



HAL
open science

Photosensitizing and antioxidant activities of humic substances: A case study of beta(2)-adrenoceptor agonist terbutaline

L. Zhou, M. Sleiman, S. Halladja, C. Ferronato, J. Chovelon, Claire Richard

► To cite this version:

L. Zhou, M. Sleiman, S. Halladja, C. Ferronato, J. Chovelon, et al.. Photosensitizing and antioxidant activities of humic substances: A case study of beta(2)-adrenoceptor agonist terbutaline. *Journal of Photochemistry and Photobiology A: Chemistry*, 2020, 391 (—), 10.1016/j.jphotochem.2020.112360 . hal-02510669

HAL Id: hal-02510669

<https://hal.science/hal-02510669>

Submitted on 21 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 Photosensitizing and Antioxidant Activities of Humic Substances: A case study
2 of β 2-adrenoceptor Agonist Terbutaline

3

4 Lei Zhou ^{a,b,c*}, Mohamad Sleiman ^b, Sabrina Halladja ^{b,d}, Corinne Ferronato ^e, Jean-Marc Chovelon ^e,
5 Claire Richard ^{b,*}

6

7 ^a State Environmental Protection Key Lab of Environmental Risk Assessment and control on Chemical
8 Processes. School of Resources & Environmental Engineering, East China University of Science and
9 Technology, Shanghai 200237, China

10 ^b Université Clermont Auvergne, CNRS, Sigma-Clermont, Institut de Chimie de Clermont-Ferrand, F-
11 63178 Aubière, France

12 ^c Shanghai Institute of Pollution Control and Ecological Security, Shanghai 200092, PR China

13 ^d Département de Chimie, Université de Skikda, 21000, Algeria

14 ^e Univ Lyon, Université Claude Bernard Lyon 1, CNRS-UMR 5256, IRCELYON, F-69626, 2 Avenue
15 Albert Einstein, Villeurbanne, France

16

17 *Corresponding authors.

18 Email addresses:

19 zhoulei@ecust.eud.cn (L. Zhou);

20 claire.richard@uca.fr (C. Richard).

21

22

23 Abstract

24 Humic substances (HS) play an important role in the phototransformation of micro-pollutants in
25 surface waters and a significant part of this reactivity is often attributed to oxidant triplet excited states
26 ($^3\text{HS}^*$). The present study aims to understand why $^3\text{HS}^*$ were found to be negligibly involved in the
27 HS-mediated phototransformation of β 2-adrenoceptor agonist terbutaline (TBL) while this chemical
28 shows a high reactivity towards triplet 3-carboxybenzophenone ($^3\text{CBP}^*$), a typical HS model sensitizer.
29 To clarify this apparent discrepancy, several experiments were conducted. We first confirmed that
30 TBL can be easily oxidized by triplet riboflavin ($^3\text{RF}^*$), another sensitizer ($k = (1.7 \pm 0.4) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$).
31 Afterwards, we studied the effect of TBL on the HS-mediated phototransformation of 2,4,6-
32 trimethylphenol (TMP), chosen for its high reactivity with $^3\text{HS}^*$. TBL was found to enhance the rate
33 of TMP loss. This result is rationalized by postulating that (i) TMP and TBL are both oxidized by
34 $^3\text{HS}^*$ to yield the phenoxyl radicals $\text{TMP}_{\cdot\text{H}}$ and $\text{TBL}_{\cdot\text{H}}$, respectively, and (ii) $\text{TBL}_{\cdot\text{H}}$ is further reduced
35 by TMP which generates additional $\text{TMP}_{\cdot\text{H}}$. Fitting the experimental data gives $k = (1.8 \pm 0.5) \times 10^9$
36 $\text{M}^{-1} \text{ s}^{-1}$ for the reaction between $^3\text{HS}^*$ and TBL and DFT calculations further support the conclusion
37 that $\text{TBL}_{\cdot\text{H}}$ can be reduced by a lot of phenols. Hence, in the case of TBL, the oxidant effect of $^3\text{HS}^*$ is
38 counterbalanced by the antioxidant properties of HS.

39 **Key Words:** Humic substances; Laser flash photolysis; Substituted phenols; Antioxidant; DFT
40 calculation.

41

42 1. Introduction.

