Unexpected culprit of increased estrogenic effects: Oligomers in the photodegradation of preservative ethylparaben in water

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Abstract

Widespread occurrence of emerging organic contaminants (EOCs) in water have been explicitly associated with adverse effects on human health, therefore representing a major risk to public health. Especially the increased toxicity is frequently observed during the photodegradation of EOCs in natural water, and even wastewater treatment plants. However, the culprit of increased toxicity and formation mechanism has yet to be recognized regarding the estrogenic activity. In this study, by combining laboratory experiments with quantum chemical calculations, the induction of human estrogenic activity was investigated using the yeast two-hybrid reporter assay during the photodegradation of preservatives ethylparaben (EP), along with identification of toxic products and formation mechanisms. Results showed that the increase in estrogenic effect was induced by photochemically generated oligomers, rather than the expected OH-adduct. The maximum estrogenic activity corresponded to the major formation of oligomers, while OH-adducts were less than 12%. Two photochemically generated oligomers were found to contribute to estrogenic activity, produced from the cleavage of excited triplet state molecules and subsequent radical-radical reactions. Computational toxicology results showed that the increased estrogenic activity was attributed to oligomer [4-Hydroxy-isophthalic acid 1-ethyl ester 3-(4-hydroxy-phenyl)] and its EC50 was lower than that of the parent EP. In contrast, OH-adducts exhibited higher EC50 values than the parent EP, while still possessing estrogenic activity. Therefore, more attention should be paid to these photodegradation products of EOCs, including OH-adducts.

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1. Introduction

Due to the widespread occurrence of emerging organic contaminants (EOCs) in water, there has been much attention paid to their environmental behaviors and adverse effects on human health (Fenner et al., 2013). Especially it is frequently observed the increased toxicity during the photodegradation of EOCs in natural water (Gao et al., 2019; Rozas et al., 2016), and even in real effluent wastewater (An et al., 2015; Klamerth et al., 2010), although EOCs can be successfully photodegraded to negligible concentration. Therefore, the culprit of increased toxicity, and their formation mechanisms is of increasing concern during the photodegradation of EOCs in water.

Parabens are an important group of EOCs in aquatic environment, which for more than a century have been widely used as preservatives in food additives (Freese et al., 1973), cosmetics and pharmaceutical products (Bledzka et al., 2014; Boberg et al., 2010). In particular, parabens with short chain esters are the most commonly used, such as methylparaben, ethylparaben (EP), n-propylparaben and n-butylparaben (Soni et al., 2002). It has been estimated that the annual global consumption of parabens reaches 8000 tons (Ramaswamy et al., 2011). Accordingly, parabens are detected ubiquitously in various aquatic environments (e.g. wastewater, surface water, and drinking water) with the concentration range of 0.3–30,000 ng/L (Haman et al., 2015), and even human body samples (Guo and Kannan, 2013; Karthikraj et al., 2017; Paul et al., 2005; Raza et al., 2018). In particular, recent studies have suggested that parabens have the potential for carcinogenicity, teratogenicity and reproductive toxicology, as well as adipogenesis (Adoamnei et al., 2018; Darbre and Harvey, 2008; Fransway et al., 2019). Both in vivo and in vitro results show that parabens have the potential to exert estrogenic activity and could
bind to estrogenic receptors, causing unwanted effects at concentra-
tions far below those required to induce acute toxicity (Ramaswamy et al., 2011). Furthermore, adverse effects could be enhanced by photochemical irradiation of parabens (Fransway et al., 2019; Lee et al., 2017). Therefore, the photochemical degra-
dation mechanism of parabens in aquatic environments and the conse-
quence of these degradation mechanisms on health effects, are of high concern.

