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Complete List of Authors:	Milstead, Reid; Willamette University, Chemistry Nance, Keeton; Willamette University, Chemistry Tarnas, Kulananalu; Willamette University, Chemistry Egelhofer, Kira; Willamette University, Chemistry; University of Oregon, Chemistry and Biochemistry Griffith, David R.; Willamette University, Chemistry

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Reid P. Milstead, Keeton T. Nance, Kulananalu S. Tarnas, Kira E. Egelhofer, and David R. Griffith*

Department of Chemistry, Willamette University, Salem, OR, USA

* *Corresponding author*

Environmental Significance Statement

Halogenated estrogens have been detected in treated wastewater, yet the environmental fate of these moderately potent endocrine-disrupting chemicals is poorly understood. Our study investigated the photochemical degradation of halogenated estrogens under natural sunlight. We found that estrogen photolysis rates increased as the number of halogen substituents on estrogens and the pH of the water increased. Overall, our results suggest that halogenated estrogens degrade rapidly by direct photolysis at pH 7, a finding that has important implications for removing halogenated estrogens from treated wastewater and characterizing the overall flux of estrogen to natural waters.

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Department of Chemistry, Willamette University, Salem, OR, USA

* *Corresponding author*

Abstract

Halogenated estrogens are thought to be moderately potent endocrine-disrupting compounds that are formed during chlorine-based wastewater disinfection processes and may represent a significant fraction of the total amount of estrogen delivered from wastewater treatment plants to receiving waters. Yet we lack key information about the photochemical degradation of halogenated estrogens, a process that has important implications for UV-based wastewater treatment and environmental fate modeling. To better understand halogenated estrogen degradation in aquatic environments, we studied the direct photolysis of 17β-estradiol (E2), 2-bromo-17β-estradiol (monoBrE2), 2,4-dibromo-17β-estradiol (diBrE2), and 2,4-dichloro-17β-estradiol (diClE2) as well as the indirect photolysis of diBrE2 under natural solar irradiance. We found that direct photolysis rate constants increased with halogenation as pK_a values decreased and molar absorptivity spectra shifted toward higher wavelengths. Compared to E2, quantum yields were threefold larger for monoBrE2, but 15-32% smaller for the dihalogenated forms. The rate of diBrE2 (pK_a ~ 7.5) photolysis was strongly influenced by pH. At pH 7, diBrE2 degraded on minute time scales due to the large red-shifted molar absorptivity values and greater quantum yields of the phenolate form. Degradation rates were only slightly different in the presence of Suwannee River Humic Acid (5 mg L^{-1}), and quenching experiments pointed to excited triplet state dissolved organic matter (^{3}DOM*) as the dominant reactive intermediate responsible for the indirect photolysis of diBrE2. Overall, our data suggest that halogenated estrogens are particularly susceptible to photochemical degradation at environmentally relevant pH values.

Introduction

Estrogens are essential hormones for the proper growth and development of all vertebrate species. Yet, at concentrations as low as nanogram per liter (ng L^{-1}), estrogens can act as potent endocrine disrupting compounds, causing feminization, infertility, and reduced overall sexual fitness of a range of aquatic organisms.[1-9] Estrogens exist in free, conjugated, and halogenated forms in the environment (Figure 1).[10-14] As the bioactive form, free estrogens are particularly potent.[15] Vertebrates naturally excrete estrogens as free forms as well as glucuronide and sulfate conjugates,[16, 17] which can be transformed back into free estrogens by bacteria present in water treatment systems and natural waters.[18] Chlorinated estrogens are formed when estrogens react with free chlorine (hypochlorite/hypochlorous acid) during wastewater disinfection processes.[19, 20] If bromide ions are present, free chlorine is rapidly converted to free bromine (hypobromite/hypobromous acid),[21] which can subsequently react with estrogens to form brominated derivatives.[19, 22]

Wastewater treatment plant (WWTP) effluent contains free estrogens at concentrations that range from ng L^{-1} to μg L^{-1}.[13, 18, 23-25] The distribution and concentration of estrogens in a particular effluent depends on source water chemistry, hydraulic flow rates, treatment efficiencies, and disinfection methods.[13, 18] While effluent is a well-studied and important source of free estrogens to natural waters,[14, 26] less is known about halogenated estrogens, which have been detected in WWTP effluent from Japan to the United States.[12, 20] In Boston, Massachusetts (USA), effluent contained a range of chlorinated and brominated estrogens at concentrations that matched or exceeded the free forms. MonoBrE2 was observed at particularly high levels, in part because Boston's WWTP is located near the coast and influent contains relatively high bromide ion concentrations due to seawater intrusion.[12]

Despite evidence that halogenated estrogens are present in wastewater, have the potential to bioaccumulate in fish,[27] and are thought to retain approximately 0.01% to 10% of the estrogenic potency of free estrogens,[22, 28, 29] we lack a clear understanding of halogenated estrogen distributions and the factors that control the rates at which these forms degrade in the environment. Likely environmental removal processes include flushing (*i.e.*, dilution), sorption of estrogens to solids followed by sedimentation and burial, biodegradation by microbial communities, and direct and indirect photochemical degradation.