43 Humic substances (HS) that include humic and fulvic acids are ubiquitous in soils and natural waters,
44 and can promote the degradation of organic micro-pollutants in illuminated aquatic environments [1,
45 2]. Their reactivity is however complex and not fully understood. Indeed, upon sunlit irradiation, HS is
46 capable of generating various reactive species such as hydroxyl radical (OH) [3], singlet oxygen ($^1\text{O}_2$)
47 [4], triplet excited states ($^3\text{HS}^*$) [5], oxy and peroxy radicals [6], that can accelerate the decomposition
48 of the organic micro-pollutants. Among all these reactive species, $^3\text{HS}^*$ have been found to play an

49 important role in the photochemical degradation of organic micro-pollutants, such as pesticides [7] and
50 pharmaceuticals [8]. They are generally proposed to abstract electrons or H atoms from
51 micropollutants and lead to their oxidation. However, it has been also reported that the sensitized-
52 photodegradation of chemicals such as aniline and sulfonamide antibiotics can be inhibited by HS [9-
53 11]. HS that contain phenolic/polyphenolic moieties in their complex structures [12, 13] could act as
54 antioxidants, regenerating the starting micro-pollutants (reaction 1).



56 In a former study, the photodegradation of β 2-adrenoceptor agonist terbutaline (TBL) in the presence
57 of HS was reported [14]. Our results revealed that $^3\text{HS}^*$ played a negligible role in the transformation
58 of TBL, which seemed contradictory with the high reactivity of TBL with triplet 3-
59 carboxybenzophenone ($^3\text{CBP}^*$), a typical HS model sensitizer [14]. We proposed a backwards
60 reaction process to rationalize these observations. Specifically, TBL could be oxidized by $^3\text{HS}^*$ at a
61 significant rate, but the generated intermediary radical could also be reduced by HS itself to give back
62 TBL.

63 To confirm our hypothesis according which HS oppose both photosensitizing and antioxidant
64 activities, several experiments were conducted in this study. Laser flash photolysis (LFP) was applied
65 to determine the reaction rate constant of TBL with triplet riboflavin ($^3\text{RF}^*$), which was selected as
66 another HS model sensitizer. Moreover, we studied the HS-mediated transformation of 2,4,6-
67 trimethylphenol (TMP) in the presence of TBL. TMP was chosen for its high reactivity with $^3\text{HS}^*$ and
68 used to confirm and evaluate the interaction between $^3\text{HS}^*$ and TBL through competitive reactions
69 [15-17]. Finally, density functional theory (DFT) calculations were performed to evaluate the
70 antioxidant ability of a series of substituted phenols that could be potentially present in HS.

71 2. Experimental

72 2.1 Chemicals and reagents

73 Terbutaline hemisulfate (TBL, 98%), 2,4,6-trimethylphenol (TMP, 99%), riboflavin (RF, 98%) and
74 humic acids (HS, reference number 53680) were purchased from Sigma-Aldrich. HPLC grade

75 acetonitrile (ACN) was supplied by Fisher Chemical. Other reagents were at least of analytical grade
76 and used as received without further purification. Milli-Q water (18.2 MΩ cm) was obtained from a
77 Millipore Milli-Q system.

78 2.2 Photodegradation experiments and analyses

79 A vertical cylindrical reactor (id = 14 mm) was used in this study. The air-saturated solutions were
80 irradiated in a device equipped with three “black light” Mazda 125 W lamps (90 % of radiant energy
81 emitted at 365 nm). The radiant flux was equal to 5×10^8 Einstein $\text{cm}^{-3} \text{s}^{-1}$ which was measured directly
82 using a VLC-3W radiometer. Aliquots of 1 mL were withdrawn after selected irradiation times and
83 further analyzed by HPLC. The decay of TMP alone or in the presence of TBL was monitored until
84 conversion extents comprised between 55 and 85%. The rates of TMP consumption were measured at
85 conversion extents comprised between 12 and 30%. The concentration of TMP was measured by
86 HPLC using a Waters HPLC apparatus equipped with a 2996 photodiode array detector (PAD), an
87 auto sampler and a reverse-phase C₁₈ Nucleodur, Macherey-Nagel column (5 μm, 250×4.6 mm). The
88 flow rate was set at 1 mL min⁻¹, and the mobile phase was a mixture of 60 % methanol and 40% water
89 acidified with phosphoric acid (0.1%).

90 Laser flash photolysis (LFP) experiments were carried out using a Quanta Ray GCR 130-01Nd: YAG
91 laser and an Applied Photophysics station previously described [18]. Solutions containing TBL and
92 RF were irradiated at 355 nm, using the third harmonic of the laser. A red filter was placed between
93 the cell and the photomultiplier to avoid parasitic signals. The rate constant of reaction of TBL with
94 ³RF* (absorbance of 0.5 at 355 nm) was measured by monitoring the decay of ³RF* at 650 nm. The
95 rate of ³RF* disappearance in the absence of oxygen was equal to:

$$96 \quad -\frac{d[{}^3\text{RF}^*]}{dt} = (k_d + k[\text{TBL}]][{}^3\text{RF}^*] \quad (\text{Eq 1})$$

97 where k_d is the rate constant of deactivation of ³RF* and k represents the bimolecular rate constant of
98 reaction between TBL and ³RF*. $k_d + k[\text{TBL}]$ is the apparent first order decay rate constant of ³RF*
99 that was obtained by fitting the experimental data using an exponential decay. The reactivity of the

100 phenoxyl radical issued from TBL with oxygen was studied by exciting TBL (10^{-3} M) at 266 nm. The
101 phenoxyl radical decay was monitored over 940 μ s at 440 nm, the maximum of absorption of the
102 transient [19]. The solutions were saturated with air or with oxygen. The fitting of the transient decay
103 was done between 50 and 400 μ s.

