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2 Photosensitized degradation of acetaminophen in natural organic matter

3 solutions: The role of triplet states and oxygen

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Abstract

The photolysis of acetaminophen, a widely used pharmaceutical, in simulated natural organic matter solutions was investigated. The triplet states of natural organic matter ($^3\text{NOM}^*$) were found to play the dominant role in its photodegradation, while the contributions from hydroxyl radicals and singlet oxygen were negligible. Dissolved oxygen (DO) plays a dual role. From anaerobic to microaerobic (0.5 mg/L DO) conditions, the degradation rate of acetaminophen increased by 4-fold. That suggests the involvement of DO in reactions with the degradation intermediates. With increasing oxygen levels to saturated conditions (26 mg/L DO), the degradation rate became slower, mainly due to DO's quenching effect on $^3\text{NOM}^*$. Superoxide radical ($\text{O}_2^{\bullet-}$) did not react with acetaminophen directly, but possibly quenched the intermediates to reverse the degradation process. The main photochemical pathways were shown to involve phenoxyl radical and N-radical cations, finally yielding hydroxylated derivatives, dimers and nitrosophenol. A reaction mechanism involving $^3\text{NOM}^*$, oxygen and $\text{O}_2^{\bullet-}$ is proposed.

Keywords: acetaminophen; photodegradation; oxygen; triplet states organic matter

1. Introduction

Acetaminophen (paracetamol), chemically named N-acetyl-p-aminophenol, is one of the most-used over-the-counter pharmaceuticals. It is employed as a fever reducer and a pain reliever in many conditions such as headache, muscle aches, arthritis and so on (Newson et al., 2000). Like other popular pharmaceuticals, acetaminophen and its metabolites are released into aquatic environments through wastewater treatment plants. Previous studies have reported that it is ubiquitous at nanogram and even milligram per liter concentrations in surface waters and even in ground water (Fairbairn et al., 2016; Ma et al., 2016; Paíga and Delerue-Matos 2016). Although the possible interactions of acetaminophen with organisms living in the environment are not well documented, it is suspected to affect aqueous ecology. During the chlorination processes widely used to disinfect drinking water and wastewater, significant amounts of acetaminophen are likely to be transformed into 1, 4-benzoquinone and N-acetyl-p-benzoquinone imine (NAPQI), both fairly toxic (Bedner and MacCrehan 2006). In human medicine, acetaminophen forms a small amount of NAPQI, which could result in liver and kidney damage (Mauldin and Savarie 2010). Acetaminophen's wide prevalence and potential toxicity have increased pressure to investigate its transformation and fate in surface water (De Laurentiis et al., 2014; Liang et al., 2016; Peuravuori 2012).

In general, biodegradation, sorption and photodegradation are the three major processes removing pharmaceuticals from natural waters (Carstens et al., 2013; Li et al., 2016; Onesios and Bouwer 2012; Tixier et al., 2003). Acetaminophen's relatively high water solubility and low partition coefficient account for its partitioning into the water phase, with relatively little left in sediments (Ternes et al., 2002). Sorption alone is responsible for only about 30% of the loss of aqueous-phase acetaminophen after 15 days (Yamamoto et al., 2009). Biodegradation could be an effective loss pathway, but its time scale runs to months (Li et al., 2014). Several studies have recently suggested that photodegradation could be important and competitive with biodegradation.

