

EXPERIMENTAL AND THEORETICAL INSIGHTS INTO PHOTOCHEMICAL TRANSFORMATION KINETICS AND MECHANISMS OF AQUEOUS PROPYLPARABEN AND RISK ASSESSMENT OF ITS DEGRADATION PRODUCTS

TAICHENG AN,^{*†} HANSUN FANG,^{†||} GUIYING LI,[†] SHILONG WANG,[‡] and SIDE YAO[§][†]State Key Laboratory of Organic Geochemistry and Guangdong Key Laboratory of Environmental Resources Utilization and Protection, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, People's Republic of China[‡]School of Life Science and Technology, Department of Chemistry, Tongji University, Shanghai, People's Republic of China[§]Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai, People's Republic of China^{||}University of Chinese Academy of Sciences, Beijing, People's Republic of China

(Submitted 9 January 2014; Returned for Revision 4 April 2014; Accepted 29 April 2014)

Abstract: The kinetics and mechanisms of ultraviolet photochemical transformation of propylparaben (PPB) were studied. Specific kinetics scavenging experiments coupled with quantum yield determinations were used to distinguish the roles of various reactive species induced by self-sensitized and direct photolysis reactions, and the excited triplet state of PPB (³PPB*) was identified as the most important species to initiate the photochemical degradation of PPB in aquatic environments. The computational results of time-resolved absorption spectra proved that ³PPB* is a highly reactive electron acceptor, and a head-to-tail hydrogen transfer mechanism probably occurs through electron coupled with proton transfer. Physical quenching by, or chemical reaction of ³PPB* with, O₂ was confirmed as a key step affecting the initial PPB transformation pathways and degradation mechanisms. The transformation products were identified and the toxicity evolutions of PPB solutions during photochemical degradation under aerobic and anaerobic conditions were compared. The results indicate that anaerobic conditions are more likely than aerobic conditions to lead to the elimination and detoxification of PPB but less likely to lead to PPB mineralization. *Environ Toxicol Chem* 2014;33:1809–1816. © 2014 SETAC

Keywords: Parabens Environmental transformation Photoenhanced toxicity Endocrine disruptors Ecotoxicology assessment

INTRODUCTION

Parabens are widely used antimicrobial preservatives of commercial products such as food, cosmetics, and pharmaceuticals [1], and they are therefore classed as pharmaceutical and personal care products (PPCPs). Although some of them have short half-lives, their continual use and release make them pseudo-persistent, and they are frequently detected in environmental matrices, including the atmosphere, water, and soil [2]. Parabens are characterized by their ester chains and include methyl-, ethyl-, propyl-, and butyl-paraben. They have been found to be endocrine-disrupting chemicals with a median effective concentration (EC₅₀) of approximately 10⁻⁶ M [3]. Thus, the conclusion can be drawn that parabens may pose some risks to human health and aquatic ecosystems.

The photochemical process generally consists of a series of free reactive species and energy transfer processes [4]. Different photolysis systems lead to specific initial reactions and degradation pathways, which determine the degradation kinetics and composition as well as the yield of photoproducts. In addition to biodegradation, it is well known that photons play an important role in the transformation of PPCPs, in either natural or artificial aquatic environments. Moreover, ultraviolet (UV) irradiation is commonly used to disinfect food and cosmetics [5], resulting in the photolysis of additives such as parabens in commercial products. It is therefore important to investigate the photo-

transformation kinetics, photoproducts, and mechanisms involved in the photolysis of parabens to help understand their fate and the risks they pose. To the best of our knowledge, only a few studies have investigated the photochemical transformation of parabens. Two of them focused mainly on the photosensitized degradation of butylparaben using simulated solar radiation as the light source without considering the photolytic process initiated by absorbing photons directly, and singlet oxygen (¹O₂) was thought to play a major role in butylparaben transformation [6]. The other studies reported the degradation kinetics of butylparaben [7]. However, the light screen or photosensitization effects on degradation kinetics raised by environmental factors, and the initial reactions involved in the photochemical transformation pathways and mechanisms, especially for photolytic pathways when parabens are absorbing photons directly, have not yet been investigated in detail. The transformation products and the fate of parabens under UV irradiation, as well as the risks posed by the photochemical transformation, have never been evaluated, and the estrogenic effects of parabens and their degradation products have not been studied.

In the present study, the kinetics and mechanisms involved in the photochemical transformation of parabens were studied by using propylparaben (PPB). The contributions of various reactive species were distinguished based on specific kinetics scavenging experiments and measured quantum yields. The transformation mechanisms initiated by self-sensitized and direct photochemical processes are proposed. The main species involved was identified by laser flash photolysis as the excited triplet state of PPB (³PPB*), which was confirmed by transient spectra from the theoretical calculations. Finally, the estrogenic activity and the acute toxicity of PPB as well as its

All Supplemental Data may be found in the online version of this article.

* Address correspondence to antc99@gig.ac.cn

Published online 2 May 2014 in Wiley Online Library
(wileyonlinelibrary.com).

DOI: 10.1002/etc.2632

phototransformation products at different time intervals were compared under aerobic and anaerobic conditions to gain insight into the role of O₂ in the photochemical transformation of PPB in water.

MATERIALS AND METHODS

Materials

Propylparaben (99% pure; see structure in Figure 1) and anthracene (>97% pure) were purchased from Tokyo Chemical Industry, 4-hydroxybenzoic acid (99% pure) was purchased from Adamas Reagent, and all other reagents were analytical grade used as received. Luminescent bacteria (*Photobacterium phosphoreum*) were obtained from the Institute of Soil Science, Chinese Academy of Science. All solutions were prepared using high-purity deionized water (18 MΩ cm; Millipore).

Apparatus and methodology for photochemical studies

Details of the kinetics and scavenging experiments of the photochemical transformation of PPB are summarized in the Supplemental Data. The effects of 4 environmental variables (concentrations of humic acid [HA], Cl⁻, NO₃⁻, and Ca²⁺) on photochemical degradation were studied using the center composite design (Supplemental Data, Table S1). The transient spectra of the intermediates produced were studied using laser flash photolysis methods, as described in Wang et al. [8].

The quantum yield of intersystem crossing of PPB (Φ_{isc}^{PPB}) was determined based on the energy transfer method by introducing anthracene (Ant) as the energy acceptor [9]. The fluorescence quantum yield (Φ_f^{PPB}) was determined from the corrected fluorescence spectra using tryptophan [10]. All detailed methods on quantum yield determination are summarized in the Supplemental Data.

Analysis methods

The absorptive ultraviolet/visible (UV/Vis) spectra were analyzed with a Hengping 756PC UV spectrophotometer, and the irradiance spectrum of light was measured with a spectrometer (USB 2000+, Ocean Optics). The fluorescence intensity of 100 μM PPB with a specific anion was determined by a fluorescence spectrophotometer with excitation wavelength at 255 nm (Zolix Instruments). The apparatus and detailed methods of high-performance liquid chromatography and ultra-performance liquid chromatography–tandem mass spectrometry are summarized in the Supplemental Data.