104 2.3 Calculations

105 Density Functional Theory (DFT) calculations were performed using the Gaussian 09 software
106 package [20]. The geometry optimization and frequency calculations were carried out by using B3LYP
107 method and 6-311G* basis set. The obtained **sums** of electronic and thermal free energies were used to
108 represent the Gibbs free energy of each chemical. The integral equation formalism polarized
109 continuum model (IEFPCM), based on the self-consistent-reaction-field (SCRF) method, was used to
110 consider the bulk solvent effect of water [21].

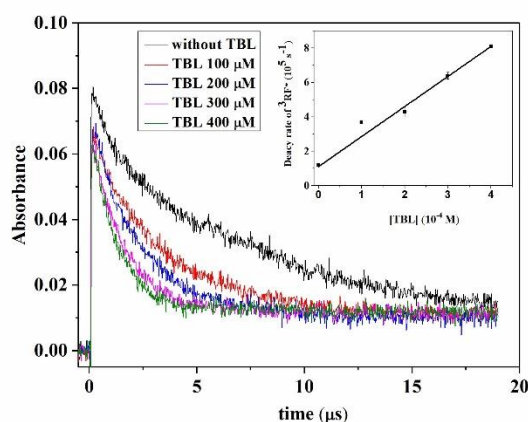
111 3. Results and discussion.

112 3.1 Reactivity of TBL towards $^3\text{RF}^*$

113 It was previously shown that the photodegradation of TBL was significantly **enhanced** by HS [14].
114 Investigating the effect of sorbic acid and oxygen, two $^3\text{HS}^*$ scavengers, led to the conclusion that
115 $^3\text{HS}^*$ played a negligible role in the HS-mediated photodegradation of TBL. **However, this result was**
116 **in contrast with the high reactivity of TBL towards triplet of 3-carboxybenzophenone ($^3\text{CBP}^*$), which**
117 **is often regarded as a typical model sensitizer of HS.**

118 To further verify the oxidability of TBL, another model sensitizer, riboflavin, was **used in this work.**
119 **The** reaction rate constant of TBL with $^3\text{RF}^*$ was also determined by LFP as performed with CBP.
120 Figure 1 shows the decay of $^3\text{RF}^*$ at 650 nm **in the absence and presence of TBL (0-400 μ M) in argon-**
121 **saturated medium.** TBL clearly accelerated the rate of $^3\text{RF}^*$ decay, the apparent first order decay rate
122 constant of $^3\text{RF}^*$ increasing from $1.2 \times 10^5 \text{ s}^{-1}$ in the absence of TBL to $8.0 \times 10^5 \text{ s}^{-1}$ in the presence of
123 400 μ M of TBL. **Using a linear regression of the first order decay rate constant of $^3\text{RF}^*$ versus TBL**

124 concentration, we could estimate the second order rate constant of reaction of TBL with $^3\text{RF}^*$ to $(1.7 \pm$
125 $0.4) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, which fits well with that found with $^3\text{CBP}^*$ $((2.7 \pm 0.3) \times 10^9 \text{ M}^{-1} \text{ s}^{-1})$.



126
127 Figure 1. Decay of $^3\text{RF}^*$ monitored at 650 nm in the absence and presence of TBL under argon-
128 saturated condition; the insert shows the linear relationship of the apparent first order decay rate
129 constant of $^3\text{RF}^*$ versus the concentration of TBL.

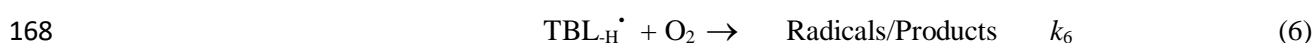
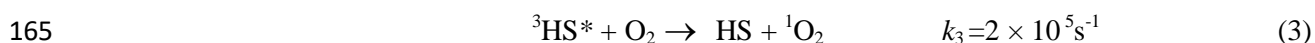
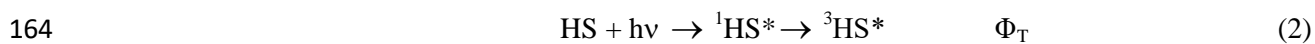
130 3.2 HS-mediated photodegradation of the mixture TBL and TMP

