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Chlorination of oxybenzone: Kinetics, transformation, disinfection byproducts formation, and genotoxicity changes

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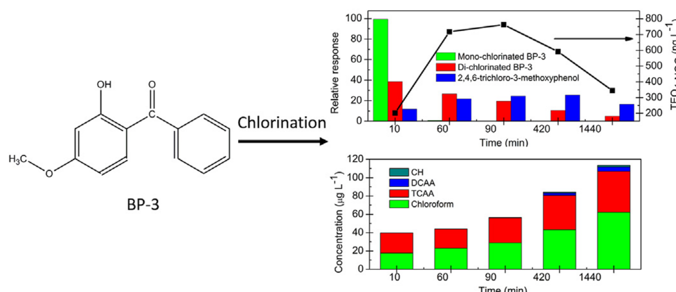
HIGHLIGHTS

- Oxybenzone reacted quickly with chlorine.
- Transformation product 2,4,6-trichloro-3-methoxyphenol was comparably stable.
- High amounts of chloroform and TCAA were formed during chlorination of oxybenzone.
- Significantly elevated genotoxicity was observed after chlorination of BP-3.

GRAPHICAL ABSTRACT



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ABSTRACT

UV filters are a kind of emerging contaminant, and their transformation behavior in water treatment processes has aroused great concern. In particular, toxic products might be produced during reaction with disinfectants during the disinfection process. As one of the most widely used UV filters, oxybenzone has received significant attention, because its transformation and toxicity changes during chlorine oxidation are a concern. In our study, the reaction between oxybenzone and chlorine followed pseudo-first-order and second-order kinetics. Three transformation products were detected by LC-MS/MS, and the stability of products followed the order of tri-chloro-methoxyphenoyl > di-chlorinated oxybenzone > mono-chlorinated oxybenzone. Disinfection byproducts (DBPs) including chloroform, trichloroacetic acid, dichloroacetic acid and chloral hydrate were quickly formed, and increased at a slower rate until their concentrations remained constant. The maximum DBP/oxybenzone molar yields for the four compounds were 12.02%, 6.28%, 0.90% and 0.23%, respectively. SOS/umu genotoxicity test indicated that genotoxicity was highly elevated after chlorination, and genotoxicity showed a significantly positive correlation with the response of tri-chloro-methoxyphenoyl. Our results indicated that more genotoxic transformation products were produced in spite of the elimination of oxybenzone, posing potential threats to drinking water safety. This study shed light on the formation of DBPs and toxicity changes during the chlorination process of oxybenzone.

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1. Introduction

UV filters are an important ingredient in personal care products to protect humans from sun exposure. They are increasingly used

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because of concern over UV irradiation and skin carcinoma. Apart from that, UV filters are also applied as additives in various plastic products to prevent yellowing and degradation (Zenker et al., 2008). UV filters frequently end up in aquatic environments as a direct or indirect consequence of human activities, directly through recreational activities (i.e., swimming in surface waters) and industrial wastewater discharges and indirectly through wastewater effluent containing UV filters (Santos et al., 2012).

Oxybenzone (BP-3) is one of the most frequently used benzophenone (BP) type UV filters. The additive amount of BP-3 in sunscreen products was reported to be as high as 10% (Kim and Choi, 2014). Many studies have confirmed the endocrine-disrupting effects of BP-3 both *in vivo* and *in vitro* (Schreurs et al., 2005; Díaz-Cruz and Barceló, 2009). Specifically, BP-3 showed significant estrogenic effects during *in vitro* studies on MCF-7 human breast cancer cells, and the relative estradiol (E2) effect can be as high as 105% (Schlumpf et al., 2001). After exposure to BP-3, significant induction of vitellogenin was observed in rainbow trout and Japanese medaka (Coronado et al., 2008). Considering the high K_{OW} value of BP-3 (Log $K_{OW} = 3.79$), it has the potential to accumulate in living creatures and pose potential threats to human health and other living creatures.

BP-3 has frequently been detected in aquatic environments, and ranks higher than other UV filters in terms of concentration level (typically in the magnitude of ng L^{-1} to μg L^{-1}) and detection frequency (Balmer et al., 2005; Kameda et al., 2011; Diaz-Cruz et al., 2012; Jurado et al., 2014; Tsui et al., 2014). One thing of great concern is that extraordinarily high levels of BP-3 were observed in tap water in Barcelona, Spain, with a maximum value of 363 μg L^{-1} (Diaz-Cruz et al., 2012). High levels of BP-3 were detected in waters used for recreational purposes. Up to 2013 ng L^{-1} BP-3 was detected in seawater near a popular beach in South Carolina, USA (Bratkovics and Sapozhnikova, 2011), and concentration in swimming pools in Greece and Germany was 2400–3300 ng L^{-1} (Lambropoulou et al., 2002), and 1200 ng L^{-1} (Zwiener et al., 2007), respectively.

Disinfection is the final protection of drinking water before it is distributed to consumers. It is effective in inactivating pathogens. However, the micropollutants which are not removed by the traditional drinking water treatment (coagulation, sedimentation, and filtration) are likely to be oxidized in the disinfection process (Westerhoff et al., 2005). Chlorine is the most commonly used disinfectant in the world. Despite its efficiency in killing pathogens, DBPs such as trihalomethanes (THMs) and haloacetic acids (HAAs) can be formed through the reaction between natural organic matter (NOM) and chlorine. Apart from the formation of DBPs from NOM, chlorine can also react with anthropogenic organic pollutants forming DBPs. Despite the fact that the concentration level of BP-3 should be low in aquatic environments, elevated levels have been observed in many waters used for recreational purposes. Thus, research on formation of DBPs is important for assessing water safety and human health impact. Moreover, other transformation products might also be formed during the chlorination process (Bedner and MacCrehan, 2005; Chen and Westerhoff, 2010; Tawk et al., 2014). In recent years, numerous studies have focused on the identification of transformation products from organic pollutants by chlorination, e.g., pharmaceuticals and personal care products (PPCPs), and possible implications to the aquatic environment since the overall toxicity of parent compounds after chlorination has been observed to increase (Hu et al., 2002; Tawk et al., 2014).