Computational methods

All calculations were performed using Gaussian 03 software [11], which was employed to calculate the triplet state energies, the vertical ionization energies, and the transient absorption spectra. The geometries and frequencies of the stationary points were determined using the B3LYP/6-31g(d,p)

basis set. Time-dependent density functional theory at the B3LYP/6-311++g(d,p) level was used to calculate the energies and intensities of the transition spectra, and the UV/Vis spectra were simulated using SWizard software [12].

Acute toxicity and the estrogenic activity assay

After *P. phosphoreum* was exposed to a treated solution for 15 min, the acute toxicity was assayed using a toxicity analyzer (Dxy-3; Nanjing Kuake) [13], and the evolution of the estrogenic activity during the photochemical transformation of PPB was investigated using an estrogenic activity assay kit, measuring the β-galactosidase activity of a recombination yeast cell based on a published method [14].

RESULTS AND DISCUSSION

Photochemical transformation kinetics

To model the photochemical transformation of PPB in natural waters under UV light, different concentrations of HA, Cl⁻, NO₃⁻, and Ca²⁺ were selected to optimize the photochemical degradation of PPB using the center composite design. Based on the complete experimental design matrix and the response factor (Supplemental Data, Table S2), a semiempirical expression (Equation 1) consisting of 15 statistically significant coefficients is obtained as follows

$$Y = \frac{1}{1000} (84 - 3.168A + 7.879B + 1.663C - 17D + 0.163AB + 5.91AC + 0.564AD - 3.39BC + 0.224BD + 1.516CD + 0.62A^2 - 4.245B^2 + 1.24C^2 + 5.464D^2) \quad (1)$$

where Y represents the photochemical degradation rate constant of PPB, and A , B , C , and D represent the concentrations of Cl⁻, NO₃⁻, Ca²⁺, and HA, respectively. The residual plot (Supplemental Data, Figure S1) shows that the model predicted the degradation rate constant of PPB very well. A Pareto chart (Supplemental Data, Figure S2) was also obtained according to the model and showed that HA and Cl⁻ concentrations affected the photochemical degradation of PPB with efficiencies of negative 62.5% and negligible 2.2%, respectively, whereas the NO₃⁻ and Ca²⁺ concentrations affected the photochemical degradation with efficiencies of positive 13.4% and negligible 0.6%, respectively. The 2-dimensional contour (Supplemental Data, Figure S3) of the 2 significant components, HA and NO₃⁻, indicated that: Increasing HA concentration would constantly depress the degradation rate constant, and NO₃⁻ would also display a negative effect toward k_1 (pseudo-first-order rate constant) when its concentration exceeded 0.70 mM. The absorption spectra of the 4 components and the emission spectra of the light source (Supplemental Data, Figure S4) showed that PPB mainly absorbed photons in the UVC region (200–300 nm), and all 4 investigated components could compete for photons with PPB. Interestingly, HA and NO₃⁻ were 2 common photosensitizers: The former would produce HO[•], ¹O₂, and the excited state, and the latter would result in HO[•] when absorbing photons [15]. However, HA significantly inhibited the degradation of PPB at all levels, and NO₃⁻ was found to be a competitor at relatively high concentrations, indicating that self-sensitized or direct photochemical effect significantly contributed to the photolysis of PPB. Hence, detailed photochemical transformation mechanisms were further investigated in a pure PPB solution.

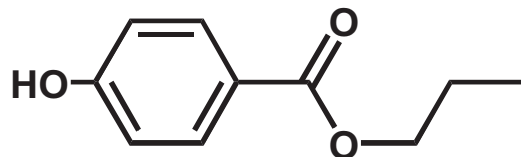


Figure 1. Molecular structure of propylparaben (PPB).

Reactive species initiating the photochemical transformation

Based on our identification of intermediates and reported information on aromatic and phenolic compounds [16], the initial photochemical degradation pathways of PPB by directly absorbing photons are shown in Figure 2. Pathway 1 is the reactions with common free oxidative species self-sensitized by PPB, whereas pathway 2 is initiated by the excitation of the PPB molecule itself through the photoionization, excited singlet state ($^1\text{PPB}^*$), and $^3\text{PPB}^*$.

As for free oxidative species, specific scavengers were used to distinguish contributions from each other: 100 mM isopropanol was used to remove HO^\bullet [17]; 1 mM NaN_3 was used to quench $^1\text{O}_2$ [18]; and O_2 was removed by bubbling N_2 to assess the roles of oxidative species such as $^1\text{O}_2$, H_2O_2 , and the superoxide anion ($\text{O}_2^{\bullet-}$). The PPB degradation kinetics curves with and without the addition of scavengers are summarized in Figure 3 (k_1 values are shown in Supplemental Data, Table S3). The PPB degradation rate constant with the addition of the HO^\bullet scavenger was found to be 0.035 min^{-1} , which was close to that for the non-scavenged solution (0.034 min^{-1}), indicating that HO^\bullet was not involved in the photochemical degradation of PPB. The rate constant decreased to 0.008 min^{-1} (i.e., by 76.5%) with the addition of NaN_3 , indicating that $^1\text{O}_2$ might play an important role. However, further study showed that excluding O_2 from the solution by bubbling N_2 could dramatically increase k_1 to as high as 0.401 min^{-1} , which is 11.8 times higher than the rate constant without the scavenger addition, and seems to contradict the assumption of the importance of $^1\text{O}_2$ mentioned above. Therefore, further evidence is needed to determine the role of oxidative species resulting from O_2 . As for $^1\text{O}_2$, the bimolecular rate constant with PPB was estimated first. The disassociation constant ($\text{p}K_a$) value of PPB is 8.24 [19]; the bimolecular rate constants for $^1\text{O}_2$ with nondissociated PPB (at pH 5.0) and dissociated PPB (PPB^- ; at pH 11.0) were determined by the competition reaction and found to be $1.7 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $2.3 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$, respectively (Supplemental Data, Figure S5A). However, the PPB degradation rate constant k_1 decreased gradually from 0.059 min^{-1} at pH 3.0 to 0.012 min^{-1} at pH 11.0, and the $[\text{PPB}^-]/[\text{PPB}]$ ratio increased from 5.8×10^{-6} to 575 (Supplemental Data, Figure S5B). The contradictory pattern between the bimolecular rate constant and the rate constant possibly implies that $^1\text{O}_2$ does not play a major role in the photochemical degradation of PPB, and the quenching effect induced by NaN_3 was obviously raised by the elimination of other reactive species. Furthermore, the PPB degradation rate constants obtained in pure $^1\text{O}_2$, H_2O_2 , and $\text{O}_2^{\bullet-}$ solution were all below 0.001 min^{-1} (Figure 3; Supplemental Data, Table S3), indicating that even if these oxidative