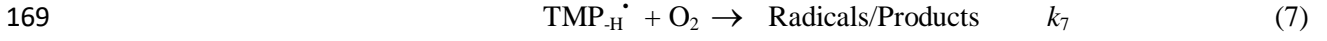
131 Based on the reaction rate constants measured between TBL and $^3\text{CBP}^*$ or $^3\text{RF}^*$, we expected that
132 TBL was able to be oxidized by $^3\text{HS}^*$. To confirm this hypothesis and to get a deeper insight into the
133 reaction between HS and TBL, we investigated the effect of TBL on the HS-sensitized
134 photodegradation of TMP. TMP was chosen because it readily can react with $^3\text{HS}^*$ and was often used
135 as a probe molecule to quantify their contribution [15, 17]. On the first hand, TBL could compete with
136 TMP for the reaction with $^3\text{HS}^*$ and potentially reduce its rate of photodegradation. On the other hand,
137 as a good H-donor, TMP might reduce the phenoxy radicals issued from TBL ($\text{TBL}_{\text{H}}^{\cdot}$), and
138 regenerate TBL.

139 In the presence of HS (20 mg L^{-1}) and in air-saturated solution, TMP ($5 \times 10^{-5} \text{ M}$) was photodegraded
140 with an initial rate of $1.5 \times 10^{-8} \text{ M s}^{-1}$ (Figure 2). The addition of TBL in the range of 1×10^{-4} - $4 \times 10^{-4} \text{ M}$
141 increased this rate until $3.7 \times 10^{-8} \text{ M s}^{-1}$ (Figure 2). To rationalize these data, we propose that reactions
142 2-8 take place. Specifically, $^3\text{HS}^*$ are formed after light absorption by HS and inter-system crossing
143 (reaction 2), Φ_{T} is the quantum yield of $^3\text{HS}^*$ formation. Afterwards, $^3\text{HS}^*$ can undergo several decay

144 pathways: deactivation by reaction with oxygen (reaction 3), competitive oxidations of TBL (reaction
145 4) and TMP (reaction 5). Values of k_3 and k_5 were determined as $2 \times 10^5 \text{ s}^{-1}$ [17] and $2 \times 10^9 \text{ M s}^{-1}$ [15],
146 respectively. Reaction 4 and 5 generate phenoxyl radicals $\text{TBL}_{\cdot\text{H}}$ and $\text{TMP}_{\cdot\text{H}}$ that can potentially be
147 involved in several reactions: they can react with oxygen, with TMP or TBL through addition or H-
148 abstraction reactions, or with other radicals [22]. At low photon rate absorption as it is the case here,
149 the phenoxyl radicals concentration is low and radical/radical re-combinations should proceed at lower
150 rates than radical/molecule reactions. Therefore, in this section, we consider that $\text{TBL}_{\cdot\text{H}}$ and $\text{TMP}_{\cdot\text{H}}$
151 will mainly react with molecules. The first possibility is a reaction with oxygen through reactions 6
152 and 7, respectively. To estimate the value of k_6 , we generated $\text{TBL}_{\cdot\text{H}}$ radicals by LFP of TBL (10^{-3} M)
153 and monitored their decay in different conditions. In air as in oxygen-saturated solutions, the decay of
154 $\text{TBL}_{\cdot\text{H}}$ was well fitted by a second order kinetics ($2k/\epsilon = 3.2 \pm 0.3 \times 10^5 \text{ s}^{-1}$, Figure SI-1, Supporting
155 Information), indicating that in these particular conditions where $\text{TBL}_{\cdot\text{H}}$ concentration was very high,
156 radical/radical reactions were dominant while reaction of $\text{TBL}_{\cdot\text{H}}$ with both O_2 ($2.6 \times 10^{-4} \text{ M}$ or 1.3×10^{-3}
157 M) and TBL (10^{-3} M) were negligible. The half-life of $\text{TBL}_{\cdot\text{H}}$ being of $2 \times 10^{-4} \text{ s}$, the absence of oxygen
158 effect on the radical decay indicates that the maximum value of $k_6[\text{O}_2]$ is about 370 s^{-1} , and k_6 smaller
159 than $2.8 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. The second possibility in the case of $\text{TBL}_{\cdot\text{H}}$ radicals is a reaction with TMP
160 (reaction 8). The exact nature of this reaction is not fully defined at this stage. It might be a H-atom
161 transfer from TMP to $\text{TBL}_{\cdot\text{H}}$, or some addition reaction of $\text{TBL}_{\cdot\text{H}}$ on TMP. In both cases, reaction 8
162 constitutes an additional pathway of TMP consumption.