Chlorination behavior of BP-3 in aquatic environments has not caused concern until recently. Negreira et al. (2008) studied chlorination kinetics of BP-3 and identified transformation products by gas chromatography - mass spectrometry (GC-MS). The first-order and second-order kinetics and the influence of pH on the degradation behavior of BP-3 were comprehensively studied, and the chloroform formation potential was also investigated by Duirk et al. (2013). Zhuang et al. (2013) investigated stability of transformation products and acute toxicity changes using tests by *Vibrio fischeri*. Lately, Manasfi et al. (2015) studied the formation patterns of brominated products of BP-3 and bromoform formation in an artificial seawater swimming pool. Chlorination kinetics, transformation products, and genotoxicity changes of the compound 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid (BP-4), which shares similar structure to BP-3, was also evaluated (Negreira et al., 2012; Xiao et al., 2013). To our knowledge, no research has ever focused on the genotoxicity changes after chlorination of BP-3, and the relationship between transformation products and toxicity has not been investigated. The formation of other DBPs from chlorination of BP-3, such as HAAs, are not known. Thus, genotoxicity changes measured using SOS/umu test and DBP formation from chlorination were evaluated in this study, as well as degradation kinetics and product identification.

2. Materials and methods

2.1. Chemicals

Oxybenzone (>98%, analytical grade), sodium hypochlorite solution (available chlorine 4.00–4.99%), ammonium acetate for mass spectrometry (>99%, eluent addictive for LC-MS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). EPA 551A, 551B halogenated volatile mixture containing trihalomethanes (THMs), haloketones (HKs), haloacetonitriles (HANs), chloral hydrate (CH) and EPA 552.2 haloacetic acids mix were purchased from Supelco (Bellefonte, PA, USA). Methyl *tert*-butyl ether (MtBE) and methanol of HPLC grade were purchased from Fisher Scientific (Geel, Belgium) and J.T. Baker (USA), respectively. Stock solution of BP-3 was prepared in methanol with a concentration of 1 g L^{-1}. The pH value was adjusted by 10 mM phosphate buffer (mixture of 10 mM disodium hydrogen phosphate dodecahydrate and 10 mM sodium dihydrogen phosphate dihydrate) in this study, and determined by a pH meter (Thermo Scientific). Ultra-pure water used in the study was produced by a Mill-Q purifier (Millipore). All the glass vials were rinsed with acetone, methanol, and ultra-pure water in sequence before use.

2.2. Chlorination kinetics of BP-3

In order to have better knowledge of the chlorination rate of BP-3, the degradation kinetics experiment was conducted in a 150 mL amber glass bottle equipped with a small magnetic stir bar to ensure homogeneity of the solution. The experiment was carried out at room temperature (approximately 24 °C), and pH was controlled at 7 ± 0.1. The initial concentration of BP-3 was 1 mg L^{-1}, and different free chlorine doses were spiked to analyze the influence of chlorine dose on the reaction kinetics. In order to ascertain that the reaction was performed under pseudo-first-order conditions, the free chlorine dose was spiked at least ten times higher than that of BP-3 (the molar ratios of Cl_2/BP-3 = 9.6, 11.2, 12.8, 13.7, and 16.0, respectively). At given time intervals, 1 mL of aliquot was sampled, and transferred to a 2 mL injection vial containing excess $Na_2S_2O_3$ to quench the residual chlorine. The samples were analyzed by LC-MS/MS as soon as possible. The kinetic study was also conducted under pH 6 and 8 to assess the influence of pH on the reaction rate.

2.3. Chlorination products identification

The products identification experiment was carried out in a

150 mL amber glass bottle at pH 7 with the initial BP-3 concentration and chlorine dose of 1 mg L^{-1} and 3 mg L^{-1}, respectively. At different time intervals, 1 mL aliquot was sampled and transferred to the injection vial containing excess $Na_2S_2O_3$ to quench the residual chlorine. The samples were stored at 4 °C until LC-MS/MS analysis.

2.4. DBP formation test

The DBP formation test was performed in several 150 mL amber glass bottles under head-space free conditions (total water volume of about 170 mL). The temperature of the reaction was kept at 20 °C, and pH was controlled at 7 using phosphate buffer. The initial concentration of BP-3 was 1 mg L^{-1}, and 3 mg L^{-1} of chlorine was added to make sure residual chlorine was approximately 1 mg L^{-1} after 24-h incubation. Different incubation time was selected to trace DBPs formation. The residual chlorine was quenched using ten times' molar equivalent concentrations of $Na_2S_2O_3$. All the water samples were stored at 4 °C before the analysis of DBPs.

2.5. Sample pretreatment for SOS/umu test

One liter of incubated water at 20 °C and pH 7 with BP-3 concentration of 1 mg L^{-1} and chlorine dose of 3 mg L^{-1} was extracted using the automatic solid phase extractor (Reeko Auto SPE-06C, USA) with Oasis HLB (200 mg, Waters) column. Chlorine was quenched and the solution pH was readjusted to 2 by sulphuric acid before sample loading. The column was preconditioned with 15 mL methanol and 15 mL pH = 2 water subsequently, and the sample loading rate was controlled at 10 mL min^{-1}. After 1-h dry of the SPE column by nitrogen gas, 5 mL of dichloromethane and 5 mL solvent of methanol:dichloromethane = 1:9 were used to elute the accumulated compounds from the column, and the elute was collected. After evaporation of most of solvents, the solvent was exchanged to dimethyl sulphoxide (DMSO) for practice of SOS/umu test.