Photo degradation is an important process for the trans-
formation of EOCs in aquatic environments, due to bond breakage and the formation of new products (Gmurek et al., 2017). Recent research has suggested that the photochemical degradation of parabens occurs quickly with exposure to UV (Gmurek et al., 2015), UV-C supported oxidants (Dhaka et al., 2018), N-doped TiO2 (Petalà et al., 2015) and photocatalytic ozonation with O3/UV/ZnO (Asgari et al., 2019). Under optimum conditions, mainly hydroxylation and dealkylation products were reported during the photocatalytic and photocatalytic degradation of parabens (Petalà et al., 2015). Hydroxylated products (OH-products) were predicted by theoretical calculations, which were found to increase the aquatic toxicity of parabens during ‘OH-induced indirect degradation in aquatic environments (Gao et al., 2014, 2016). Subsequently, this finding was validated based on the phototoxicity of parabens measured during their photocatalytic degradation in water (Hu et al., 2019), with transformation products found to be potentially more refractory and toxic to aquatic organisms than the parent compound (Frontistis et al., 2017; Gao et al., 2016; Mendez-Arriaga et al., 2008; Olmez-Hanci et al., 2015).

Estrogenic activity during transformation has rarely been assessed. However, parabens have been associated with the occurrence of breast cancer due to their estrogenic activity. A previous study finding that estrogenic activity increased during the photolytic process of propylene in water (An et al., 2014), while decreasing during its photocatalytic degradation (Fang et al., 2013). This finding indicates that photolytic products are estrogenic and could enhance estrogenic activity, which has also been observed during the photolysis of other organic pollutants. For example, photochemical degradation products that exert estrogenic activity are formed from estrone and bisphenol A (Frontistis et al., 2017; Olmez-Hanci et al., 2015; Souissi et al., 2014), although no photochemical degradation products have been identified due to the poor separation technology of intermediates and the lack of standard samples.

Generally, ring-hydroxylated products are believed to be responsible for the increased estrogenic activity, due to the similarity of their structures with estradiol (Montero et al., 2019). Based on this, the ring-hydroxylated product, 3-hydroxy-propyl paraben (3-OH-PPB), was suspected to be responsible for the increased estrogenic activity observed during paraben photolysis (An et al., 2014), although this remains uncertain due to a lack of direct experimental evidence. In contrast, the decreased estrogenic activity was observed during the photocatalytic degradation of ethyl-
paraben (Frontistis et al., 2017) and propyl parabens (Fang et al., 2013), in which ring-hydroxylated products are the major products. These results indicate that unexpectedly, the ring-
hydroxylated products may not be a main contributor to the in-
crease in estrogenic activity.

In the present study, EP was selected as a typical paraben in aquatic environment, and the concentrations were detected as high as 0.15 and 9.9 μg/L in freshwater water and wastewater treatment plants (Haman et al., 2015), respectively. The estrogenic activity evolution was determined during the photo-
formation processes using the yeast two-hybrid reporter assay, with analysis of the factors responsible for the increase in estrogenic activity during the transformation process. Particularly, the degradation products were isolated at different times by semi-
preparative high-performance liquid chromatography and identi-
fied with high performance liquid chromatography quadrupole-
time of flight tandem mass spectrometry (HPLC-TOF-MS), with confirmation by comparison with commercially available. Furthermore, on the basis of the data from laser flash photolysis and quantum chemical calculation, the formation pathways of degradation product and tentative degradation mechanisms in water were proposed.

2. Materials and methods

2.1. Chemicals

EP was obtained from Tokyo Chemical Industry (Japan) (99% purity). Hydroxybenzoic acid was supplied by Adamas Reagent, Ltd. (purity > 99%). 3,4-Dihydroxy-benzoic acid ethyl ester was purchased from J&K Scientific (purity > 98%). Isopropanol and acetone were purchased from ANPEL Laboratory Technologies Inc. (Shanghai, China) (purity > 99%). Triethanolamine was purchased from General-reagent (Shanghai, China) (purity > 78%). The estrogenic activity assay kit was provided by the research center for eco-
vironmental sciences, Chinese Academy of Sciences. All solutions were prepared with high-purity deionized water (18.2 MΩ cm) (Millipore Corp., USA) and high-purity nitrogen (N2) was used in the specific experiment to investigate the reactive species in the photodegradation reaction systems.