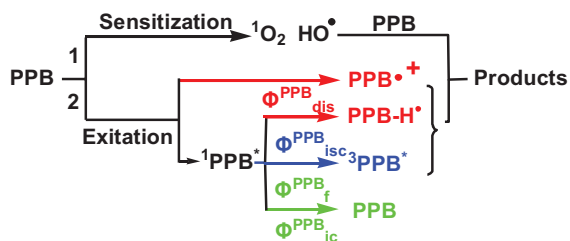


Figure 2. Initial photochemical reaction mechanisms and degradation pathways for propylparaben (PPB) under ultraviolet irradiation. $\text{PPB}^{\bullet+}$ = radical cation of PPB; PPB_{dis} = photodissociation; PPB-H^\bullet = phenoxyl radical of PPB; PPB_{isc} = intersystem crossing; PPB_{f} = fluorescent emission; PPB_{ic} = internal conversion; $^1\text{PPB}^*$ = excited singlet state of PPB; $^3\text{PPB}^*$ = excited triplet state of PPB. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

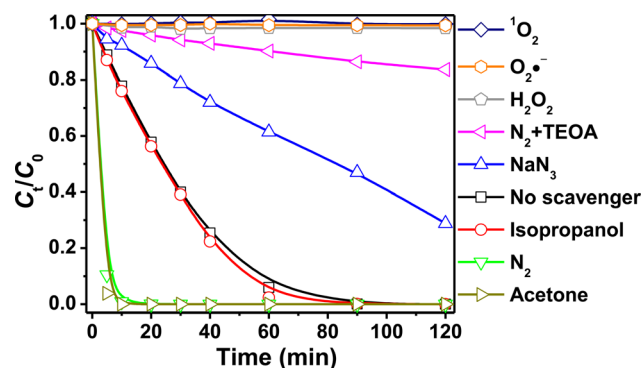


Figure 3. Degradation kinetics of 100 μM propylparaben (PPB) in the dark (10 μM H_2O_2 and 1 μM KO_2), by $^1\text{O}_2$ (80 μM Rose Bengal under visible light) and under ultraviolet irradiation (in scavenger-free, 100 mM isopropanol, nitrogen saturated, 1 mM NaN_3 , 10 mM triethanolamine [TEOA], nitrogen saturated, and 10% acetone solutions). C_t = PPB concentration at time t ; C_0 = initial PPB concentration. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

species could be formed by phenolic as well as aromatic carbonyl compounds [20], they play negligible roles during the self-sensitized photochemical degradation of PPB.

Kinetics studies have proved that efficiently removing O_2 increases the degradation rate constant, as mentioned above, because O_2 is a well-known efficient quencher of the excited triplet state [21]. Therefore, we tentatively assumed that the initial PPB photochemical degradation step would probably occur through $^3\text{PPB}^*$. To validate this assumption, the PPB solution was degassed with N_2 and added to 10 mM triethanolamine (TEOA), which acted as an excited state quencher, to indirectly confirm the contribution of the excited molecular species [22]. The $\text{N}_2 + \text{TEOA}$ significantly decreased the rate constant to 0.002 min^{-1} (Figure 3), suggesting that the excited molecular species contributed 94.1% of the photochemical degradation of PPB. Moreover, when 10% (volume fraction) acetone was introduced as a triplet state sensitizer [23], an extremely high rate constant, 0.654 min^{-1} , was obtained, confirming our assumption that the excited triplet state is mainly responsible for the photochemical transformation of PPB. However, the kinetics might be insufficient to determine the exact contribution of specific species to the initial photochemical degradation, because of the light screening effect of additives and the lack of appropriate scavengers for some species, such as the excited singlet state and radical cations. Therefore, the quantum yield of each photochemical process was also estimated and double confirmed in detail using the later laser flash photolysis results.

According to Figure 2, the initial degradation quantum yields from photoexcited PPB molecules consisted of 4 parts: photodissociation ($\Phi_{\text{dis}}^{\text{PPB}}$), intersystem crossing ($\Phi_{\text{isc}}^{\text{PPB}}$), fluorescent emission ($\Phi_{\text{f}}^{\text{PPB}}$), and internal conversion ($\Phi_{\text{ic}}^{\text{PPB}}$). To distinguish each process, the transient spectra of 500 μM PPB in acetonitrile under N_2 and O_2 saturated conditions were compared (Figure 4). The photodissociation was composed of the photoionization of PPB and bond homolysis of $^1\text{PPB}^*$, which can result in the radical cation ($\text{PPB}^{\bullet+}$) and the phenoxyl radical (PPB-H^\bullet), respectively, as was previously found for phenolic compounds [24]. To better understand the formation mechanisms of specific intermediates, all the calculated $^3\text{PPB}^*$, $\text{PPB}^{\bullet+}$, and PPB-H^\bullet spectra were also compared and summarized in the inset of Figure 4. The $\text{PPB}^{\bullet+}$ and PPB-H^\bullet would not be eliminated by O_2 , which could even enhance the yield of radical cations by acting as the electron acceptor if the reactions really

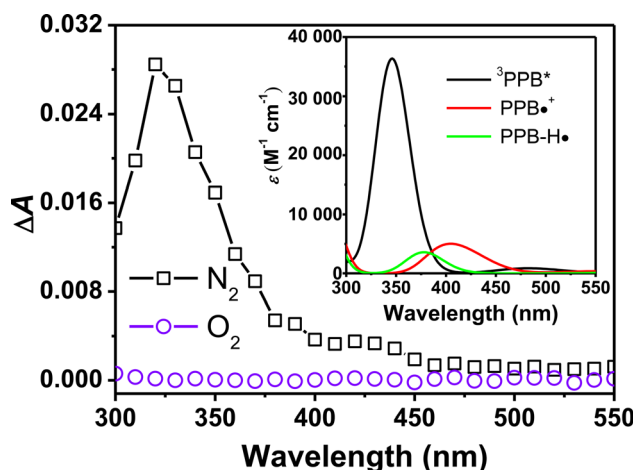


Figure 4. Transient absorption spectra of 500 μM propylparaben (PPB) in N_2 and O_2 saturated solution at 0.5 μs . The inset represents the calculated absorption spectra of transient intermediates. ΔA = transient absorption value; ${}^3\text{PPB}^*$ = excited triplet state of PPB; $\text{PPB}^{\bullet+}$ = radical cation of PPB; PPB-H^{\bullet} = phenoxyl radical of PPB. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