2.6. Analytical method

2.6.1. Instrumental analysis of BP-3 and degradation byproducts

BP-3 and its chlorination byproducts were detected using Triple Quad LC-MS/MS (LC 1290, MS 6460, Agilent Technologies) with a high-pressure binary pump, a thermostated LC column compartment, and an autosampler. The separation of chlorination products was accomplished by the LC system equipped with a C18 column (2.1 mm × 50 mm × 2.5 μm, Waters XBridge, Ireland), and the detection of BP-3 and products was performed by the MS system with an electrospray ion (ESI) source. The mobile phases were pure methanol and 0.5 mM ammonium acetate in ultra-pure water. The flow rate was set at 0.2 mL min^{-1}, and gradient elution procedures were as follows: methanol was 5% at first, increased to 80% in 1 min, increased to 100% in 2 min, decreased to 5% in 6.1 min, and kept for 0.4 min. Post-run time was 2 min. The temperature of the column was controlled at 30 °C. MS scanning in the positive or negative mode was used to identify the chlorination products, and multiple reaction mode (MRM) was used to quantify BP-3 and semi-quantify products. Two product ions were selected in the MRM mode.

2.6.2. Instrumental analysis of DBPs

Chloroform, chloral hydrate (CH) and chloropropanones were analyzed following USEPA method 551.1 (USEPA, 1995), and were determined by gas chromatography (Agilent 7890A, Santa Clara, USA) equipped with a DB-1 capillary column (30 m × 0.25 mm × 0.25 μm, Agilent, USA) and an electron capture detector (Agilent, USA). Chloroacetic acids (CAAs) were analyzed following USEPA method 552.3 (USEPA, 2003), and the instrument used was the same as that for chloroform, except that gas chromatography was equipped with a DB-1701 capillary column (30 m × 0.25 mm × 0.25 μm, Agilent, USA).

2.6.3. SOS/umu test

SOS/umu test was conducted to examine the genotoxicity changes in the chlorination process, and SOS/umu test was performed according to the method described in the previous report (Yan et al., 2014). Each sample was done in triplicates. DMSO and 4-nitroquinoline (4-NQO) were treated as negative and positive control, respectively. β-galactosidase activity was calculated according to the following equation: β-galactosidase activity (unit) = 1000 × (A420−1.75 × A570)/(20 × 0.0667 × A595). Here, A420, A570, and A595 were the absorbance at 420 nm, 570 nm, and 595 nm, respectively. The number 20 and 0.0667 represented reaction time and dilution factor, respectively. Induction ratio (IR) for 4-NQO and samples was achieved by dividing sample β-galactosidase activity by that of DMSO. A sample was considered positive only when a significantly linear relationship of dose-response was observed. Furthermore, 4-NQO equivalent genotoxicity can be quantitatively calculated by dividing the slope of the linear regression between response and the sample volume by the linear

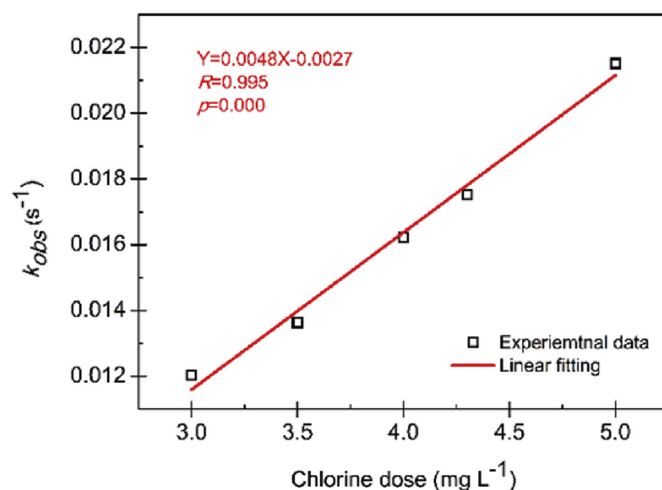

Fig. 1. Second-order reaction rate of BP-3 with chlorine at pH 7: $[BP\text{-}3]_0$ = 1 mg L^{-1}, [phosphate] = 10 mM.

Table 1
Parameters of pseudo-first-order reaction of BP-3 with different chlorine/BP-3 molar ratios at pH 7, and those at different pH conditions with chlorine/BP-3 molar ratio of 9.6.

pH = 7						$[Cl_2]$/BP-3 = 9.6			
$[Cl_2]$/BP-3	9.6	11.2	12.8	13.7	16.0	pH	6	7	8
k_{obs} (s^{-1})	0.012	0.014	0.016	0.018	0.022	k_{obs} (s^{-1})	0.002	0.012	0.024
$t_{1/2}$ (s)	43	38	33	31	26	$t_{1/2}$ (s)	286	43	16
R	0.996	0.997	0.999	0.999	0.999	R	0.991	0.996	0.997

k_{obs}: pseudo-first-order reaction rate coefficient; $t_{1/2}$: half-time; R: correlation coefficient.

regression between response and amount of 4-NQO.

3. Results and discussion

3.1. Chlorination kinetics of BP-3

In order to effectively understand the reaction rate of BP-3 with chlorine, the chlorination kinetic experiment was conducted under conditions where chlorine dose was greater than of BP-3. Parameters of the pseudo-first-order reaction of BP-3 with different chlorine doses at pH 7 are shown in Table 1. The pseudo-first-order kinetics for the degradation of BP-3 were observed with the correlation coefficients (R) all greater than 0.996 when BP-3 was treated with different chlorine doses at pH 7. BP-3 was degraded quickly by chlorine, and the half-time of BP-3 varied from 43 s to 26 s with chlorine doses ranging between 3 and 5 mg L^{-1}. Furthermore, the effect of pH on the chlorination rate of BP-3 was also evaluated, and the corresponding parameters are shown in Table 1. The pseudo-first-order degradation rate increased rapidly with the increasing pH, and the reaction rate under the chlorine dose of 3 mg L^{-1} was 0.13, 0.45, and 1.44 min^{-1} at pH 6, 7, and 8, respectively. Considering very similar pKa of BP-3 and chlorine (7.6 and 7.5, respectively), the higher reaction rate at higher pH might be attributed to the preferential reaction between non-protonated forms (Negreira et al., 2008). The elevated reaction rate at higher pH in this study was consistent with the findings by Negreira et al. (2008) and Duirk et al. (2013).