60 In general, photochemical processes include both direct and indirect photodegradation. Direct
61 photolysis requires that the pollutant's absorption spectrum overlaps with that of solar radiation.
62 Indirect photodegradation would occur in the presence of photosensitizers, which in natural
63 photodegradation are often some natural organic matter (NOM), nitrite and nitrate. The NOM
64 absorbs sunlight to form a singlet excited state ($^1\text{NOM}^*$). Immediately a small fraction of the
65 $^1\text{NOM}^*$ transfers to a triplet excited state ($^3\text{NOM}^*$) through intersystem crossing (ISC), and $^3\text{NOM}^*$
66 then reacts with dissolved oxygen to produce singlet oxygen ($^1\text{O}_2$) by energy transfer (Latch and
67 McNeill 2006; Sun et al., 2015; Zhang et al., 2014). Intramolecular electron transfer between
68 electron donors and acceptors inside $^1\text{NOM}^*$ or $^3\text{NOM}^*$ produces reducing radical intermediates,
69 which further react with dissolved oxygen to yield superoxide anions ($\text{O}_2^{\bullet-}$) (Garg et al., 2011; Zhang
70 et al., 2012). The mechanism for photochemical formation of $\text{HO}^\bullet$ from NOM is still under
71 investigation, likely includes: (i) a H_2O_2 -dependent pathway involves Fe species and photo-generated
72 H_2O_2 , i.e., Fenton or Fenton-like reactions (Vione et al., 2014; Gligorovski et al., 2015); (ii) direct
73 photolysis of hydroxybenzoic acid derivatives (Sun et al., 2015); and (iii) the exciplex produced
74 through the interaction of an excited state of a quinone component initially present in NOM (Page et
75 al., 2011; Pochon et al., 2002).

76 With respect to acetaminophen, Vione's group has reported that the direct photolysis quantum
77 yield is $(4.57 \pm 0.17) \times 10^{-2}$, which results into a relatively slow direct photodegradation rate due to
78 the minimal overlap of acetaminophen's UV absorption spectrum with the wavelengths of solar
79 irradiation (De Laurentiis et al., 2014). They have also suggested that $\text{CO}_3^{\bullet-}$ and $^3\text{NOM}^*$ are two
80 major reactive species involved in acetaminophen's indirect degradation. However, the detailed
81 mechanisms involved are not well documented. In general, energy and electron transfer are two
82 major processes involved in the chemistry of $^3\text{NOM}^*$. Energy transfer would occur when the
83 compounds present have diene groups (Grebel et al., 2011; Song et al., 2007; Yan et al., 2014), while

electron transfer should be preferred when electron-donating functional groups are present, aniline and phenolic groups being common examples (Golanoski et al., 2012; Wenk et al., 2011).

This study focused on the photodegradation of acetaminophen in a NOM enriched solution under simulated solar irradiation. A series of experiments were conducted to explore the roles of reactive oxygen species (ROS) in photosensitized degradation. The reaction rate constants of $^1\text{O}_2$ with acetaminophen were determined over a range of pH values and were shown to be higher under alkaline conditions. The contribution of $\text{O}_2^{\bullet-}$ was eliminated by adding superoxide dismutase (SOD). The direct reaction of $^3\text{NOM}^*$ with acetaminophen was also studied, and the degradation mechanisms were elucidated through Liquid Chromatograph/Quadrupole-Time of Flight (LC-QTOF) high resolution mass spectrometry. The results suggest that electron transfer from acetaminophen to $^3\text{NOM}^*$ plays a key role. It yields an important intermediate-phenoxyl radical, which either combines to yield dimers or is decomposed by dissolved oxygen.

2. Materials and methods

2.1 Materials

Acetaminophen (ACE, 99%), furfuryl alcohol (FFA, 98%), furfuraldehyde (FAD, 99%), D_2O (99.9%), 2-propanol, Rose Bengal (RB, 90%), terephthalic acid (TA), trifluoroacetic acid (HPLC grade), superoxide dismutase (SOD) and diethylenetriaminepentaacetate (DTPA) were purchased from Sigma-Aldrich. Methanol and acetonitrile (HPLC grade) were obtained from Merck (Germany). Suwannee River NOM (SRNOM, 1R101N), Nordic Reservoir NOM (NRNOM, 1R108N), Pahokee peat fulvic acid (PPFA, 1R107F), Pony Lake fulvic acid (PLFA, 1R109F), Suwannee River fulvic acid standard I (SRFA I, 1S101F), Suwannee River fulvic acid standard II (SRFA II, 2S101F) and Suwannee River humic acid standard II (SRHA, 2S101H) were obtained from the International Humic Substances Society. 2-methyl-6-[p-methoxyphenyl]-3, 7-dihydroimidazo [1,2-a] pyrazin-3-one, and a methyl cypridina luciferin analog (MCLA) were purchased from TCI Chemicals. 2-hydroxyl

terephthalic acid (2HTA) was synthesized using a published method (Mason et al., 1994).