happened [25]. However, no significant signals were detected for the addition of O_2 , indicating that the quantum yield of photodissociation ($\Phi_{\text{dis}}^{\text{PPB}}$) would be negligible (≈ 0). An intermediate with an absorption maximum at 320 nm was identified to be ${}^3\text{PPB}^*$ because of its sensitivity to O_2 . The quantum yield of intersystem crossing of PPB ($\Phi_{\text{isc}}^{\text{PPB}}$) was determined based on the energy transfer method (Supplemental Data, Equations S3–S7) by introducing Ant as the energy acceptor. The formation curves of the triplet state of Ant (${}^3\text{Ant}^*$) were monitored in the presence of PPB (Supplemental Data, Figure S6), and the $\epsilon_{320}^{\text{PPB}}$ and $\Phi_{\text{isc}}^{\text{PPB}}$ values were determined to be 9196 $\text{M}^{-1} \text{cm}^{-1}$ and 0.274 by using $\Phi_{\text{isc}}^{\text{Ant}} = 0.66$ [26]. The $\Phi_{\text{f}}^{\text{PPB}}$ was determined to be 0.002 with tryptophan as the standard, so $\Phi_{\text{ic}}^{\text{PPB}}$ was calculated to be 0.724. All these results indicated that only ${}^3\text{PPB}^*$ could possibly initiate the photochemical transformation of PPB. The importance of ${}^3\text{PPB}^*$ also explained the negative effect of HA on the photochemical degradation of PPB. The energy gap for the excited triplet state of PPB (${}^3\text{PPB}^*$) was calculated to be 3.352 eV, which is much higher than the energy gap for dissolved organic matter (2.233–2.597 eV) [27]. According to the Sandros equation [28], the quenching rate constant by energy transfer from ${}^3\text{PPB}^*$ to dissolved organic matter is very close to the diffusion control rate constant.

Initial mechanisms for the photochemical transformation

Electron and hydrogen transfer were 2 common reaction routes for the excited triplet state [29]. Therefore, the electron affinity of ${}^3\text{PPB}^*$ was first estimated through its reaction with 3 different anions. The vertical ionization energies were calculated for the electron donating abilities of the anions [27] and found to be Cl^- (6.79 eV) > N_3^- (5.47 eV) > CO_3^{2-} (4.72 eV). The transient spectra of 500 μM PPB with 1 mM N_3^- , CO_3^{2-} , and Cl^- were recorded in an acetonitrile– H_2O solution (volume fraction 9:1, Figure 5). The results indicate that N_3^- and CO_3^{2-} decreased the ${}^3\text{PPB}^*$ absorption, but Cl^- increased the ${}^3\text{PPB}^*$ absorption. From the decay kinetics curves at 330 nm shown in the inset of Figure 5, the elimination rate constants were found to be $2.9 \times 10^5 \text{ s}^{-1}$, $9.4 \times 10^5 \text{ s}^{-1}$, $38.6 \times 10^5 \text{ s}^{-1}$, and $5.0 \times 10^5 \text{ s}^{-1}$ for H_2O , N_3^- , CO_3^{2-} , and Cl^- , respectively. The results clearly showed that ${}^3\text{PPB}^*$ could be scavenged by anions, probably via an electron donation mechanism (Figure 6) [27]. An intermediate assigned to PPB-H^{\bullet} (according to the calculated

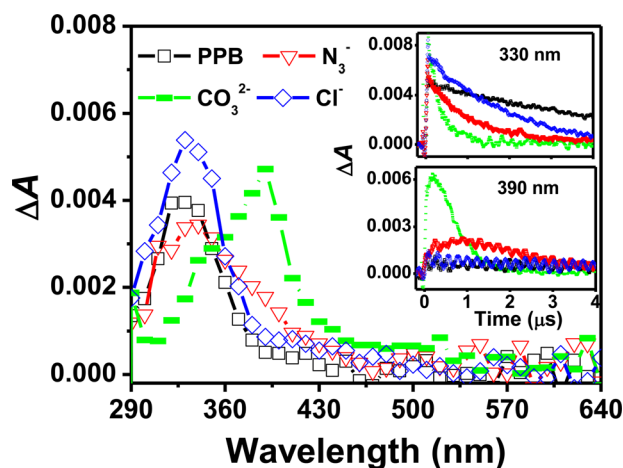


Figure 5. Transient absorption spectra of 500 μM propylparaben (PPB) and mixed solutions with 1 mM NaN_3 , Na_2CO_3 , or NaCl , saturated with N_2 at 0.5 μs . The inset shows the decay kinetics curves at 330 nm and the formation kinetics curves at 390 nm of specific solutions. ΔA = transient absorption value. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

spectra shown in Figure 5) was formed at 390 nm (Figure 5 and its inset plot), which agreed with previous reports that the phenoxyl radical always showed an absorption maximum of approximately 400 nm [30]. The PPB-H^{\bullet} was probably produced through the rapid reaction of the oxidative radicals formed (N_3^{\bullet} , $\text{CO}_3^{\bullet-}$, and $\text{Cl}_2^{\bullet-}$) with PPB by abstracting an electron [31], and the occurrence of the intermediate at 390 nm further confirmed the electron transfer between ${}^3\text{PPB}^*$ and anions in the initial reaction step, as shown in Figure 6. However, the absence of PPB-H^{\bullet} in the pure PPB solution without any other anions indicated that the electron transfer had not occurred only with ${}^3\text{PPB}^*$ and PPB.

Interestingly, the PPB-H^{\bullet} yields followed the order $\text{CO}_3^{\bullet-}$ ($E^0 = 1.59 \text{ V}$) > N_3^{\bullet} ($E^0 = 1.33 \text{ V}$) > $\text{Cl}_2^{\bullet-}$ ($E^0 = 2.41 \text{ V}$) [32], which obviously was not in agreement with the oxidation potential of these radicals. These results could be attributed to the ineffective chemical quenching by electron transfer between ${}^3\text{PPB}^*$ and anions with high vertical ionization energy, such as the formation of $\text{Cl}_2^{\bullet-}$ by ${}^3\text{PPB}^*$. Hence, the further reactions that led to the formation of PPB-H^{\bullet} by $\text{Cl}_2^{\bullet-}$ were weak, which was comparable with the negligible formation kinetics of PPB-H^{\bullet} in the solutions with Cl^- (Figure 5, inset plot). These results could be used to explain some phenomena obtained by the kinetics studies as mentioned above. That is, through electron transfer reactions, N_3^- could efficiently quench the ${}^3\text{PPB}^*$ rather than ${}^1\text{O}_2$, finally resulting in the apparent decrease of the photolysis rate constant, whereas Cl^- was a weak electron donor that would negligibly affect the photochemical transformation of PPB.