To investigate the influence of chlorine dose on BP-3 degradation efficiency, the relationship between the pseudo-first-order reaction rate coefficient (k_{obs}) and chlorine dose was analyzed, and a linear fitting line with $R > 0.995$ was achieved, indicating the reaction between BP-3 and chlorine corresponded to second-order-

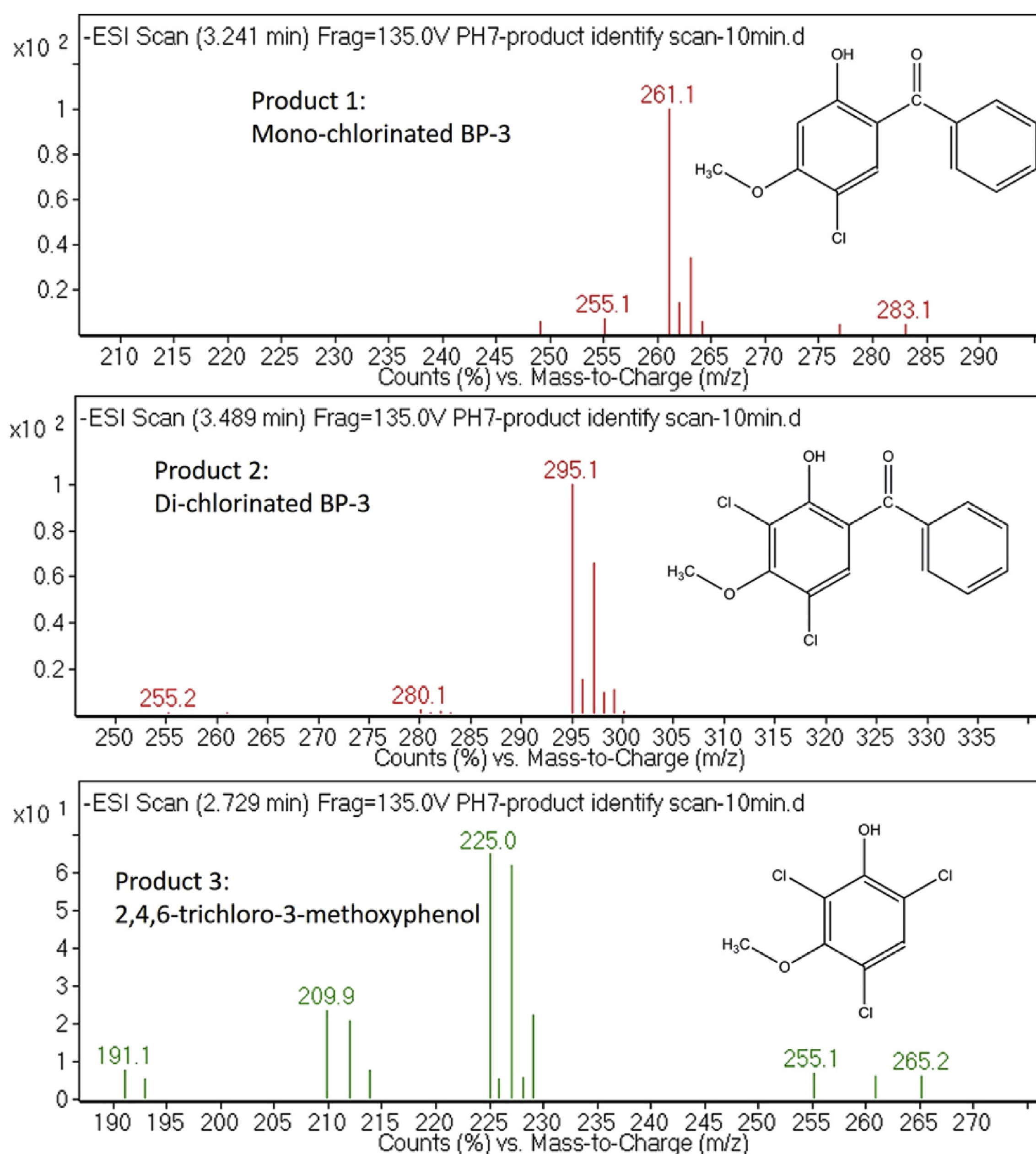

Fig. 2. MS spectra and corresponding structures of the chlorination products of BP-3.

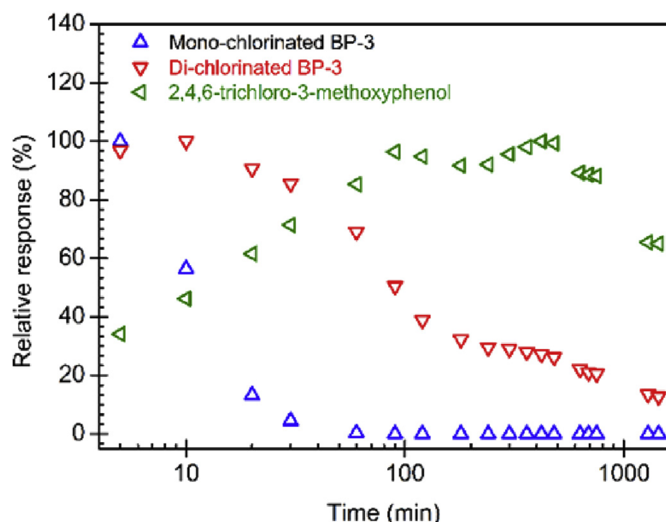

Fig. 3. Relative abundance of chlorination products over time. [BP-3] = 1 mg L^{-1}, [phosphate] = 10 mM, [Cl_2] = 3 mg L^{-1}, pH = 7.