2.2. Photochemical transformation experiments

The photochemical transformation of EP was performed in a cylindrical quartz reactor (diameter: 2.4 cm; height: 20 cm) with a double-walled cooling water jacket to maintain a constant temperature for solutions throughout all experiments. A 1000 W high-pressure mercury lamp (maximum emission wavelength of 365 nm) (Bilon, Inc., Shanghai, China) was used as a light source. After complete stirring, the lamp was turned on and 1.5 mL of reaction solution was collected at required time intervals for EP concentration analysis and the identification of photochemical degradation products. The pseudo-first-order rate constant (k) was used for the description of the photochemical degradation kinetics of EP. The total organic carbon (TOC) concentrations of the solutions were analyzed using a Shimadzu TOC-5000A TOC analyzer.

2.3. Analysis methods for EP concentration and transformation products

The EP concentration during photochemical degradation was determined by a high-performance liquid chromatography (HPLC) (Agilent 1260 series) with photodiode array detector, and an Agi-

lent C18 column (4.6 × 250 mm, 5 μm particle diameter) used for chromatographic separation at 25 °C. A 20 μL injection volume was applied with a mobile phase (0.5 mL min−1) consisting of 50% acetonitrile and 50% Milli-Q water. The detection wavelength was set to 255 nm.

The transformation products of EP were identified using HPLC-
TOF-MS (Agilent G6545B, USA). Approximately 10 μL sample vol-
umes were directly analyzed via an Agilent Eclipse Plus C18 column (2.1 × 50 mm, particle diameter of 1.78 μm) at the 0.2 mL min−1 eluent flow rate. The mobile phase consisted of acetonitrile (A) and Milli-Q water (B) with 0.07% acetic acid. The ratio of A:B was maintained at 1:9 for 2 min, changed linearly to 2:8 over 20 min and to 4:6 over 40 min, then maintained at 4:6 for 5 min. Subse-
sequently, the composition was returned to 1:9 over 45 min and maintained at this ratio for 5 min. The electrospray ionization (ESI)
mass spectrometry (MS) in the negative ion mode was operated with a fragmentor voltage of 175 V, with argon acted as a collision gas with various collision energies for the analysis of daughter ions.

2.4. Laser flash photolysis and scavenging experiments

Laser flash photolysis (LFP) was performed with a Nd:YAG laser, with a 266 nm laser pulse for a 5 ns duration with the energy of 10–15 mJ per pulse. The detection light source was a xenon lamp, with the laser and analytical light beam passing perpendicularly through a slit (1 × 10 mm) and then to a quartz cell (10 × 10 × 40 mm) for reaction. The transmitted light entered a monochromator equipped with an R955 photomultiplier. The output signals from the HP 54510 B digital oscillograph were recorded for further analysis. The assessments of all samples were performed immediately at 25 °C after preparation. For LFP, all experiments were carried out in an anaerobic environment, through deoxygenizing with high purity nitrogen.

The specific experiments were designed to investigate the contribution of the reactive species. For example, 100 mM of iso-propanol was added to quench the hydroxyl radicals (•OH), while the effect of oxygen (O2) was investigated by bubbling N2 into the solution at different O2 concentrations. Further, triethanolamine (TEOA, 10 mM) was used to quench the triplet excited state, while acetone (10%) was added to enhance the production of excited triplet states. Approximately 1 ml solution was sampled at various reaction time intervals to analyze the levels of the residual EP.