From the information outlined, it seems that the assumption that ${}^3\text{PPB}^*$ acts as an electron acceptor has been clarified. However, before drawing a final conclusion, the involvement of ${}^1\text{PPB}^*$ molecules in reactions with anions should also be ruled out. Therefore, variations in the fluorescence of 100 μM PPB

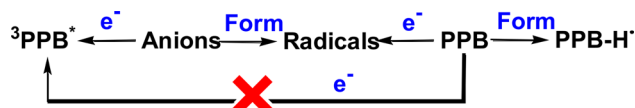


Figure 6. The series of reactions initiated by the electron transfer mechanism between the excited triplet state of propylparaben (${}^3\text{PPB}^*$) and anions. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

with the addition of 1 mM NaN_3 , Na_2CO_3 , or NaCl were also measured (Supplemental Data, Figure S7), and we found that their fluorescence intensities were all close to that of the pure PPB solution, the differences not exceeding 6%. These results excluded the possibility that the anions could react with $^1\text{PPB}^*$, probably because of the extremely short lifespan of $^1\text{PPB}^*$, further proving that $^3\text{PPB}^*$ is an exclusively active electron acceptor.

The results obtained gave insights into the assumption that electron transfer could be induced by $^3\text{PPB}^*$ intermolecularly. To explore this possibility, the vertical ionization energies of PPB and $^3\text{PPB}^*$ also were calculated and found to be 6.54 eV and 3.45 eV, respectively. Combined with the above results, the vertical ionization energies for the PPB molecules and anions were found to be in the order $\text{Cl}^- > \text{PPB} > \text{N}_3^- > \text{CO}_3^{2-} > ^3\text{PPB}^*$. Therefore, the conclusion can be drawn that the electron transfer mechanism was energetically feasible between $^3\text{PPB}^*$ and PPB or $^3\text{PPB}^*$. However, the electron transfer intermediate, $\text{PPB}-\text{H}^*$, was not observed in the pure PPB solution, indicating that another reaction mechanism might have occurred along with the electron transfer mechanism, hydrogen transfer for instance. The results obtained also indicated that using anions in a photochemical degradation system to adjust the ionic strength or the pH value should always be carefully considered, because some anions can delay or enhance the photochemical transformation of organic pollutants, as electron donors.

The hydrogen transfer reaction was further evaluated by assessing the deuterium kinetic isotope effect, because the exchangeable O-H protons in PPB could be replaced by deuterons from the large molar excess of D_2O in the solution [33,34]. Experiments were performed in N_2 -saturated H_2O and D_2O solutions with lower irradiation energy, to produce the PPB degradation kinetics curve and some formation products (Figure 7). A kinetic isotope effect on the initial PPB degradation rate constant was observed, with a ratio $r_{\text{H}_2\text{O}}/r_{\text{D}_2\text{O}}$ of 5.84. Moreover, a photoproduct was identified as 4-hydroxybenzoic acid (HB; $m/z = 137$) by comparing its mass spectrum and retention time with an authentic standard (Supplemental Data, Figure S8A), and HB was also found to have an isotope effect with $r_{\text{H}_2\text{O}}/r_{\text{D}_2\text{O}} = 18.99$ (Figure 7). The enhancement in H_2O suggested that HB was a product of a hydrogen or proton transfer reaction during the photochemical degradation of PPB.

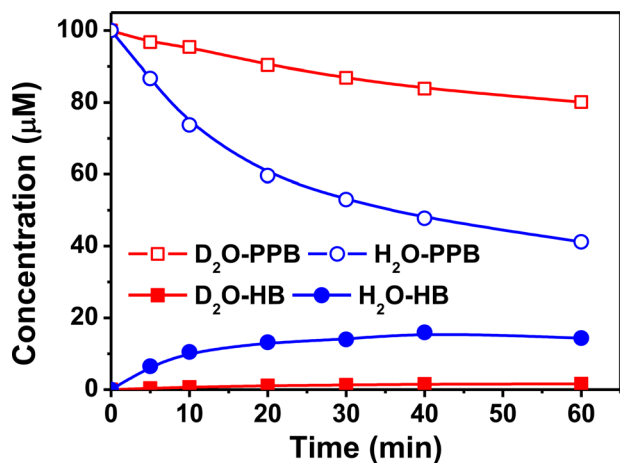


Figure 7. Evolution of the concentrations of propylparaben (PPB) and 4-hydroxybenzoic acid (HB) at different photochemical degradation time intervals in N_2 saturated D_2O (red line) or H_2O (blue line) solution under a 100-W high-pressure mercury lamp. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

To further validate the hypothesis and gain a deeper insight into the hydrogen transfer mechanism, the selected ion monitoring chromatograms of HB at 10 min were also measured under different conditions: air-water, air-acetonitrile, and N_2 -water (Figure 8A). Under all these conditions HB was formed, and its yield increased in the order air-water < air-acetonitrile < N_2 -water. Comparing the air-water and N_2 -water results showed that O_2 significantly decreased the HB yield, indicating that HB was formed directly from $^3\text{PPB}^*$. It could also be formed in the aprotic solvent acetonitrile (Figure 8A), in which the only proton donor was the PPB molecule itself, because only the hydrogen in the hydroxyl group of the PPB molecule was exchangeable with the solvent, H_2O or D_2O . Therefore, the only possible mechanism was confirmed as intermolecular head-to-tail hydrogen transfer reactions, which occurred between an aromatic carbonyl triplet and the hydroxyl group of the PPB molecule. The shoulder peak around 400 nm in Figure 4 should probably be assigned to mixed spectra of the ketyl and phenoxy radicals, formed via intermolecular hydrogen transfer, which was similar to the spectra given in a previous study [35].

The presence of O_2 could significantly quench $^3\text{PPB}^*$, so its role in the photochemical transformation of PPB was also studied, to see whether any other chemical processes were involved. Under air balanced conditions (solution exposed and stirred in air for 15 min), a new photoproduct was identified as 3-hydroxy-propyl paraben (3-PPBOH; $m/z = 195$; Supplemental Data, Figure S8B). According to previous studies, the HO-adduct product formed generally by the hydrolysis of a radical cation resulted from electron transfer between PPB molecules, or direct reaction with HO^* [36]. If these assumptions were true, the involvement of the H_2O molecule should be inevitable, and more than 1 photoproduct with $m/z = 195$ was expected to be formed