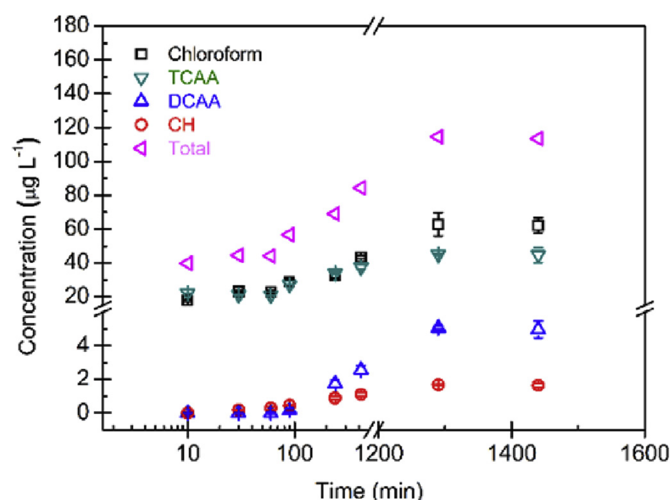

Fig. 4. Time trend of DBPs formation from chlorination of BP-3: [BP-3] = 1 mg L^{-1}, [phosphate] = 10 mM, [Cl_2] = 3 mg L^{-1}, pH = 7. Error bar represents standard deviation of three replicates.

kinetics with respect to the concentrations of BP-3 and chlorine (Fig. 1). The fitted second-order-kinetic rate was 342 L s^{-1} mol^{-1}.

3.2. Identification and stability of chlorination products

A total of 3 chlorination products were detected in the negative scan mode of LC-MS/MS, and no transformation products were detected under the positive scan mode. Their structures were proposed according to the MS spectrum, and spectra of these 3 products and corresponding structures are shown in Fig. 2.

As can be seen in Fig. 2, product 1 had isotopic m/z ratio of 3:1, which confirmed there was one chlorine in the structure. Together with m/z 261, it can be inferred that mono-chlorinated BP-3 was formed by electrophilic substitution of one hydrogen atom by chlorine atom. Both *para* and *ortho* position mono-chlorinated BP-3 were detected in the research carried out by Zhuang et al. (2013), so identification of only one product might be due to the difficulty of separating two products in this column. If only one compound was formed, the preferential substitution of chlorine occurred at *para* position because of the steric hindrance of *ortho* position (Manasfi et al., 2015). Product 2, with the isotopic ratio of 9:6:1 and a molecular weight of 262, was di-chlorinated BP-3 with another hydrogen being substituted by chlorine at *ortho* position because *meta* position is usually inactivated in the process of electrophilic substitution. The molecular weight of product 3 was 226, and had an isotopic ratio of 27:27:9:1, which was consistent with the characteristic of tri-chlorinated product. Product 3 was 2,4,6-trichloro-3-methoxyphenol, and it was formed by Baeyer-Villiger rearrangement of the carbonyl group of BP-3, hydrolysis, decarboxylation, and chlorination successively (Negreira et al., 2012; Xiao et al., 2014).

Because no commercial products of these chlorination products were available, the relative response of the quantitative ion in the MRM mode was used to trace the variation of these products during the 24-h reaction. The m/z used to quantify and semi-quantify these products in the MRM mode was selected based on the most abundant product ions in the product ion scan mode. The relative abundance of the 3 chlorinated products over time is shown in Fig. 3. Mono-chlorinated BP-3 formed quickly in large amounts in the first 5 min, and its following reduction conformed to pseudo-first-order kinetics with the half time of 4.6 min (not shown), which meant mono-chlorinated BP-3 was very unstable. The peak of di-chlorinated BP-3 reached at about 10 min, and its reduction rate was much slower than that of mono-chlorinated BP-3. Nearly 69% and 13% of di-chlorinated BP-3 still existed after the 1-h and 24-h reactions, respectively. Product 2,4,6-trichloro-3-methoxyphenol was steadily formed, and reached its peak after the chlorination period of 8 h. Then it slightly decreased, and 67% of 2,4,6-trichloro-3-methoxyphenol still remained after 24 h. Product 2,4,6-trichloro-3-methoxyphenol is a chlorination product that can remain for a long time in the chlorinated BP-3 aquatic system.

3.3. Formation of DBPs from BP-3

To evaluate DBPs formation from BP-3, concentrations of chloroform, chloral hydrate (CH), chloropropanones, and chloroacetic acids along the reaction time were measured. Among these DBPs, four DBPs including chloroform, CH, dichloroacetic acid (DCAA), trichloroacetic acid (TCAA) were detected. The individual and sum concentrations of these DBPs (total) over time were shown in Fig. 4. They showed an increasing trend in the first 20 h, and stayed stable afterward. The highest concentration of chloroform, TCAA, DCAA, and CH was 62.87, 44.94, 5.07, and 1.68 μg L^{-1}, respectively. In order to assess formation of DBPs from BP-3, the DBP/BP-3 molar yields

Table 2
Parameters of linear regression between water volume and induction ratio (IR).