2.2 Photochemical experiments

The lighting system was a solar simulator (Suntest XLS⁺) with a 1700W xenon lamp and a solar filter which produces light with wavelengths above 290 nm. The absolute irradiance spectrum of the solar simulator shown in Figure SI-1 of the supplementary material was recorded using an Ocean Optics USB-4000 spectrometer. The absolute intensity of irradiation between 300 and 400 nm was fixed at 40W/m², and the chamber temperature was controlled at 25 ± 1 °C.

Twenty mL of 20 µM acetaminophen solution was irradiated in a closed quartz vessel with silica gel stopper under simulated solar irradiation with a 10 mg L⁻¹ (as DOC) solution of SRNOM added. The solutions were buffered with 5.0 mM phosphate buffer to pH 7 except in the tests evaluating pH effects. A 0.5 mL aliquot of the solution was withdrawn at periodic intervals over a 60-hour irradiation period and each aliquot was analyzed using High Performance Liquid Chromatography (HPLC) with photodiode array detector (PAD) (Agilent 1260). All of the irradiation experiments were conducted in at least two or more replicates.

The role of each ROS was assessed using the selective quenchers 2-propanol (26 mM) for HO[•] and 75 units per milliliter of SOD for superoxide. A subset of the experiments was conducted in D₂O to enhance any ¹O₂ effects. To evaluate the role of ³NOM* and dissolved oxygen, tests with different dissolved oxygen concentrations were conducted. Gas with different volume ratios of oxygen, nitrogen and argon, including 100% argon (DO<0.1 mg/L), 100% nitrogen (DO=0.5 mg/L), 10% oxygen +90% nitrogen (DO=4.1 mg/L), 20% oxygen +80% nitrogen (DO=7.2 mg/L), 40% oxygen +60% nitrogen (DO=12.2 mg/L), 80% oxygen +20% nitrogen (DO=20.3 mg/L) and 100% oxygen (DO=26.5 mg/L) was obtained directly from a gas company (Guangzhou Junduo Co., Ltd., China). It was purged into the solution throughout the tests and the concentration of dissolved oxygen was measured by a portable DO meter (Mettler Toledo, Switzerland) and maintained during

the tests.

2.3 Detection of ROS concentrations

¹O₂. The selective probe compound furfuryl alcohol (FFA, 0.03mM) was employed to measure the steady state concentration of ¹O₂ ([¹O₂]_{ss}). The steady state concentration can be calculated as follows:

$$\frac{d[\text{FFA}]}{dt} = k_{i_{\text{O}_2, \text{FFA}}} [\text{FFA}] [{}^1\text{O}_2]_{ss} \quad (2)$$

Here, [¹O₂]_{ss} was determined by dividing the observed FFA degradation rate by the reaction rate constant of FFA with ¹O₂ (8.3 × 10⁷ M⁻¹ s⁻¹). (Latch et al., 2003) and the residual concentration of FFA can be determined by HPLC-PAD (Agilent 1260). The mobile phase was 35% acetonitrile and 65% H₂O, both containing 0.05% trifluoroacetic acid (TFA). The flow rate was 0.7 mL min⁻¹. The detection wavelength was 214 nm, and the column was thermostated at 30°C. The injection volume was 20 µL.

HO•. To explore the steady state concentration of HO• in the NOM enriched solutions ([HO•]_{ss}), varied concentrations of TA were employed to trap the radical and produce 2HTA, as illustrated in equation (3).

The concentration of 2HTA was detected by HPLC with a fluorescence detector (excitation wavelength 315 nm; emission wavelength 425 nm) (Agilent 1260). The mobile phase consisted of 35% methanol (v/v) and 65% H₂O (with 0.05% trifluoroacetic acid, v/v) at a flow rate of 1.0 mL min⁻¹.