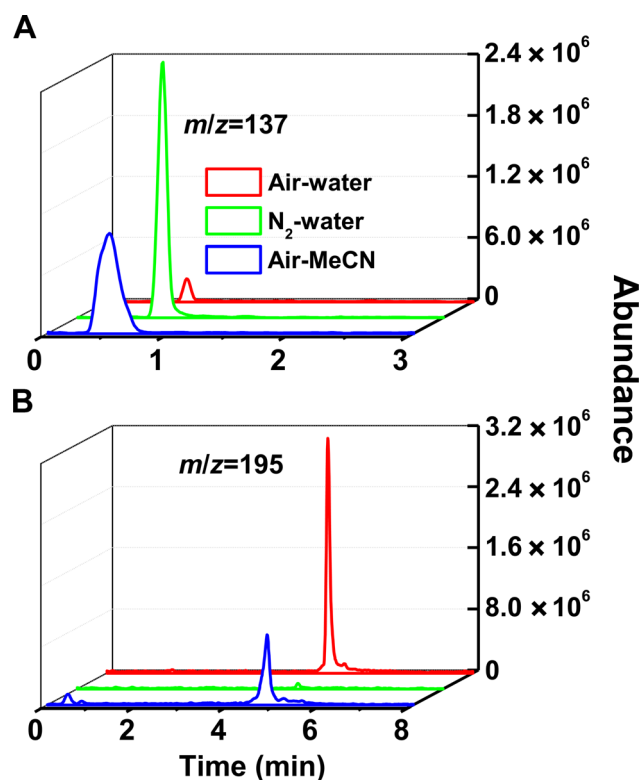


Figure 8. Selected ion monitoring chromatograms for (A) $m/z = 137$ and (B) $m/z = 195$ of propylparaben (PPB) degraded in air-water, N_2 -water, and air-acetonitrile in electrospray ionization (ESI^-) mode. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

during the photochemical transformation. However, the photo-product 3-PPBOH was only obtained in air–water and air–acetonitrile, but not in N₂–water, within 10 min (Supplemental Data, Figure 8B). That is, 3-PPBOH was formed even in dry acetonitrile, and its yield was related to the existence of O₂ rather than H₂O. Moreover, 3-PPBOH was the only photoproduct with an *m/z* of 195, ruling out the involvement of HO[•] radical or H₂O in the PPB^{•+} reaction. Therefore, 3-PPBOH probably originated from the direct reaction of ³PPB* with O₂, with a collision complex [37], which was tentatively assigned to a transient endoperoxide [24].

To summarize, ³PPB* was found to be an efficient electron acceptor that could be quenched by anions such as NaN₃, and the photochemical transformation was initiated through an intermolecular reaction mechanism. No evidence was found for the formation of the PPB-H[•] intermediate, while definitive evidence—for example, the isotope effect and the photoproducts formed—proved that hydrogen transfer was an important initial reaction mechanism. Thus a tentative mechanism initiated by ³PPB* is proposed for the direct photolysis of PPB: Electron transfer coupled with proton transfer, occurring through the transfer of the hydroxyl group hydrogen to the aromatic carbonyl of another molecule. The reaction mechanism could also be seen as a kind of self-sensitized reaction: ³PPB* acting as the sensitizer reacted with itself or the ground state PPB molecules. Besides intermolecular reaction, O₂, which acts as both a physical and chemical quencher of the excited triplet state, could also easily react with ³PPB*. Therefore, the initial reaction mechanism (Supplemental Data, Scheme S1), the steady-state photoproduct composition, and transformation rate constant all depended on the O₂ concentration in water.

Toxicity evolution during the photochemical transformation

Considering the important role of O₂ in the degradation pathways and the different O₂ levels in the reaction solutions, the photochemical transformations of PPB under air and in the N₂ degassed solutions were monitored using a UV/Vis spectrophotometer (Supplemental Data, Figure S9A and B). Changes in the pH were also obtained, as shown in the Figure insets. Under aerobic conditions, 3 isosbestic points were observed, at 217 nm, 233 nm, and 284 nm, within 30 min, indicating the transformation of PPB to its photoproducts. The mineralization of PPB was observed after 40 min from the decrease in the absorbance at all wavelengths. The pH values first decreased gradually until 90 min, and then increased slowly, indicating the accumulation and then mineralization of acidic products, with *pK_a* values below that of PPB, into CO₂ and H₂O. Under anaerobic conditions, the absorption peaked at 257 nm and decreased dramatically within 5 min. Two isosbestic points were obtained at 217 nm and 233 nm, from 10 min to 60 min, and another at 278 nm was observed between 10 min and 120 min, indicating the constant accumulation of photoproducts in solution. Little variation of absorbance spectra and pH values after 90 min suggested the formation of refractory photoproducts. The results obtained implied that PPB was degraded quickly but was not mineralized under anaerobic conditions, and the opposite was the case under aerobic conditions.

The identification and quantification of all photoproducts are expensive. Hence, bioassay methods are generally used to estimate the detoxification process [38]. Because PPB is characterized by its antimicrobial and estrogenic activities, luminescent bacteria and transgenic yeast were used to evaluate the toxicity evolution of the degradation mixtures with an initial PPB concentration of 100 μM (Figure 9A and B). The

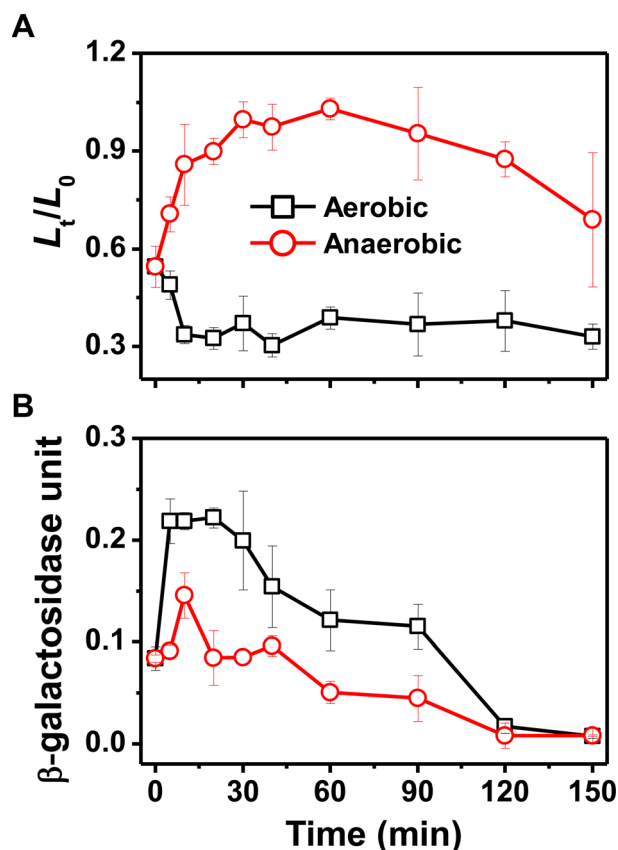


Figure 9. (A) Acute toxicity and (B) estrogenic activity in solutions collected at different photochemical degradation time intervals, with 100 μM propylparaben (PPB) as the initial concentration, under aerobic (black line) and anaerobic (red line) conditions. L_t = luminescent value at time t; L₀ = initial luminescent value.

luminescent bacteria results showed that, when photochemical degradation was initiated, photoproducts with higher acute toxicities than PPB were formed and accumulated under aerobic conditions until 150 min, although all of the PPB was degraded. The increased toxicity might have originated from the formation of 3-PPBOH, because hydroxyl addition generally enhances the toxicity of aromatic organic pollutants [38]. In contrast, toxicity dramatically decreased under anaerobic conditions within 10 min, and showed almost no inhibition effect until 60 min. The decreased toxicity could be the result of the formation of less toxic products, like HB [39]. However, the toxicity increased again after 60 min, promoted mainly by the secondary PPB photoproducts.