2.5. Estrogenic activity assessment

During the photochemical degradation of EP, estrogenic activity was evaluated using the yeast estrogenic assay according to previously described methods (Wang et al., 2010; Zhang et al., 2014). Briefly, the yeast cells were grown at 30 °C with continual agitation at 150–200 rpm for 24–48 h to achieve logarithmic growth. Approximately 20 μl of serial dilutions of chemicals were combined with 160 μl of the medium containing the yeast cells and 20 μl of blank medium. Subsequently, 200 μl of the test solution was transferred to a 96-well plate incubated at 30 °C for 4 h with 800 rpm in a thermo-shaker (Heidolph, incubator 1000, Germany). The cell density was assessed at 600 nm using ultraviolet spectrophotometry (Spectrophotometer, Unico UV-2000; Shanghai, China). Following this, 150 μl of the test solution was removed and different reagents were added to the remaining solution in the sequence as instructed by the kit manufacturer’s instructions (State Key Laboratory of Environmental Aquatic Chemistry, China). Finally, the solution absorbance was recorded at 420 nm. Each sample was analyzed in quadruplicate and each assay was carried out more than three times.

2.6. Quantum chemistry calculations and computational toxicology

The electronic structures of EP and its products were optimized using Gaussian 09 software (Frisch et al., 2009). The hybrid density functional B3LYP method with the 6-31G(d,p) basis set was used, and the solvent effect was simulated using the continuum solvation model (CPCM). Moreover, the vibrational frequencies of EP and its products were calculated at the same level to identify the most stable of all optimized structures. In addition, the potential estrogenic effects of EP and its photochemical degradation products were theoretically evaluated using the VirtualToxLab package based on multi-dimensional quantitative structure-activity relationship, as early described in references (Vedani et al., 2012, 2015; Vedani and Smiesko, 2009). The binding affinity to the estrogen receptors was expressed as the 50% inhibitive concentration (IC50 value).

3. Results and discussion

3.1. The evolution of estrogenic activity during photochemical degradation

The photochemical degradation of EP in water was performed both under visible light and UV irradiation, with the results illustrated in Fig. 1. Approximately 500 μM EP was found to be completely removed under UV irradiation within 90 min, while only 10% was photochemically degraded under visible light irradiation at the same concentration. The data suggested that EP could be rapidly degraded in water under UV light exposure. Furthermore, the curves of the photochemical degradation of EP fitted well with the pseudo-first-order kinetics equation (inset of Fig. 1) and a rate constant of 6.70 ± 0.76 × 10⁻² min⁻¹ was obtained with a half-time of 10.2 min.

Furthermore, the evolution of estrogenic activity during the photochemical degradation of EP was also evaluated with the yeast two-hybrid reporter assay transfected with the human estrogen receptor α (a Saccharomyces cerevisiae-based lac-Z (β-galactosidase)). Firstly, this method was performed to estimate the estrogenic activity of E2, an established natural estrogen with very potent estrogenic activity. The deduced EC50 value was 2.04 ± 0.15 × 10⁻¹⁰ M for E2 (Fig. S1), which is consistent with previous observations (EC50 2.3 × 10⁻¹⁰ M) (Gaido et al., 1997), suggesting the reliability of this method for evaluating the estrogenic activity. Furthermore, the estrogenic activity evolution during the photochemical degradation of EP was also investigated as shown Fig. 2. It is noticeable that the estrogenic activity initially increased and then decreased, with maximum estrogenic activity at 40 min of irradiation. This phenomenon indicated that the photochemical products had higher estrogenic activity than the original compound EP and that products might include hydroxylated parabens (OH-EP). It is generally thought that OH-adducts on a benzene ring might increase the estrogenic activity of the original organic pollutants. It has previously been reported (Mboula et al., 2015), that the phenol group was not destroyed during the photocatalysis of E2 and the estrogenic effect remained in the corresponding solution. The persistently existed estrogenic activity during the photocatalysis is believed to be owing to the presence of the phenol group in the intermediates. Thus, the OH-adduct products are considered to be a possible reason for the increase of estrogenic activity.