	0 min	10 min	1 h	1.5 h	7 h	24 h
Equation	Y = 0.0098X + 0.5758	Y = 0.0381X + 0.523	Y = 0.1837X + 0.704	Y = 0.1888X + 0.7149	Y = 0.1509X + 0.807	Y = 0.0906X + 0.8482
R	0.911	0.954	0.997	0.991	0.995	0.972
p	0.089	0.046*	0.003**	0.009**	0.005**	0.028*

Y: IR; X: water volume (mL); *R*: correlation coefficient; *p*: significance value; *: significant correlation at 0.05 level; **: significant correlation at 0.01 level.

were calculated. The maximum DBP/BP-3 molar yields for the four compounds were 12.02%, 6.28%, 0.90%, and 0.23%, respectively. Duirk et al. (2013) previously reported a higher chloroform/BP-3 molar yield of 22.1%. Their experiment was conducted at pH 8 with a chlorine/BP-3 ratio of 50, while the pH condition in this study was 7 with a lower chlorine/BP-3 ratio of 10. These two different conditions might explain the formation difference of chloroform from BP-3. No research ever analyzed the formation of other chlorinated DBPs than chloroform from BP-3. Attention must be paid to the formation and high yields of DBPs (especially chloroform and TCAA) from BP-3 in the water used for recreational activities. Recently, occurrence of bromoform and bromal hydrate (BH) were reported in artificial bromide-rich seawater reacted with chlorine in a study by Manasfi et al. (2015).

3.4. *Genotoxicity evaluation of chlorination of BP-3*

As discussed previously, BP-3 was easily degraded by chlorine, and readily transformed into chlorination products including DBPs such as chloroform and TCAA, so it was of great importance to assess the toxicity changes by conducting a toxicology experiment. In this study, SOS/umu genotoxicity test was adopted to understand genotoxicity changes after chlorination of BP-3. The parameters of correlation between water volume and IR, and 4-NQO equivalent genotoxicity ($TEQ_{4\text{-}NQO}$) for different reaction periods were shown in Table 2 and Fig. 5 (A), respectively. Significantly positive correlations were observed between IR and water volume for samples after all the five different reaction periods ($p < 0.05$) except for original BP-3.

As was seen from Fig. 5 (A), BP-3 exhibited no genotoxicity, which was consistent with the earlier study in which BP-3 showed no genotoxic effect during both *in vivo* and *in vitro* tests. (Abramsson-Zetterberg and Svensson, 2011). Genotoxicity was highly elevated after chlorination of BP-3. Genotoxicity increased first, and then decreased. Among the selected time intervals, maximum $TEQ_{4\text{-}NQO}$ was reached at 1 h and 1.5 h with $TEQ_{4\text{-}NQO}$ of 718 ng L^{-1} and 763 ng L^{-1}, respectively. $TEQ_{4\text{-}NQO}$ still remained in a high level of 344 ng L^{-1} after 24-h chlorination. $TEQ_{4\text{-}NQO}$ level after chlorination of BP-3 (original concentration of 1 mg L^{-1}) in this study was one order of magnitude higher than that of cefazolin (original concentration of over 2 g L^{-1}) (Li et al., 2013), and was comparable with that in finished water from some drinking water treatment plants in China with total organic carbon (TOC) being 1–5 mg L^{-1} (Wang et al., 2011). However, $TEQ_{4\text{-}NQO}$ concentration three orders of magnitude higher (level of 10^2 μg L^{-1}) was observed in the reverse osmosis (RO) concentrate from a municipal wastewater reclamation reverse osmosis system (Sun et al., 2014). Genotoxicity change in this study was different from that tested by *Vibrio fischeri*, which showed toxicity after chlorination was in the same level as the original water sample (Zhuang et al., 2013). Thus, supplementary toxicity tests may be necessary to fully understand the potential toxic effect during transformation of compounds.

Genotoxicity difference was observed after different chlorination periods (Fig. 5A). In the first 1.5 h, mono-chlorinated BP-3 decreased to less than 1%, and the abundance of di-chlorinated BP-3 showed a decreasing trend while that of 2,4,6-trichloro-3-methoxyphenol kept increasing. This indicated that the increasing trend of genotoxicity might be related with that of 2,4,6-trichloro-3-methoxyphenol. The dominant role of 2,4,6-trichloro-3-methoxyphenol in determining genotoxicity could also be reflected from much lower $TEQ_{4\text{-}NQO}$ after 10-min of reaction (202 ng L^{-1}) when di-chlorinated BP-3 reached its maximum while 2,4,6-trichloro-3-methoxyphenol took a small proportion. As was shown in Fig. 5 (B), a significantly positive relationship between 2,4,6-trichloro-3-methoxyphenol response and genotoxicity was achieved.

It is worthwhile to note that the steady increase of chloroform and TCAA did not contribute significantly to the overall variance of genotoxicity compared with 2,4,6-trichloro-3-methoxyphenol. To evaluate genotoxicity of DBPs, genotoxicity test of THMs (mixture of chloroform, bromodichloromethane, dibromochloromethane, and bromoform) and HAAs (mixture of monochloroacetic acid, monobromoacetic acid, dichloroacetic acid, trichloroacetic acid, bromochloroacetic acid, bromodichloroacetic acid, dibromoacetic acid, chlorodibromoacetic acid, and tribromoacetic acid) was carried out respectively following the same procedure, and no genotoxicity was observed for either of them. Thus, THMs and HAAs were not accountable for genotoxicity changes. Actually, another compound with similar structure 2,4,6-trichloro-phenol, which is assigned as a probable carcinogen in humans by USEPA (http://www3.epa.gov/ttn/atw/hlthef/tri-phen.html), was proved to induce structural and numerical aberrations *in vitro* (Armstrong et al., 1993) and cause carcinogenesis by inducing point mutations in the somatic genome (Yin et al., 2009). Thus, there was possibility that 2,4,6-trichloro-methoxyphenol could also cause toxic effects. However, it was not clear that 2,4,6-trichloro-3-methoxyphenol was responsible for genotoxicity changes due to existence of unknown intermediate products. For example, the