163





171 In the absence of TBL, assuming the **quasi stationaty state (q.s.s.)** hypothesis, the steady state
 172 concentration of $^3\text{HS}^*$, $[\text{}^3\text{HS}^*]_{\text{ss}}$, is equal to:

173
$$[\text{}^3\text{HS}^*]_{\text{ss}} = I_a \Phi_T \frac{1}{k_3 + k_5[\text{TMP}]} \quad (\text{Eq 2})$$

174 where I_a is the light intensity absorbed by HS, Φ_T , is the quantum yield of $^3\text{HS}^*$ formation and $[\text{TMP}]$
 175 the concentration of TMP The initial decay rate of TMP (r_1) is therefore equal to:

176
$$r_1 = -\frac{d[\text{TMP}]}{dt} = I_a \Phi_T \frac{k_5[\text{TMP}]_0}{k_3 + k_5[\text{TMP}]_0} \quad (\text{Eq 3})$$

177 **Where $[\text{TMP}]_0$ is the initial TMP concentration.** When TBL is added to the solution, $[\text{}^3\text{HS}^*]_{\text{ss}}$ is equal
 178 to:

179
$$[\text{}^3\text{HS}^*]_{\text{ss}} = I_a \Phi_T \frac{1}{k_3 + k_4[\text{TBL}] + k_5[\text{TMP}]} \quad (\text{Eq 4})$$

180 and the initial TMP loss rate (r_2) is equal to:

181
$$r_2 = \left(-\frac{d[\text{TMP}]}{dt}\right)_{\text{TBL}} = I_a \Phi_T \frac{k_5[\text{TMP}]_0}{k_3 + k_4[\text{TBL}]_0 + k_5[\text{TMP}]_0} + k_8[\text{TMP}]_0[\text{TBL}_{\cdot\text{H}}]_{\text{ss}} \quad (\text{Eq 5})$$

182 where $[\text{TBL}_{\cdot\text{H}}]_{\text{ss}}$ represents the steady-state concentration of $\text{TBL}_{\cdot\text{H}}$, which can be obtained by the
 183 following equation (Eq 6):

184
$$k_8[\text{TMP}][\text{TBL}_{\cdot\text{H}}]_{\text{ss}} + k_6[\text{O}_2][\text{TBL}_{\cdot\text{H}}]_{\text{ss}} = I_a \Phi_T \frac{k_4[\text{TBL}]}{k_3 + k_4[\text{TBL}] + k_5[\text{TMP}]} \quad (\text{Eq 6})$$

185 and $[\text{TBL}_{\cdot\text{H}}]_{\text{ss}}$ is equal to:

186
$$[\text{TBL}_{\cdot\text{H}}]_{\text{ss}} = I_a \Phi_T \frac{k_4[\text{TBL}]}{k_3 + k_4[\text{TBL}] + k_5[\text{TMP}]} \times \frac{1}{k_6[\text{O}_2] + k_8[\text{TMP}]} \quad (\text{Eq 7})$$

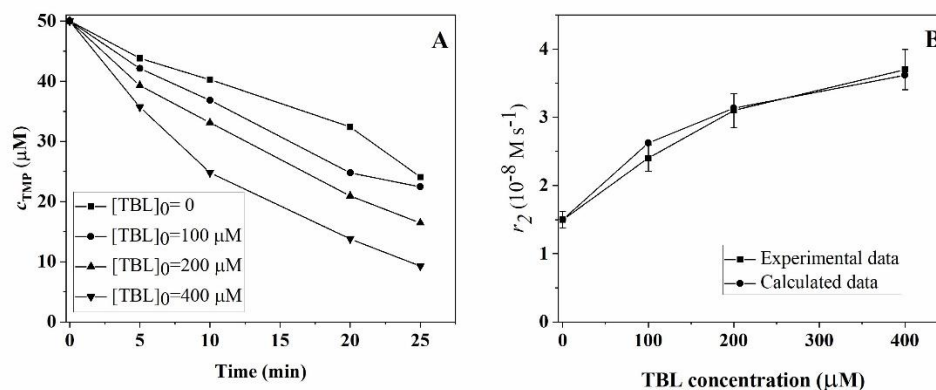
187 Finally, one gets:

$$188 \quad r_2 = \frac{I_a \Phi_T}{k_3 + k_4[\text{TBL}]_0 + k_5[\text{TMP}]_0} \times \left(k_5[\text{TMP}]_0 + \frac{k_8[\text{TMP}]_0 \times k_4[\text{TBL}]_0}{k_8[\text{TMP}]_0 + k_6[\text{O}_2]} \right) \quad (\text{Eq 8})$$