The estrogenic activity was found to increase from the beginning of photolysis and then to disappear at 150 min, under both aerobic and anaerobic conditions, implying that the primary photoproducts were estrogenic or could enhance the estrogenic effect of PPB. However, both the toxic transformation products and the estrogenic original compound PPB could effectively be detoxified with a prolonged transformation time. Compared with parabens, HB has been confirmed as an inactive endocrine component [40], and the increase in estrogenic activity under both conditions should not be the result of HB. In addition, the estrogenic intensity was found to be higher under aerobic than under anaerobic conditions, indicating that the photoproducts originating from O₂ are more estrogenic. However, because of the lack of detailed information about the transformation mixtures and the authentic 3-PPBOH standard, it is still hard to distinguish the contributions from other photoproducts. These

results indicate that aerobic conditions are more favorable than anaerobic conditions for PPB mineralization, although they result in some higher risk photoproducts, which can eventually be eliminated because of the constant mineralization processes. In contrast, anaerobic conditions are highly efficient in eliminating the parent compound and reducing the overall toxicity but produce some products that are resistant to UV light.

CONCLUSIONS

Kinetics studies suggested that PPB could be photochemically degraded when absorbing UV light. Then the center composite design was carried out to model the effects of 4 environmental variables (HA, Cl^- , NO_3^- , and Ca^{2+}); all the environmental factors were found to compete for photons with PPB. The reactive species and the initial photochemical transformation mechanisms were then studied in a pure PPB solution. Both the scavenging experiments and the quantum yield measurements suggested that the initial photolysis of PPB was dominated by $^3\text{PPB}^*$ rather than by other self-sensitized free oxidative species. Further studies combined with the experimental and the calculated results implied that $^3\text{PPB}^*$ was a reactive electron acceptor, and electron transfer coupled with proton transfer with a head-to-tail intermolecular reaction was proposed as the main transformation pathway. Then O_2 was proved to be not only the physical triplet quencher, but also a direct reactant with $^3\text{PPB}^*$ to form a complex. Furthermore, the composition of steady-state photoproducts and the toxicity evolution also depended on the level of O_2 concentration in solution. The results showed that the anaerobic condition was more efficient in eliminating the parent compound and decreasing toxicity, but was less effective in the mineralization of transformation photoproducts.

SUPPLEMENTAL DATA

Tables S1–S3.
Figures S1–S9.
Scheme S1. (1.9 MB DOC).

Acknowledgment—The present study is no. 1863 from the Guangzhou Institute of Geochemistry, Chinese Academy of Sciences. The study was financially supported by the Chinese Academy of Sciences Knowledge Innovation Program (KZCX2-YW-QN103), the Science and Technology R&D Fund of Shenzhen City (JC201005250054A) and Guangdong Province (2012A032300010), the National Natural Science Foundation of China (40973068), and the Earmarked Fund of the State Key Laboratory of Organic Geochemistry (SKLOG2012A01). The authors thank T.T. Qian for her kind help in conducting laser experiments and for useful discussions.