During the photochemical degradation of EP in water, the typical OH-adduct (3,4-Dihydroxy-benzoic acid ethyl ester, or 3-(Fig. 1. The photochemical degradation curve and TOC evolution of EP degradation (500 μM) with 1000W UV light and visible light. The inset shows a plot of pseudo-first-order rate constant vs. EP concentration.)
OH-EP (for short), was indeed detected by HPLC-TOF-MS and confirmed by comparison with standard substances (Fig. 3). However, it was observed that the evolution of estrogenic activity in the degradation solution was inconsistent with the formation of OH-adducts during the photochemical degradation of EP. The peak area of 3-OH-EP initially increased, reaching a maximum at 20 min of irradiation, while the estrogenic activity was slightly reduced in comparison with the original EP (Fig. 2). During the photochemical degradation reaction, the OH-adduct was further decomposed and almost completely depleted (<12%) after 40 min of irradiation, while the solution continued to exhibit maximum estrogenic activity. Thus, these data implied that the OH-adduct 3-OH-EP was not responsible for the increase in estrogenic activity during the photochemical degradation of EP in water.

In order to confirm the estrogenic activity of OH-adducts, the dose-response curves were established for 3-OH-EP and EP as a control (Fig. S2). The EC50 value of 3-OH-EP was found to be 2.32 × 10⁻⁴ M, which is an order of magnitude higher than that of EP (1.35 × 10⁻⁵ M) (Fig. S3). Therefore, it can be concluded that the estrogenic activity of the OH-adduct 3-OH-EP is lower than that of the parent compound EP. This finding further confirmed the above-mentioned experiment that the increase in estrogenic activity during the photochemical degradation of EP could not be attributed to the formation of the OH-adduct product, 3-OH-EP. Therefore, in order to identify the core mechanism, it is essential to identify the photochemical products formed during the degradation of EP in water, especially the degradation products at 40 min of irradiation.

### 3.2. Identification of photochemical transformation products

In order to explore the direct cause for the increase in estrogenic activity during the photochemical degradation of EP, further analysis of the photochemical degradation products was performed. Total ion current (TIC) chromatograms and the fragmentation patterns of each product are shown in Figs. S4 and S5, respectively. Also, the structures of degradation intermediates are summarized in Table S2. As shown by the identified products after 40 min of irradiation (Fig. 3), four main chromatographic peaks were obtained, with the retention times of 10.6, 32.0, 34.9, and 53.3 min, respectively. The peak at 34.9 min was identified as the parent EP compound by LC-TOF-MS analysis and comparison with standard substances. Similarly, the transformation product A with a peak at 10.6 min was identified as p-hydroxybenzoic acid (HB). Based on the measurement of estrogenic activity (Fig. S6) and previously reported literatures (Watanabe et al., 2013; Zhu and Wei, 2019), HB was confirmed as an inactive endocrine component, and therefore, the increase in estrogenic activity cannot be attributed to HB formation.

Accordingly, the remaining degradation products B (32.0 min) and C (53.3 min) were investigated more thoroughly. As shown in Fig. 2, the peak areas of products B and C increased initially and then declined with the photochemical transformation of EP. Products B and C were the main components in the degradation solution at 40 min and therefore, might account for the increase in estrogenic activity. The structures of B and C were inferred from the results of HPLC-TOF-MS analysis (Fig. S5-B, C). Product B had a [M-H]- of 301.0720 and the molecular formula of C16H14O6. Analysis of the fragment ions m/z 273.0405, 229.0506 and 185.0608, indicated the loss of an –COO group. Also, the mass difference between the fragment ion m/z 273.0405 and parent ion m/z 301.0720 matched with the loss of C2H4. Therefore, product B was rationally identified as the photochemically generated oligomer 4-Hydroxy-isophthalic acid 1-ethyl ester 3-(4-hydroxy-phenyl)-ethyl-[COO group. Also, the mass difference between the fragment ion m/z 273.0405 and parent ion m/z 301.0720 matched with the loss of C2H4. Therefore, product B was rationally identified as the photochemically generated oligomer 4-Hydroxy-isophthalic acid 1-ethyl ester 3-(4-hydroxy-phenyl) (Fig. 2). Similarly, product C had a [M-H]- of 329.1028 and the molecular formula of C16H14O6 containing two EP structures. Therefore, product C was also identified as the photochemically generated oligomer 4-Hydroxy-3-[2-(4-hydroxy-phenoxyacarbonyl)-ethyl]-benzoic acid ethyl ester (Fig. 2).