The initial formation rate of 2HTA can be expressed as in equation (4):

$$R_{2HTA} = \frac{d[2HTA]}{dt} = \eta k_{HO\bullet,TA}[TA][HO\bullet] \quad (4)$$

where η is the reaction yield, taken as 28% (Charbouillot et al., 2011) and $k_{HO\bullet,TA}$ is the bimolecular reaction rate constant, taken as $3.3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ (Mark et al., 1998). Meanwhile, the $[HO\bullet]$ can also be expressed as:

$$[HO\bullet] = \frac{R_{HO\bullet}}{S + k_{HO\bullet,TA}[TA]} \quad (5)$$

Where S represents the scavenging rate constant for $HO\bullet$ by SRNOM and $R_{HO\bullet}$ is the $HO\bullet$ formation rate. In the absence of TA, equation (5) can be simplified as $[HO\bullet] = \frac{R_{HO\bullet}}{S}$.

Combining equations (4) and (5) one gets:

$$R_{2HTA} = \eta k_{HO\bullet,TA}[TA] \frac{R_{HO\bullet}}{S + k_{HO\bullet,TA}[TA]} \quad (6)$$

Figure SI-2 shows how R_{2HTA} could vary as a function of $[TA]$. When $[TA] \rightarrow 0$, it would give the slope of the tangent to the curve (the red dotted line). Therefore $[HO\bullet]$ can be calculated as $(4.3 \pm 0.1) \times 10^{-16} \text{ M}$.

Superoxide radical anion. A flow injection analysis system (Waterville Analytical, Waterville, ME) was employed to detect the steady state $O_2^{\bullet-}$ concentration ($[O_2^{\bullet-}]_{ss}$). 2-Methyl-6-[p-methoxyphenyl]-3,7-dihydroimidazo[1,2-a]pyrazine-3-one (MCLA) was used as a chemiluminescent probe that reacts selectively with $O_2^{\bullet-}$. The test solution was $1.0 \mu\text{M}$ MCLA in 0.05 M acetate buffer and the pH was adjusted to 5.5 by adding HCl. The MCLA solution and the sample solution were pumped into the mixing cell and the reaction product gave a chemiluminescence signal at 455 nm which was detected using a photomultiplier. The experimental details can be found in our previous publication (Zhang et al. 2014)

The system was calibrated by adding a known volume of superoxide standard solution to obtain samples with different concentrations. In brief, the superoxide standard solutions were

prepared by photolysis of a mixed solution of 12 M absolute ethanol, 41 M acetone and 15 μ M DTPA at pH 12 using 1mM borate buffer. The mixture was irradiated with a UV₂₅₄ lamp and superoxide was determined by measuring the UV absorbance at 240 nm and dividing $\epsilon_{240\text{nm}}$ (2350 M⁻¹ cm⁻¹) (Rose et al., 2008; Rusak et al., 2011). No solvent interference has been observed during the preparation of superoxide standard solutions. The fitted standard curves are shown in Figure SI-3.

2.4 Reaction rate constant of ¹O₂ with acetaminophen

Rose Bengal (0.1 mM) was used as a photosensitizer for ¹O₂ production. Acetaminophen (20 μ M) and FAD (100 μ M) were added to achieve the competition kinetics under irradiation by simulated sunlight. The reaction rate constant of ¹O₂ and acetaminophen can be obtained using the following formula:

$$\frac{k_{1O_2, \text{acetaminophen}}}{k_{1O_2, \text{FAD}}} = \frac{\ln([\text{acetaminophen}]/[\text{acetaminophen}]_0)}{\ln([\text{FAD}]/[\text{FAD}]_0)} \quad (7)$$

where, $k_{1O_2, \text{FAD}}$ was taken as $8.4 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ (Gollnick and Griesbeck 1985). The loss of acetaminophen was monitored along with the loss of FAD using HPLC-PAD as mentioned above.

2.5 Analytical methods for acetaminophen and the photodegradation products

The acetaminophen concentrations were analyzed using a 1260 series Agilent HPLC system equipped with a C18 column (4.6 $\times$ 75 mm, 1.8 μ m particle size). The mobile phase consisted of 65% H₂O (with 0.05% TFA, v/v) and 35% methanol (v/v) with a flow rate of 1.0 mL/min. The photodiode array detector was set at 244 nm.