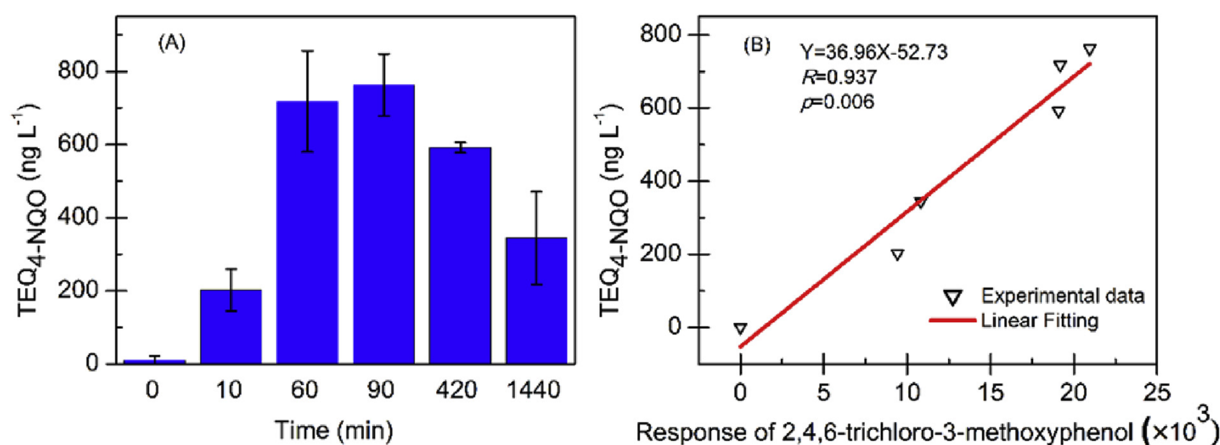

Fig. 5. Genotoxicity at different chlorination time of BP-3: [BP-3] = 1 mg L^{-1}, [phosphate] = 10 mM, [Cl_2] = 3 mg L^{-1}, pH = 7, and error bar represents standard deviation of three replicates (A); linear correlation between $TEQ_{4\text{-}NQO}$ and response of 2,4,6-trichloro-3-methoxyphenol (B).

corresponding ester products were not detected in the current study, but they were reported by Negreira et al. (2012) using LC-QqTOF-MS. This study was a preliminary investigation of the relationship between transformation products and toxicity, and further research needs to be done to find out which product is responsible for toxicity increase.

4. Conclusions

As one kind of emerging contaminants, UV filters are raising great concern. BP-3 is one of the most widely used UV filters in the world, and its behavior in the water treatment process has not received attention until recently. Although the kinetics study and product formation of BP-3 during chlorination has previously been studied, little effort was devoted to studying associated DBP formation and toxicity changes. This study investigated degradation kinetics, chlorination products, DBP formation, and genotoxicity changes. The degradation of BP-3 by chlorine occurred very quickly, and followed pseudo-first-order and second-order kinetics. Three chlorination products were detected by LC-MS/MS, and 2,4,6-trichloro-3-methoxyphenol was the most stable chlorination product. This result was of great concern because genotoxicity showed a significantly positive correlation with 2,4,6-trichloro-3-methoxyphenol response. Relatively high amounts of chloroform and TCAA were detected after 24-h chlorination of BP-3, and their maximum DBP/BP-3 molar yields were 12.02% and 6.28%, respectively. Our study strengthens the understanding of the fate of BP-3 during chlorination process, and provides evidence that chlorination products with comparatively higher genotoxicity can be formed, posing potential threats to drinking water safety and consumer health. Further research needs to be conducted to explore the relationship between products and toxicity changes.