189 **Eq 8** confirms that if reaction 8 did not exist, TBL would decrease the rate of TMP loss due to the
190 competition between TBL and TMP for the reaction with $^3\text{HS}^*$, which is not confirmed
191 experimentally. Therefore, reaction 8 needs to be considered **in this study**. Using Eq 8, we attempted
192 to fit experimental data of Figure 2. **For simplification, we first made the hypothesis that $k_6[\text{O}_2]$ is**
193 **negligible behind $k_8[\text{TMP}]$** . As $k_6[\text{O}_2]$ is $< 370 \text{ s}^{-1}$, this implies that $k_8[\text{TMP}] > 3700 \text{ s}^{-1}$ and thus $k_8 >$
194 $7.3 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$, considering that $[\text{TMP}]_0 = 50 \text{ }\mu\text{M}$. The experimental data were well fitted using $k_4 =$
195 $(1.8 \pm 0.5) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ which is in accordance with the rate constant of reaction with $^3\text{CBP}^*$ and
196 $^3\text{RF}^*$. If $k_6[\text{O}_2]$ is not negligible behind $k_8[\text{TMP}]$, then the consumption of TMP through reaction 8 is
197 less important and k_4 value has to be increased. The value of $(1.8 \pm 0.5) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ is therefore a
198 minimal value.

199 Concerning the nature of reaction 8, we postulated that the reaction is a H-atom transfer between
200 $\text{TBL}_{-\text{H}}\cdot$ and TMP to yield TBL and $\text{TMP}_{-\text{H}}\cdot$ **because** TMP is a good H-atom donor. The H-atom
201 transfer between $\text{TBL}_{-\text{H}}\cdot$ and TMP was further confirmed by DFT calculation estimating the
202 difference of Gibbs free energies before and after this reaction (ΔG). The result with $\Delta G (-0.26 \text{ eV})$
203 < 0 indicates the spontaneity of this reaction from the thermodynamic point of view, supporting our
204 hypothesis that $\text{TBL}_{-\text{H}}\cdot$ can be reduced by TMP and induce its degradation.

205



206

207 Figure 2. Photodegradation of TMP in the presence of HS and different concentrations of TBL (A);
 208 Experimental and calculated decay rates of TMP (in M s^{-1}) under different conditions (B) ($[\text{TMP}]_0 =$
 209 $50 \mu\text{M}$, $[\text{HS}]_0 = 20 \text{ mg L}^{-1}$, $\text{pH} = 8.0$, air-saturated condition).

210 3.3 Antioxidant ability of phenolic moieties

211 To explain the negligible involvement of $^3\text{HS}^*$ in the HS-mediated photodegradation of TBL, we
 212 postulate that like TMP, HS may have the capacity to reduce $\text{TBL}_{\cdot\text{H}}$, as described in reaction 9,
 213 regenerating TBL.



215 The mentioned antioxidant properties of HS have been documented in the case of compounds such as
 216 aniline and sulfonamide antibiotics [9-11]. They are linked to H-atom donor properties, and mainly
 217 attributed to HS phenolic and polyphenolic moieties [12, 13, 23]. Janssen et al. have used mono- and
 218 dihydroxylated cinnamic acids as proxies of some of the hydroquinonic and phenolic moieties in HS
 219 [24]. They found that these analogues of redox-active moieties could suppress the indirect photolysis
 220 of tryptophan with rate constants ranging from 9.7×10^6 to $2.6 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$. After the H-atom
 221 abstraction, HS will undergo irreversible follow-up reactions [25].

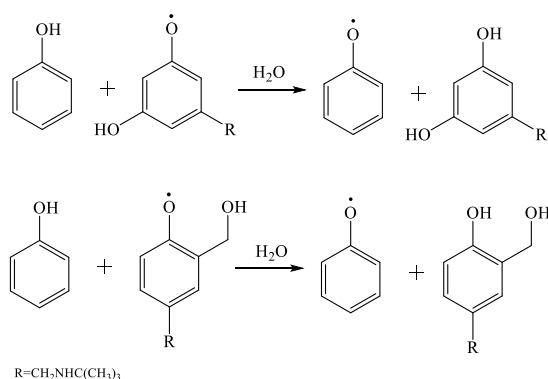
222 Table 1. DFT calculation results for the possibilities of H-abstraction reactions of different substituted
 223 phenols with $\text{TBL}_{\cdot\text{H}}$ (ΔG_1) and $\text{SAL}_{\cdot\text{H}}$ (ΔG_2).