Photoproducts were examined via high-resolution LC-QTOF using an Agilent 1290-6540 mass spectrometer equipped with a Zobrax C18 column (2.1 $\times$ 100 mm, 1.8 μ m particle size). The spectrometer was used in positive electrospray ionization mode with time-of-flight mass scanning. The MS settings were especially optimized as follows: nebulizer gas at 60psi, capillary voltage at 3000V, and the fragmentor at 160V. The sample volume was 20 μ L, and all the samples were

analyzed in quadruplicate in order to ensure the accuracy and repeatability of the mass spectroscopy. The Mass Profiler Professional statistical software suite (Agilent) was used to assist with the identification of the products.

3. Results and discussion

3.1 The role of ROS in the indirect photolysis of acetaminophen

The direct photolysis of acetaminophen at pH 7.0 under simulated solar irradiation was very slow ($k = (2.50 \pm 0.20) \times 10^{-6} \text{ s}^{-1}$). The rate was enhanced four-fold in the presence of SRNOM ($k = (1.04 \pm 0.04) \times 10^{-5} \text{ s}^{-1}$), as is shown in Figure 1. The rates with all seven NOM isolates (SRNOM, NRNOM, PPFA, PLFA, SRFA I, SRFA II, SRHA), are shown in Figure SI-4. The fastest decay was observed in the PLFA solution ($t_{1/2} \sim 6.7 \text{ h}$), while NRNOM was the least reactive ($t_{1/2} \sim 17.5 \text{ h}$). The difference in decay rates among the different NOM solutions could be due to the diverse photo-reactivity of NOM. Still, NOM is considered an active photosensitizer, and it generates ROS such as $\text{HO}^\bullet$, $^1\text{O}_2$, and $^3\text{NOM}^*$ under solar irradiation (Bodhipaksha et al., 2015; Zeng and Arnold 2012). To explore the role of these ROS in the degradation of acetaminophen, SRNOM was used in further investigations.

(Insert Figure 1)

Role of $\text{HO}^\bullet$. Addition of excess 2-propanol (26 mM) did not change acetaminophen's degradation rate, suggesting that $\text{HO}^\bullet$ makes little contribution to the degradation process (Figure 1). Although 2-propanol could potentially interfere with acetaminophen's direct photolysis by scavenging its excited states (Vione et al., 2010), such an inhibiting effect would not be important here due to the low importance of the acetaminophen direct photolysis. The reaction rate constant between $\text{HO}^\bullet$ and acetaminophen has been reported as $1.7 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ (Yang et al., 2009). The steady state $\text{HO}^\bullet$ concentration in the SRNOM solution tested was $(4.3 \pm 0.1) \times 10^{-16} \text{ M}$ (Figure SI-2). Therefore the contribution of $\text{HO}^\bullet$ to the observed degradation was only 7% at pH 7 (Table 1), which

confirms an insignificant role for HO[•] in the decay of acetaminophen.

(Insert Table 1)

Role of ¹O₂. Singlet oxygen has a longer lifetime in D₂O than in H₂O, thus increased acetaminophen loss would be expected if ¹O₂ is important in the photodegradation process. As Figure 1 shows, the decay rate of acetaminophen in D₂O/SRNOM was $(1.47 \pm 0.03) \times 10^{-5} \text{ s}^{-1}$, which was slightly faster than in H₂O. This indicates that ¹O₂ makes only a minor contribution to the loss of acetaminophen.

The reaction rate constant of ¹O₂ with acetaminophen was investigated at pH 5–10, and the data are shown in Figure SI-5. At pH 7 the reaction rate constant was $3.35 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ using Rose Bengal as a photosensitizer and FAD as the reference compound. The pH increase greatly enhanced the reaction rate constant, which at pH 10 reached $2.26 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$. The pK_a value of acetaminophen is 9.5. At higher pH, acetaminophen occurs exist in the form of phenolate ion, which generally has a higher reaction rate constant with ¹O₂ due to the high electron density on the aromatic ring (Schwarzenbach et al., 2005; Yang et al., 2013). The steady state ¹O₂ concentration at pH 7 was $4.82 \times 10^{-13} \text{ M}$, and it did not vary much at the other tested pHs. The contribution from ¹O₂ to the observed degradation was 1.5% at pH 7 and 9.2% at pH 10. As suggested by the quencher results, ¹O₂ was not the major species accounting for the loss of acetaminophen.

