicity increased and then decreased with the increasing FAC dosages in neutral system, while the acute toxicity increased straightly in alkaline system. In order to explore the relationship between the toxicity and products formation, one of intermediates P5 in alkaline chlorination system was prepared, and the acute toxicity was measured. Fig. S7 shows the toxicity results of 4HB and P5, the toxicity of P5 is much higher than 4HB, which imply that the formation of P5 has positive contribution to the toxicity increase in 4HB chlorination system. By analyzing the relationship between the toxicity and products distribution (Fig. S8), the formation of P5 has a significant contribution to the toxicity increase. Of course, the combinatorial effects of all the

products in the reaction mixtures would not be ignored.

3.6. Environmental significance

The previous experiments were conducted in pure water matrix, while if the transformation can be repeated in real ambient water matrix is still unknown. Therefore, two ambient water samples were collected from suburban Beijing, China. One sample was effluent of a sewage treatment plant (concentration of dissolvable organic carbon: 5.1 mg L⁻¹; concentration of ammonia nitrogen: 0.2 mg L⁻¹; pH: 8.2), and the other was lake water (concentration of dissolvable organic carbon: 12.6 mg L⁻¹; concentration of ammonia nitrogen: 0.1 mg L⁻¹; pH 7.7). The 4HB (1 μM) was spiked into water samples and chlorination disinfection experiments were conducted (FAC dose: 15-equiv, ca. 1.5 mg L⁻¹, similar to the FAC dose used in the chlorination disinfection practices; period: 1 h). As shown in Fig. 5, P5 was detected within both water samples after chlorination treatment, while P2 was only found in the lake water sample after chlorination treatment. The occurrence of chlorinated products indicated that the similar transformation mechanisms of 4HB can be repeated in practical water chlorination disinfection process, and the potential risk should be concerned due to the formation of toxic chlorinated products.

4. Conclusions

4HB can be rapidly transformed in chlorination disinfection treatment at various pH values and FAC dosages. Seven products were identified out of eight detected transformation products. Three types of reactions such as chlorination substitution, Baeyer-Villiger oxidation, ester hydrolysis were involved in chlorination disinfection process. The pH conditions affected the occurrence of reaction types, and FAC dosages affected the extent of reactions. Especially, the acute toxicity had a significant increase during the chlorination process, implying the formation of chlorinated products with higher toxicities relative to 4HB. The 3,5-dichloro-4-HB had positive contribution to the toxicity increase in chlorination system. Moreover, the transformation of 4HB had also been confirmed by the occurrence of major toxic chlorinated product 3,5-dichloro-4-HB in real chlorination disinfection practices. The present study would throw more light on the transformation characteristics of BPs UV filters in chlorination disinfection

Fig. 4. Acute toxicity of reaction mixtures of 4HB chlorination system at three pH values (pH 4.6; pH 7.5; pH 11.0).

Fig. 5. The major products detected within chlorination systems of ambient water matrices spiked with 4HB (Experimental conditions: 4HB: 1 μ M, FAC: 15 μ M; reaction time: 1 h; pH: 8.2 (effluent of sewage treatment plant) and 7.7 (lake water)).

practices, and provide a scientific evidence for optimizing the operating parameters for chlorination disinfection process.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.chemosphere.2016.04.005>.

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