Furthermore, the predicted estrogenic activity (IC50) of the product B was calculated as 1.29 μM, which was lower than that of the original EP, indicating that the estrogenic activity of the product B was higher than that of the parent compound. Thus, the adverse effects on human health involving estrogenic activity of the two kinds of degradation products B and C should pay more attention in the further study.
3.3. Contributions of reactive species

In order to better understand the formation mechanism of these toxic products during the photochemical transformation of EP in water, it is necessary to comprehensively assess the dominant reactive species and their contribution to EP photochemical degradation. The specific scavengers’ experiments were sophisticatedly designed, and the degradation kinetics curves in the presence and absence of scavengers are demonstrated in Fig. 4. The experimental conditions, purpose, pseudo-first-order rate constant \( k \) and half-life for EP under different set of experimental condition are summarized in Table S1. Firstly, the photochemical degradation of EP in water was not suppressed with addition of isopropanol (the \(^*\)OH scavenger), suggesting that \(^*\)OH was not the main reactive species involved in the photochemical transformation of EP. However, the transformation of EP was clearly suppressed with addition of TEOA, which served as a quencher of the excited state (\(^*\)EP\(_t\)). That is, the rate constant remarkably reduced from 0.067 min\(^{-1}\) (no scavenger) to 0.005 min\(^{-1}\), indicating that the excited molecular species (\(^*\)EP\(_t\)) devoted to 75% of the photochemical degradation of EP in water, and that the reaction might be mostly induced by the excited triplet state (\(^*\)EP\(_t\)). Moreover, \( O_2 \) is an established efficient quencher of the excited triplet state molecules, as well as being capable of reacting with excited triplet state \(^*\)EP\(_t\), leading to the generation of oxygen-containing reactive species including superoxide anions (\( O_2^- \)) and singlet oxygen (\(^*\)O\(_2\)). Therefore, the exclusion of \( O_2 \) was performed by bubbling \( N_2 \) through the solution, to explore the roles of excited triplet state and oxygen-containing reactive species. In the absence of \( O_2 \), the rate constant dramatically increased to a maximum of 0.127 min\(^{-1}\), which was 2 times higher than that in the presence of \( O_2 \). This further indicated that the contribution of the excited triplet state molecule was more important than that of oxygen-containing reactive species, confirming the significant role of excited triplet state \(^*\)EP\(_t\). To further establish the role of \(^*\)EP\(_t\) in this system, 10% (volume fraction) acetone was introduced as a sensitizer of triplet state, resulting in a rate constant of 0.324 min\(^{-1}\), which was increased by nearly 4-fold compared to the EP solution without acetone. This result further confirmed that excited triplet state \(^*\)EP\(_t\) was the main contributor of the photochemical degradation of EP.