REFERENCES

- Meeker JD, Cantonwine DE, Rivera-Gonzalez LO, Ferguson KK, Mukherjee B, Calafat AM, Ye XY, Del Toro LVA, Crespo-Hernandez N, Jimenez-Velez B, Alshawabkeh AN, Cordero JF. 2013. Distribution, variability, and predictors of urinary concentrations of phenols and parabens among pregnant women in Puerto Rico. *Environ Sci Technol* 47:3439–3447.
- Ramirez N, Marce RM, Borrull F. 2010. Development of a thermal desorption-gas chromatography-mass spectrometry method for determining personal care products in air. *J Chromatogr A* 1217:4430–4438.
- Schultis T, Metzger JW. 2004. Determination of estrogenic activity by LYES-assay (yeast estrogen screen-assay assisted by enzymatic digestion with lyticase). *Chemosphere* 57:1649–1655.
- Ryan CC, Tan DT, Arnold WA. 2011. Direct and indirect photolysis of sulfamethoxazole and trimethoprim in wastewater treatment plant effluent. *Water Res* 45:1280–1286.
- Erofeev MV, Kieft IE, Sosnin EA, Stoffels E. 2006. UV excimer lamp irradiation of fibroblasts: The influence on antioxidant homeostasis. *IEEE T Plasma Sci* 34:1359–1364.
- Gmurek M, Miller JS. 2012. Photosensitized oxidation of a water pollutant using sulphonated porphyrin. *Chem Pap* 66:120–128.
- Bledzka D, Gmurek M, Olak-Kucharczyk M, Miller JS, Ledakowicz S. 2011. Photodegradation of n-butylparaben in natural water from Sulejow Reservoir. *Ecol Chem Eng S* 18:517–525.
- Wang M, Cheng LL, Zhu H, Li K, Wu QS, Yao SD, Wang SL. 2009. Characterization of the transient species generated in the photoexcitation of benzoic acid, 2-hydroxy-, 2-D-ribofuranosylhydrazide. *J Photochem Photobiol A* 208:104–109.
- Monadjemi S, El Roz M, Richard C, Ter Halle A. 2011. Photoreduction of chlorothalonil fungicide on plant leaf models. *Environ Sci Technol* 45:9582–9589.
- Lakowicz JR. 2006. *Principles of Fluorescence Spectroscopy*, 3rd ed. Springer, New York, NY, USA.
- Frisch MJ, Truck GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Zakrzewski VG, Montgomery JA, Stratmann JRE, Burant JC, Dapprich S, Millam JM, Daniels AD, Kudin KN, Strain MC, Farkas O, Tomasi J, Barone V, Cossi M, Cammi R, Mennucci B, Pomelli C, Adamo C, Clifford S, Ochterski J, Petersson GA, Ayala PY, Cui Q, Morokuma K, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Cioslowski J, Ortiz JV, Boboul AG, Stefnov BB, Liu G, Liashenko A, Piskorz P, Komaromi L, Gomperts R, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Gonzalez C, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Andres JL, Gonzalez C, Head-Gordon M, Replogle ES, Pople JA. 2003. GAUSSIAN 03, Revision A.1. Gaussian, Pittsburgh, PA, USA.
- Gorelsky SI. 2010. SWizard program. University of Ottawa, Ottawa, Canada. [cited 2014 March 13]. Available from: <http://www.sg-chem.net/>.
- Fang HS, Gao YP, Li GY, An JB, Wong PK, Fu HY, Yao SD, Nie XP, An TC. 2013. Advanced oxidation kinetics and mechanism of preservative propylparaben degradation in aqueous suspension of TiO_2 and risk assessment of its degradation products. *Environ Sci Technol* 47:2704–2712.
- Li GY, Zu L, Wong PK, Hui XP, Lu Y, Xiong JK, An TC. 2012. Biodegradation and detoxification of bisphenol A with one newly-isolated strain *Bacillus* sp GZB: Kinetics, mechanism and estrogenic transition. *Bioresour Technol* 114:224–230.
- Razavi B, Ben Abdelmelek S, Song W, O'Shea KE, Cooper WJ. 2011. Photochemical fate of atorvastatin (lipitor) in simulated natural waters. *Water Res* 45:625–631.
- Hermann R, Mahalaxmi GR, Jochum T, Naumov S, Brede O. 2002. Balance of the deactivation channels of the first excited singlet state of phenols: Effect of alkyl substitution, sterical hindrance, and solvent polarity. *J Phys Chem A* 106:2379–2389.
- Yang H, An TC, Li GY, Song WH, Cooper WJ, Luo HY, Guo XD. 2010. Photocatalytic degradation kinetics and mechanism of environmental pharmaceuticals in aqueous suspension of TiO_2 : A case of beta-blockers. *J Hazard Mater* 179:834–839.
- Li MY, Cline CS, Koker EB, Carmichael HH, Chignell CF, Bilski P. 2001. Quenching of singlet molecular oxygen ($\text{O}(^1\text{O}_2)$) by azide anion in solvent mixtures. *Photochem Photobiol* 74:760–764.
- Labat L, Kummer E, Dallet P, Dubost JP. 2000. Comparison of high-performance liquid chromatography and capillary zone electrophoresis for the determination of parabens in a cosmetic product. *J Pharmaceut Biomed* 23:763–769.
- Anastasio C, Faust BC, Rao CJ. 1996. Aromatic carbonyl compounds as aqueous-phase photochemical sources of hydrogen peroxide in acidic sulfate aerosols, fogs, and clouds. 1. Non-phenolic methoxybenzaldehydes and methoxyacetophenones with reductants (phenols). *Environ Sci Technol* 31:218–232.
- Halladja S, Ter Halle A, Aguer JP, Boukamh A, Richard C. 2007. Inhibition of humic substances mediated photooxygenation of furfuryl alcohol by 2,4,6-trimethylphenol. Evidence for reactivity of the phenol with humic triplet excited states. *Environ Sci Technol* 41:6066–6073.
- Brogliola MF, Bertolotti SG, Previtali CM. 2005. Excited states quenching of phenosafranin dye by electron donors. *J Photochem Photobiol A* 170:261–265.
- Latch DE, Packer JL, Stender BL, VanOverbeke J, Arnold WA, McNeill K. 2005. Aqueous photochemistry of triclosan: Formation of 2,4-dichlorophenol, 2,8-dichlorodibenzo-p-dioxin, and oligomerization products. *Environ Toxicol Chem* 24:517–525.
- Jin FM, Leitch J, vonSonntag C. 1995. The photolysis ($\lambda=254$ nm) of tyrosine in aqueous solutions in the absence and presence of oxygen. The reaction of tyrosine with singlet oxygen. *J Photochem Photobiol A* 92:147–153.
- Fasnacht MP, Blough NV. 2002. Aqueous photodegradation of polycyclic aromatic hydrocarbons. *Environ Sci Technol* 36:4364–4369.

26. Montalti M, Credi A, Prodi L, Teresa Gandolfi M. 2006. *Handbook of Photochemistry*, 3rd ed. Blackwell, Oxford, UK.
27. Zhang SY, Chen JW, Qiao XL, Ge LK, Cai XY, Na GS. 2010. Quantum chemical investigation and experimental verification on the aquatic photochemistry of the sunscreen 2-phenylbenzimidazole-5-sulfonic acid. *Environ Sci Technol* 44:7484–7490.
28. Sandros K. 1964. Transfer of triplet state energy in fluid solutions. 3. Reversible energy transfer. *Acta Chem Scand* 18:2355–2374.
29. Boscá F, Miranda MA. 1998. New trends in photobiology (invited review): Photosensitizing drugs containing the benzophenone chromophore. *J Photochem Photobiol B* 43:1–26.
30. Gadosy TA, Shukla D, Johnston LJ. 1999. Generation, characterization, and deprotonation of phenol radical cations. *J Phys Chem A* 103:8834–8839.
31. Nicolaescu AR, Wiest O, Kamat PV. 2003. Radical-induced oxidative transformation of quinoline. *J Phys Chem A* 107:427–433.
32. Li GY, Liu XL, Zhang HM, An TC, Zhang SQ, Carroll AR, Zhao HJ. 2011. In situ photoelectrocatalytic generation of bactericide for instant inactivation and rapid decomposition of Gram-negative bacteria. *J Catal* 277:88–94.
33. Markle TF, Rhile IJ, Mayer JM. 2011. Kinetic effects of increased proton transfer distance on proton-coupled oxidations of phenol-amines. *J Am Chem Soc* 133:17341–17352.
34. Huvaere K, Skibsted LH. 2009. Light-induced oxidation of tryptophan and histidine. Reactivity of aromatic n-heterocycles toward triplet-excited flavins. *J Am Chem Soc* 131:8049–8060.
35. Das PK, Encinas MV, Scaiano JC. 1981. Laser flash-photolysis study of the reactions of carbonyl triplets with phenols and photochemistry of para-hydroxypropiophenone. *J Am Chem Soc* 103:4154–4162.
36. Kurata T, Watanabe Y, Katoh M, Sawaki Y. 1988. Mechanism of aromatic hydroxylation in the Fenton and related reactions— one-electron oxidation and the NIH shift. *J Am Chem Soc* 110:7472–7478.
37. Fasnacht MP, Blough NV. 2003. Mechanisms of the aqueous photodegradation of polycyclic aromatic hydrocarbons. *Environ Sci Technol* 37:5767–5772.
38. Mendez-Arriaga F, Esplugas S, Gimenez J. 2008. Photocatalytic degradation of non-steroidal anti-inflammatory drugs with TiO₂ and simulated solar irradiation. *Water Res* 42:585–594.
39. Soni MG, Carabin IG, Burdock GA. 2005. Safety assessment of esters of p-hydroxybenzoic acid (parabens). *Food Chem Toxicol* 43:985–1015.
40. Routledge EJ, Parker J, Odum J, Ashby J, Sumpter JP. 1998. Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. *Toxicol Appl Pharmacol* 153:12–19.