Substituted phenol	Position	ΔG_1 (eV)	ΔG_2 (eV)	Substituted phenol	Position	ΔG_1 (eV)	ΔG_2 (eV)
Phenol	-	-0.147	0.142	TMP	-	-0.260	0.030
	<i>ortho</i> -	-0.076	0.213		<i>ortho</i> -	-0.479	-0.189
-CH ₃	<i>meta</i> -	-0.046	0.243	-NH ₂	<i>meta</i> -	0.031	0.320
	<i>para</i> -	-0.083	0.206		<i>para</i> -	-0.547	-0.258
	<i>ortho</i> -	-0.012	0.278		<i>ortho</i> -	0.411	0.700
-OCH ₃	<i>meta</i> -	0.050	0.339	-COOH	<i>meta</i> -	0.064	0.353
	<i>para</i> -	-0.241	0.049		<i>para</i> -	0.127	0.416
	<i>ortho</i> -	-0.015	0.274		<i>ortho</i> -	-0.010	0.279
-CHO	<i>meta</i> -	0.066	0.355	-NHCOCH ₃	<i>meta</i> -	0.041	0.330
	<i>para</i> -	0.120	0.410		<i>para</i> -	-0.142	0.147
	<i>ortho</i> -	0.092	0.381		<i>ortho</i> -	0.191	0.480
-Cl	<i>meta</i> -	0.054	0.343	-CN	<i>meta</i> -	0.124	0.413
	<i>para</i> -	-0.036	0.253		<i>para</i> -	0.108	0.397

224

225 To further support our conclusion of HS's antioxidant properties on TBL_{-H}•, DFT calculations were
226 performed to evaluate the reaction of a series of substituted phenols with TBL_{-H}• (Scheme 1), as listed
227 in Table 1. These results suggest that many substituted phenols moieties in HS are potentially able to
228 reduce TBL_{-H}• ($\Delta G_1 < 0$), depending on the substituted groups and their positions. For instance, all the
229 three methyl-phenols could reduce TBL_{-H}•. Moreover, similar calculations were also conducted in the
230 case of salbutamol (SAL), a structurally related chemical of TBL, to verify the reliability of the
231 proposed reduction mechanism. As reported previously [14], SAL that exhibited relative high
232 reactivity towards ³HS* ($>10^9 \text{ M}^{-1} \text{ s}^{-1}$) was photodegraded much faster than TBL in the same
233 experimental conditions ruling out a significant importance of the reduction of SAL_{-H}•. As expected,
234 calculation results (ΔG_2 in Table 1) reveal that most of the selected phenols are not able to reduce

235 SAL_{-H}[•] to SAL, indicating that such process was less favourable, in accordance with former
236 conclusions [14].



238 Scheme 1. Proposed reduction pathways of TBL_{-H}[•] and SAL_{-H}[•] by phenols.

239 4. Conclusion.

240 The present study was designed to answer why ³HS* appeared to be negligibly involved in the
241 photochemical transformation of TBL while TBL readily reacts towards ³CBP*, a typical HS model
242 sensitizer. Studying the HS photosensitized degradation of TBL/2,4,6-trimethylphenol revealed that
243 TBL accelerated the photodegradation of the phenolic probe. This behavior was rationalized by
244 postulating a set of reactions in which ³HS* oxidized both TBL and TMP while the radical issued from
245 TBL reacts with TMP, regenerating TBL and consuming TMP. In addition, DFT calculation results
246 further confirmed that TBL_{-H}[•] can be reduced by a lot of phenols *via* H abstraction. The results
247 obtained in this work could explain the poor photodegradation of TBL in the presence of HS, which
248 would be mainly due to the regeneration processes of TBL by the phenol moieties of HS. The present
249 study provides very useful information about the photochemical behavior of TBL in natural waters
250 containing HS, and would also help us to understand the photosensitivity and antioxidant activities of
251 HS.

252 Acknowledgements

253 The authors gratefully acknowledge the financial support from the National Natural Science
254 Foundation of China (Grant No. 21806037).

255 **References**

- 256 [1] R.G. Zepp, G.L. Baughman, P.F. Schlotzhauer, Comparison of photochemical behavior of various
257 humic substances in water: I. Sunlight induced reactions of aquatic pollutants photosensitized by
258 humic substances, *Chemosphere* 10 (1981) 109-117.
- 259 [2] R.G. Zepp, G.L. Baughman, P.F. Schlotzhauer, Comparison of photochemical behavior of various
260 humic substances in water: II. Photosensitized oxygenations, *Chemosphere* 10 (1981) 119-126.
- 261 [3] W.R. Haag, J. Hoigné, Photo-sensitized oxidation in natural water via. OH radicals, *Chemosphere*
262 14 (1985) 1659-1671.
- 263 [4] D.E. Latch, K. McNeill, Microheterogeneity of singlet oxygen distributions in irradiated humic
264 acid solutions, *Science* 311 (2006) 1743-1747.
- 265 [5] K. McNeill, S. Canonica, Triplet state dissolved organic matter in aquatic photochemistry: reaction
266 mechanisms, substrate scope, and photophysical properties, *Environmental Science: Processes &*
267 *Impacts* 18 (2016) 1381-1399.
- 268 [6] W.J. Cooper, R.G. Zika, R.G. Petasne, A.M. Fischer, Sunlight-induced photochemistry of humic
269 substances in natural waters: major reactive species, ACS Publications 1989.
- 270 [7] C.K. Remucal, The role of indirect photochemical degradation in the environmental fate of
271 pesticides: a review, *Environmental Science: Processes & Impacts* 16 (2014) 628-653.
- 272 [8] J.K. Challis, M.L. Hanson, K.J. Friesen, C.S. Wong, A critical assessment of the photodegradation
273 of pharmaceuticals in aquatic environments: defining our current understanding and identifying
274 knowledge gaps, *Environmental Science: Processes & Impacts* 16 (2014) 672-696.
- 275 [9] J. Wenk, U. Von Gunten, S. Canonica, Effect of dissolved organic matter on the transformation of
276 contaminants induced by excited triplet states and the hydroxyl radical, *Environmental science &*
277 *technology* 45 (2011) 1334-1340.
- 278 [10] J. Wenk, S. Canonica, Phenolic antioxidants inhibit the triplet-induced transformation of anilines
279 and sulfonamide antibiotics in aqueous solution, *Environ Sci Technol* 46 (2012) 5455-5462.