Role of ³NOM*. Since both HO[•] and ¹O₂ are only trivial contributors to the photosensitized degradation of acetaminophen, ³NOM* is perhaps significant. The influence of ³NOM* was investigated, and the results are shown in Figure 2. As O₂ is known to be a triplet quencher, (deoxygenated) nitrogen- and oxygen-purged solutions were used to modify the ³NOM* concentrations. As the oxygen concentration increased from 0 to 0.5 mg/L, the acetaminophen decay rates increased sharply. The loss of acetaminophen was the fastest at an oxygen concentration of 0.5 mg/L. Furthermore, significant inhibition was observed for oxygen purged solutions from 0.5 to

12mg/L, beyond which the degradation remained constant in oxygen-saturated conditions. This indicates that the role of dissolved oxygen in the photosensitized degradation is intricate. To explain it, the mechanism illustrated in Scheme 1 is proposed. In general dissolved oxygen acts as quencher for triplet excited states, in good agreement with the results observed in the oxygen-purged conditions. With increasing oxygen concentration the steady state concentration of $^3\text{NOM}^*$ would be expected to drop and the decay rate of acetaminophen to attenuate. However, the degradation remained stable in excess oxygen, suggesting that the contribution of $^1\text{O}_2$ gradually stand out and offset the decreased $^3\text{NOM}^*$. The enhanced degradation rates from argon to micro-aerobic conditions suggest that molecular oxygen is also involved in the degradation mechanism but that it probably reacts with the photochemically-induced intermediates. Previous studies have suggested that $^3\text{NOM}^*$ reacts with phenolic compounds to yield phenoxyl radical, as illustrated in Scheme 1 (Canonica et al., 1995; Golanoski et al., 2012). The phenoxyl radical can be further decomposed by oxygen. That could be the rate-limiting step at low oxygen levels. With increasing oxygen level the rate-limiting step could switch to the electron transfer step involving $^3\text{NOM}^*$ and acetaminophen.

(Insert Figure 2, Scheme 1)

Role of $\text{O}_2^{\bullet-}$. The $\text{O}_2^{\bullet-}$ steady state concentrations ($[\text{O}_2^{\bullet-}]_{\text{ss}}$) in the acetaminophen/NOM solutions were measured during the irradiation. They were in the range of 10^{-9} to 10^{-8} M, and the values decreased linearly when the initial concentration of acetaminophen increased from 0 to 3.3 μM , as shown in Figure 3a. Meanwhile, $[\text{O}_2^{\bullet-}]_{\text{ss}}$ was below the detection limit at ($<10^{-10}$ M) in the 20 μM acetaminophen NOM solutions. This indicates that $\text{O}_2^{\bullet-}$ was quenched by either acetaminophen or its photoproducts (the intermediates). Kozmér has reported that the bimolecular reaction rate constant of phenol with $\text{O}_2^{\bullet-}$ is as slow as $(2.7 \pm 1.2) \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ (Kozmér et al., 2014). Considering the similarity of acetaminophen with phenol, that rate constant might be employed to estimate the role of $\text{O}_2^{\bullet-}$ in the photosensitized degradation of acetaminophen. Based on the multiplication of

[O₂^{•-}]_{ss} and the bimolecular reaction rate constant, the contribution of [O₂^{•-}]_{ss} in the photodegradation of acetaminophen was estimated to be less than 3%. The reaction of acetaminophen with O₂^{•-} can therefore be neglected and the O₂^{•-} assumed to react primarily with the degradation intermediates.

To further elucidate the role of O₂^{•-}, SOD, converting O₂^{•-} to H₂O₂, was added to reduce the O₂^{•-} concentration. Figure 3b illustrates that the acetaminophen decay rate increased with increasing SOD doses. A likely explanation is that the phenoxyl radical, which could be formed upon acetaminophen oxidation by ³NOM^{*}, would quickly react with O₂^{•-} to regenerate acetaminophen and molecular oxygen (Wuttig et al., 2013). With increasing SOD that back reaction would be reduced, leading to up to 4-fold increases in the decay rate of acetaminophen.