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References

Abramsson-Zetterberg, L., Svensson, K., 2011. 4-methylbenzophenone and benzophenone are inactive in the micronucleus assay. Toxicol. Lett. 201, 235–239.
Armstrong, M.J., Galloway, S.M., Ashby, J., 1993. 2,4,6-trichlorophenol (TCP) induces chromosome breakage and aneuploidy in-vitro. Mutat. Res. 303, 101–108.
Balmer, M.E., Buser, H.-R., Müller, M.D., Poiger, T., 2005. Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from swiss lakes. Environ. Sci. Technol. 39, 953–962.
Bedner, M., MacCrehan, W.A., 2005. Transformation of acetaminophen by chlorination produces the toxicants 1,4-benzoquinone and n-acetyl-p-benzoquinone imine. Environ. Sci. Technol. 40, 516–522.
Bratkovics, S., Sapozhnikova, Y., 2011. Determination of seven commonly used organic UV filters in fresh and saline waters by liquid chromatography-tandem mass spectrometry. Anal. Methods 3, 2943–2950.
Chen, B.Y., Westerhoff, P., 2010. Predicting disinfection by-product formation potential in water. Water Res. 44, 3755–3762.
Coronado, M., De Haro, H., Deng, X., Rempel, M.A., Lavado, R., Schlenk, D., 2008. Estrogenic activity and reproductive effects of the UV-filter oxybenzone (2-hydroxy-4-methoxyphenyl-methanone) in fish. Aquat. Toxicol. 90, 182–187.
Díaz-Cruz, M.S., Barceló, D., 2009. Chemical analysis and ecotoxicological effects of organic UV-absorbing compounds in aquatic ecosystems. Trends Anal. Chem. 28, 708–717.
Diaz-Cruz, M.S., Gago-Ferrero, P., Llorca, M., Barcelo, D., 2012. Analysis of UV filters in tap water and other clean waters in spain. Anal. Bioanal. Chem. 402, 2325–2333.
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.
Hu, J.Y., Aizawa, T., Ookubo, S., 2002. Products of aqueous chlorination of bisphenol A and their estrogenic activity. Environ. Sci. Technol. 36, 1980–1987.
Jurado, A., Gago-Ferrero, P., Vàzquez-Suñé, E., Carrera, J., Pujades, E., Díaz-Cruz, M.S., Barceló, D., 2014. Urban groundwater contamination by residues of UV filters. J. Hazard. Mater 271, 141–149.
Kameda, Y., Kimura, K., Miyazaki, M., 2011. Occurrence and profiles of organic sunblocking agents in surface waters and sediments in Japanese rivers and lakes. Environ. Pollut. 159, 1570–1576.
Kim, S., Choi, K., 2014. Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: a mini-review. Environ. Int. 70, 143–157.
Lambropoulou, D.A., Giokas, D.L., Sakkas, V.A., Albanis, T.A., Karayannis, M.I., 2002. Gas chromatographic determination of 2-hydroxy-4-methoxybenzophenone and octyldimethyl-p-aminobenzoic acid sunscreen agents in swimming pool and bathing waters by solid-phase microextraction. J. Chromatogr. A 967, 243–253.
Li, L.P., Wei, D.B., Wei, G.H., Du, Y.G., 2013. Transformation of cefazolin during chlorination process: products, mechanism and genotoxicity assessment. J. Hazard. Mater 262, 48–54.
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., Canosa, P., Rodriguez, I., Ramil, M., Rubi, E., Cela, R., 2008. Study of some UV filters stability in chlorinated water and identification of halogenated by-products by gas chromatography-mass spectrometry. J. Chromatogr. A 1178, 206–214.
Negreira, N., Rodriguez, I., Rodil, R., Cela, R., 2012. Assessment of benzophenone-4 reactivity with free chlorine by liquid chromatography quadrupole time-of-flight mass spectrometry. Anal. Chim. Acta 743, 101–110.
Santos, A.J.M., Miranda, M.S., Esteves da Silva, J.C.G., 2012. The degradation products of UV filters in aqueous and chlorinated aqueous solutions. Water Res. 46, 3167–3176.
Schlumpf, M., Cotton, B., Conscience, M., Haller, V., Steinmann, B., Lichtensteiger, W., 2001. In vitro and in vivo estrogenicity of UV screens. Environ. Health Perspect. 109, 239–244.
Schreurs, R.H.M.M., Sonneveld, E., Jansen, J.H.J., Seinen, W., van der Burg, B., 2005. Interaction of polycyclic musks and UV filters with the estrogen receptor (ER), androgen receptor (AR), and progesterone receptor (PR) in reporter gene bioassays. Toxicol. Sci. 83, 264–272.
Sun, Y.X., Gao, Y., Hu, H.Y., Tang, F., Yang, Z., 2014. Characterization and biotoxicity assessment of dissolved organic matter in RO concentrate from a municipal wastewater reclamation reverse osmosis system. Chemosphere 117, 545–551.
Tawk, A., Deborde, M., Labanowski, J., Gallard, H., 2014. Chlorination of the β-triketone herbicides tembotrione and sulcotrione: kinetic and mechanistic study, transformation products identification and toxicity. Water Res. 76, 132–142.
Tsui, M.M.P., Leung, H.W., Lam, P.K.S., Murphy, M.B., 2014. Seasonal occurrence, removal efficiencies and preliminary risk assessment of multiple classes of organic UV filters in wastewater treatment plants. Water Res. 53, 58–67.
USEPA 551.1, 1995. Determination of Chlorination Disinfection Byproducts, Chlorinated Solvents, and Halogen Pesticides/Herbicides in Drinking Water by Liquid Extraction and Gas Chromatography with Electron-capture Detection. National Exposure Research Laboratory Office of Research and Development, Washington, DC.
USEPA 552.3, 2003. Determination of Haloacetic Acids and Dalapon in Drinking Water by Liquid-liquid Microextraction Derivatization and Gas Chromatography with Electron-capture Detection. Technical Support Center Office of Ground Water and Drinking Water, Washington, DC.
Wang, D.S., Xu, Z.Z., Zhao, Y.M., Yan, X.M., Shi, J., 2011. Change of genotoxicity for raw and finished water: role of purification processes. Chemosphere 83, 14–20.
Westerhoff, P., Yoon, Y., Snyder, S., Wert, E., 2005. Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes. Environ. Sci. Technol. 39, 6649–6663.
Xiao, M., Wei, D.B., Li, L.P., Liu, Q., Zhao, H.M., Du, Y.G., 2014. Formation pathways of brominated products from benzophenone-4 chlorination in the presence of bromide ions. J. Environ. Sci. 26, 2387–2396.
Xiao, M., Wei, D., Yin, J., Wei, G., Du, Y., 2013. Transformation mechanism of benzophenone-4 in free chlorine promoted chlorination disinfection. Water Res 47, 6223–6233.
Yan, Y., Jiang, W.W., Li, N., Ma, M., Wang, D.H., Wang, Z.J., Rao, K.F., 2014. Assessing of genotoxicity of 16 centralized source-waters in China by means of the SOS/umu assay and the micronucleus test: initial identification of the potential genotoxicants by use of a GC/MS method and the QSAR toolbox 3.0. Mutat. Research-Genetic Toxicol. Environ. Mutagen. 763, 36–43.
Yin, D.Q., Zhu, H.K., Hu, P., Zhao, Q.S., 2009. Genotoxic effect of 2,4,6-trichlorophenol on P53 gene in zebrafish liver. Environ. Toxicol. Chem. 28, 603–608.
Zenker, A., Schmutz, H., Fent, K., 2008. Simultaneous trace determination of nine organic UV-absorbing compounds (UV filters) in environmental samples. J. Chromatogr. A 1202, 64–74.
Zhuang, R.S., Zabar, R., Grbovic, G., Dolenc, D., Yao, J., Tisler, T., Trebse, P., 2013. Stability and toxicity of selected chlorinated benzophenone-type UV filters in waters. Acta Chim. Slov. 60, 826–832.
Zwiener, C., Richardson, S.D., Marini, D.M.D., Grummt, T., Glauner, T., Frimmel, F.H., 2007. Drowning in disinfection byproducts? Assessing swimming pool water. Environ. Sci. Technol. 41, 363–372.