280 [11] S. Kouras-Hadef, A. Amine-Khodja, S. Halladja, C. Richard, Influence of humic substances on
281 the riboflavin photosensitized transformation of 2, 4, 6-trimethylphenol, *Journal of Photochemistry*
282 and *Photobiology A: Chemistry* 229 (2012) 33-38.

283 [12] J.T. Nurmi, P.G. Tratnyek, Electrochemical properties of natural organic matter (NOM), fractions
284 of NOM, and model biogeochemical electron shuttles, *Environmental science & technology* 36 (2002)
285 617-624.

286 [13] M.C. Foti, Antioxidant properties of phenols, *J. Pharm. Pharmacol.* 59 (2007) 1673-1685.

287 [14] L. Zhou, M. Sleiman, L. Fine, C. Ferronato, P. de Sainte Claire, E. Vulliet, J.-M. Chovelon, G.
288 Xiu, C. Richard, Contrasting photoreactivity of β 2-adrenoceptor agonists Salbutamol and Terbutaline
289 in the presence of humic substances, *Chemosphere* (2019).

290 [15] S. Halladja, A. Ter Halle, J.-P. Aguer, A. Boulkamh, C. Richard, Inhibition of humic substances
291 mediated photooxygenation of furfuryl alcohol by 2, 4, 6-trimethylphenol. Evidence for reactivity of
292 the phenol with humic triplet excited states, *Environ Sci Technol* 41 (2007) 6066-6073.

293 [16] C. Richard, S. Canonica, Aquatic phototransformation of organic contaminants induced by
294 coloured dissolved natural organic matter, *Environmental Photochemistry Part II*, Springer2005, pp.
295 299-323.

296 [17] S. Canonica, U. Jans, K. Stemmler, J. Hoigne, Transformation kinetics of phenols in water:
297 photosensitization by dissolved natural organic material and aromatic ketones, *Oceanographic*
298 *Literature Review* 12 (1995) 1061.

299 [18] F. Bonnichon, C. Richard, G. Grabner, Formation of an α -ketocarbene by photolysis of aqueous
300 2-bromophenol, *Chem. Commun.* (2001) 73-74.

301 [19] L. Zhou, M. Sleiman, C. Ferronato, J.-M. Chovelon, P. de Sainte-Claire, C. Richard, [Sulfate](#)
302 [radical induced degradation of \$\beta\$ 2-adrenoceptor agonists salbutamol and terbutaline: Phenoxyl radical](#)
303 [dependent mechanisms](#), *Water Res.* 123 (2017), 715-723.

304 [20] M. Frisch, G. Trucks, H. Schlegel, G. Scuseria, M. Robb, J. Cheeseman, G. Scalmani, V. Barone,
305 B. Mennucci, G. Petersson, Gaussian 09, Revision A, Gaussian, Inc., Wallingford CT (2009).

306 [21] J. Tomasi, B. Mennucci, R. Cammi, Quantum mechanical continuum solvation models, *Chem*
307 *Rev* 105 (2005) 2999-3093.

308 [22] P. Neta, J. Grodkowski, Rate Constants for Reactions of Phenoxyl Radicals in Solution, J. Phys.
309 Chem. Ref. Data 34 (2005) 110-199.

310 [23] S. Canonica, M. Freiburghaus, Electron-rich phenols for probing the photochemical reactivity of
311 freshwaters, Environ Sci Technol 35 (2001) 690-695.

312 [24] E.M.-L. Janssen, P.R. Erickson, K. McNeill, Dual roles of dissolved organic matter as sensitizer
313 and quencher in the photooxidation of tryptophan, Environ Sci Technol 48 (2014) 4916-4924.

314 [25] M. Aeschbacher, C. Graf, R.P. Schwarzenbach, M. Sander, Antioxidant properties of humic
315 substances, Environ Sci Technol 46 (2012) 4916-4925.

316

317

318