(Insert Figure 3)

3.2 Acetaminophen's photodegradation products

In order to provide mechanistic insight into the reactions and the photochemical fate of acetaminophen, the products of indirect photodegradation were identified using LC-QTOF system. That provided exact m/z values relating to the chemical formulas, as shown in Table 2 that also reports product acronyms.

(Insert Table 2)

Based on the analysis of the mass spectra, the P₃₀₀ photoproduct was identified as corresponding to a dimer molecule with a relative mass error of 1.0 ppm. This could be attributed to the initial reaction of ³NOM^{*} with acetaminophen forming a phenoxyl radical, which is generally considered to be a long-lived intermediate (Canonica and Freiburghaus 2001). Therefore the self-combination of phenoxyl radicals could be observed yielding either dihydroxybiphenyls or phenoxyphenols (De Laurentiis et al., 2013). This proposed mechanism might also be involved in the oligomerization of phenols and is similar to the expected pathways in the formation of humic-like substances in the atmosphere (De Laurentiis et al., 2013; Liu et al., 2012). Furthermore the phenoxyl

radical would be oxidized by dissolved oxygen to yield carbonyl-peroxyl radicals. Carbonyl-peroxyl radicals are also subject to self-combination, producing tetroxide intermediates which can then decompose to give a variety of products (von Sonntag and Schuchmann 1997). In this case a product with a molecular weight (MW) of 165 was observed to accumulate in the solutions, as shown in Scheme 2. It is important to realize that carbonyl-peroxyl radicals also react with $\text{HO}_2^\bullet$ or $\text{O}_2^{\bullet-}$ to produce a pair of peroxide products $\text{P}_{183\text{a}}$ and $\text{P}_{183\text{b}}$. Both $\text{P}_{183\text{a}}$ and $\text{P}_{183\text{b}}$ display similar formation rates under simulated solar irradiation. They could also undergo dimerization and eliminate one H_2O_2 to form P_{332} .

(Insert Scheme 2)

Furthermore the electron transfer processes involving $^3\text{NOM}^*$ could also occur at the acetamide group to produce a N-radical cation (Scheme 2), which could be stabilized by the neighboring aromatic ring and carbonyl group. It would further combine with $\text{O}_2^{\bullet-}$ and hydrolyze, yielding the peroxide product of MW 141, and then dehydrate to give a product of MW 123.

The photodegradation products detected (using LC-MS) as a function of irradiation time are illustrated in Figure 4. Since the products have similar structures, their response factors (peak intensity/molecule) can be assumed to be similar. If so, the peak intensities shown in Figure 4 are indicative of the relative yields. The acetaminophen dimers were the major products initially, reaching a maximum concentration after 5min of irradiation. Those initial products are readily degraded upon continued irradiation. Reduction of carbonyl-peroxyl radicals by $\text{O}_2^{\bullet-}$ is also a significant reaction pathway. Electron transfer involving N atoms can also occur; 4-nitrosophenol (P_{123}) is expected to be a major accumulated product.

(Insert Figure 4)

Conclusions

These results obtained in this work suggest that reaction with $^3\text{NOM}^*$ is a major route for the

photosensitized degradation of acetaminophen in sunlit aquatic environments. The half-life of acetaminophen is between 6.7 and 17.5 hours depending on the type of NOM. The other photodegradation pathways for acetaminophen—direct photolysis and reactions with $^1\text{O}_2$ and $\text{HO}^\bullet$ radicals—are of lesser importance due to the light screening effects and the low rate constants of these species' reactions with acetaminophen. Dissolved oxygen plays a dual role. It is involved in reactions with the degradation intermediates and also quenches the excited state of NOM constituents. Therefore, the degradation of acetaminophen in different layers of a river or lake will vary greatly and depending on the dissolved oxygen concentration. Eight degradation products have been identified using LC-QTOF MS, and the degradation pathways involve the reaction of $^3\text{NOM}^*$, $\text{O}_2^{\bullet-}$, and dissolved oxygen.