3.4. Transient intermediates and photochemical degradation mechanism

The transient intermediates and the degradation mechanisms in water were further investigated using laser flash photolysis. As Fig. 5 shows, two characteristic absorption peaks were observed at 320 and 640 nm. According to the observation of similar spectra of the photochemical degradation of various structural analog (Cheng et al., 2009; Zhang et al., 2019), the absorption peak in the region 600–800 nm corresponded to hydrated electrons and the maximum absorption at 320 nm was tentatively attributed to several transient intermediates, including \(^*\)EP\(_t\), phenoxyl radical (\(^*\)EP–H) and the radical cation (EP\(^{+}\)). Unlike the \(^*\)EP\(_t\), the transient intermediates "EP–H and EP\(^{+}\)" could not be quenched by \( O_2 \), instead enhancing the formation of EP\(^{+}\) by acting as an electron acceptor. Therefore, for this reaction to occur, the absorption peak at 320 nm should be enhanced, although it disappeared completely in the presence of \( O_2 \) (Fig. S7). This observation indicated that the "EP–H and EP\(^{+}\)" could not be generated and that the absorption peak at 320 nm could be attributed only to \(^*\)EP\(_t\). Furthermore, this conclusion was confirmed by the exclusion of \( O_2 \) from solution with \( N_2 \) bubbling (Fig. S7), with the direct evidence from these assays indicating that only \(^*\)EP\(_t\) initiated the photochemical degradation of EP. As shown from Fig. 5, the transient intermediate \(^3\)EP\(_t\) was generated and then increased within the short time period of 35 ns, followed by rapid decay. In order to investigate the subsequent transformation mechanism in water, quantum chemical calculations were also performed and the reaction energies of all possible pathways for bond cleavage are shown in Fig. S8. The calculated results show that only the cleavage of the O–CH\(_2\)CH\(_3\) bond was an exothermic process with a reaction energy \( \Delta G \) of \(-5.86 \text{ kcal/mol} \), while the energies of other bonds cleavage reactions were endothermic with positive \( \Delta G \) values of 1.15–27.36 kcal/mol. These data indicated that the O–CH\(_2\)CH\(_3\) bond of \(^3\)EP\(_t\) was more likely to be broken than other bonds, leading to the formation of transformation products in water.

Based on the aforementioned results, the photochemical degradation mechanism in water was proposed as shown in Fig. 6. The excited singlet state of EP (\(^1\)EP\(_t\)) was initially formed under EP irradiation and then further transformed to an excited triplet state.
due to instability of $^{3}\text{EP}^{\ast}$. Furthermore, the transformation of $^{3}\text{EP}^{\ast}$ mainly occurred via the cleavage of the $\text{O}−\text{CH}_2\text{CH}_3$ bond, resulting in the formation of carboxylic radicals. Simultaneously, $^{1}\text{O}_2$ can be generated from $\text{O}_2$ in aerobic environments, with $^{1}\text{O}_2$ formation was confirmed by electron paramagnetic resonance (EPR) (Fig. S9). The transformation product 3-OH-EP was formed through the reaction of EP with $^{1}\text{O}_2$, rather than via a reaction with $\bullet\text{OH}$. In addition to the formation of HB with non-estrogenic activity, the carboxylic radicals could result in the formation of oligomers B and C, causing an increase in estrogenic activity during the photochemical transformation of EP in water systems.

4. Conclusions

Understanding the photochemical transformations of EOCs in water and the consequential effect on the adverse effects to human health, is highly significant. Therefore, using a typical preservative EP as an example, the evolution of estrogenic activity during photochemical degradation in water was studied, with results showing that the estrogenic activity increased with ongoing degradation duration. Interestingly, the expected OH-adduct was found not to be responsible for the increase in estrogenic activity, exhibiting an EC$_{50}$ value an order of magnitude higher than that of the original EP parent compound. This result provides new insights into the estrogenic activity of OH-adducts, altering the conventional understanding that the OH-adduct is responsible for the increase in estrogenic activity as compared with the original parent compound. These results explain previous results showing the continual decrease of estrogenic activity during the photocatalytic degradation of parabens in water, due to the main product being OH-adducts. In contrast, increased estrogenic activity during photochemical degradation is due to the formation of photochemically generated oligomers in water.

Despite the recent increase in research interest into the degradation products of EOCs in water, several gaps in knowledge remain regarding their adverse effects and the specific identification of products inducing an increase in toxicity. The limited available literature currently suggests that transformation products could be persistent and retain toxicity after the original EOC is eliminated completely in water. Thus, the environmental and health impact of degradation products together with the original parent EOCs in water should be paid more attention in future studies and risk assessments.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors appreciate the financial supports from National Natural Science Foundation of China (41425015, 41977365, and 41731279), Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program (2017BT01Z032), Natural Science Foundation of Guangdong, China (2016A030310120), Science and Technology Program of Guangzhou, China (201804010128), and Leading Scientific, Technical and Innovation Talents of Guangdong Special Support Program (2016TX032094). We want to thank the support from National Supercomputer Center in Guangzhou.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2020.115745.

References