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470 **Scheme 1.** Proposed initial step in the indirect photodegradation of acetaminophen in NOM-enriched
 471 solutions.

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475 **Scheme 2.** Identification of the degradation products of $^3\text{NOM}^*$'s reaction with acetaminophen

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479 **Table 1. The percentage contributions of ROS during photochemical transformation of**
 480 **acetaminophen.**

Type			Contribution %
direct	Rate (s ⁻¹)	2.50×10 ⁻⁶	24
¹ O ₂	k _{IO₂· ACE} (M ⁻¹ s ⁻¹)	3.35×10 ⁵	1.4
	[¹ O ₂] _{ss} (M)	4.82×10 ⁻¹³	
	k _{IO₂· ACE} ×[¹ O ₂] _{ss} (s ⁻¹)	1.61×10 ⁻⁷	
HO•	k _{OH, ACE} (M ⁻¹ s ⁻¹)	1.7×10 ⁹	7.0
	[HO•] _{ss} (M)	4.3×10 ⁻¹⁶	
	k _{OH, ACE} ×[•OH] _{ss} (s ⁻¹)	7.31×10 ⁻⁷	
O ₂ ^{• -}	k _{O₂^{• -}, ACE} (M ⁻¹ s ⁻¹)	2.7×10 ^{3 a}	< 2.6
	[O ₂ ^{• -}] _{ss} (M)	<10 ⁻¹⁰	
	k _{O₂^{• -}, ACE} ×[O ₂ ^{• -}] _{ss} (h ⁻¹)	<2.7×10 ⁻⁷	
³ [NOM]*			>65

481 ^a estimated value from $K_{HO_2\bullet + phenol} = (2.7 \pm 1.2) \times 10^3 M^{-1}s^{-1}$

482 Acetaminophen = 20 μM , SRNOM = 10 mg/L DOC, pH = 7

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Table 2. Identified photochemical transformation products of acetaminophen.

	Theoretical	Measured	Chemical	Mass error
	<i>m/z</i>	<i>m/z</i>	Formula	(ppm)⁴⁸⁸
Acetaminophen	152.0706	152.0708	C ₈ H ₉ NO ₂	1.3
P ₃₀₀	301.1183	301.1186	C ₁₆ H ₁₆ N ₂ O ₄	1.0
P ₂₅₈	259.1077	259.1087	C ₁₄ H ₁₄ N ₂ O ₃	3.9
P _{183a}		184.0603		-0.5
	184.0604		C ₈ H ₉ NO ₄	
P _{183b}		184.0600		-2.2
P ₁₆₅	166.0499	166.0496	C ₈ H ₇ NO ₃	-1.8
P ₃₃₂	333.1081	333.1084	C ₁₆ H ₁₆ N ₂ O ₆	0.9
P ₁₄₁	142.0499	142.0494	C ₆ H ₇ NO ₃	-3.5
P ₁₂₃	124.0393	124.0389	C ₆ H ₅ NO ₂	-3.2

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491 **Figure 1.** Photolysis of acetaminophen (20 μM) in the absence and presence of SRNOM (10 mg/L
 492 DOC) under simulated sunlight irradiation at pH 7.0. The results of selective quencher experiments
 493 illustrating the effects of 2-propanol ($\bullet\text{OH}$ quencher) and D_2O (a singlet oxygen promoter) are also
 494 shown.

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506 **Figure 2.** Photolysis of acetaminophen (20 μM) in the presence of SRNOM (10 mg/L DOC) under
507 simulated sunlight irradiation at pH 7.0 with various dissolved oxygen concentrations.

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Figure 3. (a) The steady state concentrations of $O_2^{\bullet-}$ in SRNOM solutions with different initial acetaminophen concentrations and (b) the effect of superoxide dismutase (an $O_2^{\bullet-}$ quencher).

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518 **Figure 4.** Reaction byproducts (listed by MW) from the photosensitized degradation of
519 acetaminophen (PA means Peak Area.)

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521 **Graphic abstract**

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