Photochemical degradation (photolysis) is likely an important removal mechanism in sunlit surface waters. Direct photolysis occurs when a compound absorbs a photon of light, leading directly to degradation. The direct photolysis rate constant is determined, in part, by the number of photons that can be absorbed by the compound of interest, a value that can be estimated using the molar absorptivity spectrum of the compound and the relevant irradiance spectrum. The direct photolysis rate constant also depends on the fraction of absorbed photons that lead to degradation events and is represented by the quantum yield (Φ). Indirect photolysis is the result of the compound of interest reacting with photochemically produced reactive intermediates (PPRIs) such as hydroxyl radical (•OH), singlet oxygen ($^1O_2$), and excited triplet state dissolved organic matter (^{3}DOM*), which are formed when sunlight interacts with natural organic matter or nitrate ions.[30-32]

Previous studies of free estrogen photodegradation have found that direct photolysis plays a minor role in overall photolysis rates compared to indirect processes.[33, 34] This trend is not surprising because free estrogens absorb very little light at wavelengths longer than 290 nm (Figure 2), where natural sunlight irradiance is important. In contrast, the electronic characteristics of halogenated estrogens, much like chlorinated triclosan,[35] should permit greater absorbance of light above 290 nm, and thus, we hypothesized that direct photolysis may be an important environmental removal process for halogenated estrogens.

The direct photodegradation of free estrogens, such as 17β-estradiol (E2), has been studied extensively under simulated artificial sunlight. Literature rate constants and quantum yields for estrogens are highly variable. Reported direct photolysis half-lives ($t_{1/2}$) of free estrogens under simulated sunlight or UV lamps range from 23 min[36] to 126 h.[37] The only study to use natural sunlight found much longer half-lives ($t_{1/2}$ ~ 40 - 75 d),[38] an effect that is likely due to differences in the power and wavelength distributions of simulated versus natural sunlight.[39]

Direct photolysis quantum yields of E2 reported in the literature also varied over two orders of magnitude (Φ = 0.0033 - 0.10 mol Einstein^{-1}) under different irradiation conditions and wavelength ranges.[40-43] For example, Lin and Reinhard[41] and Chowdhury *et al.*[42] conducted their experiments using a xenon arc lamp and a radiometer, while Mazellier *et al.*[40] used a mercury lamp combined with a phenol actinometer. Given that solar simulators and UV lamps are an imperfect approximation for "typical" sunlight spectra, the quantum yields and rate constants

determined using these artificial light sources have limitations for modeling photolysis in natural aquatic systems.

The present study was therefore designed to characterize the photochemical degradation of free and halogenated estrogens under natural solar irradiance. We employed chemical actinometry along with rooftop sunlight exposure experiments to determine photolysis rate constants, quantum yields, and the relative importance of indirect processes as a function of pH and halogen substituent identity and quantity. These findings will help improve our ability to predict the photochemical fate of estrogens and other phenolic contaminants in aquatic systems.

Experimental

Chemicals, glassware, and pH measurements

17β-estradiol (E2, ≥ 98%) was purchased from Sigma-Aldrich. 2-bromo-17β-estradiol (monoBrE2) and 2,4-dibromo-17β-estradiol (diBrE2) were purchased from Steraloids Inc. 2,4-dichloro-17β-estradiol (diClE2) was provided by Dr. Hiroshi Matsufuji. Optima grade methanol (MeOH; 99.9%; 0.2 micron filtered) was purchased from Fisher Scientific. *p*-nitroacetophenone (PNAP; 98%), *p*-nitroanisole (PNA; 97%), and CHROMASOLV grade pyridine (PYR; ≥ 99.9%) were purchased from Sigma-Aldrich. Hydrochloric acid (HCl; trace metal grade) and sodium hydroxide (NaOH; >98%) were obtained from Fisher Scientific. Compounds used in quenching experiments included *trans*-*trans*-2,4-hexadienoic acid (sorbic acid; >99%; Sigma-Aldrich), L-histidine (>99%; Sigma-Aldrich) and isopropyl alcohol (Optima grade; Fisher Scientific). Suwannee River Humic Acid (SRHA; standard II) was purchased from the International Humic Substances Society. Nitrogen gas was ultra high purity grade and ultrapure deionized water was obtained from a Cascade Scientific ELGA LC134 purification unit.

All glassware and quartz tubes were baked at 450 °C for 5 h or cleaned by triplicate rinsing in ultrapure water, methanol, and dichloromethane. The pH measurements were made using an Accumet 1003 meter (Fisher Scientific) with an Ag/AgCl electrode or an Orion 710A pH/ISE meter (Thermo Scientific) with an Orion Ross high precision pH electrode, calibrated at pH 4, 7, and 10.

Estrogen photolysis solutions

Solutions used in photolysis experiments were prepared in ultrapure water at a nominal concentration of 1 mg L^{-1} from either stock solutions in methanol or directly from pure crystalline standards. The latter were covered in foil and thoroughly mixed for at least 72 h to ensure complete dissolution. The former contained 0.1-0.2% methanol by volume and were only used in direct photolysis experiments from Feb 2015, Sep 2015, and Nov 2015. Actinometer solutions for experiments lasting more than 24 h contained 10 μM PNAP and 50 mM pyridine in ultrapure water. Actinometer solutions for shorter time scale experiments (*e.g.*, diBrE2 at pH 7) contained 10 μM PNA and 5-12 mM pyridine. These concentrations were chosen to closely match the degradation rate of estrogens under each condition.[44] Indirect photolysis solutions contained 5 mg L^{-1} of standard Suwannee River Humic Acid (SRHA). Solution pH was adjusted to pH 4, 7, 12, or 13 using HCl and NaOH, and pH was stable to within ± 0.5 over the course of photolysis experiments. The chloride ion concentration in any photolysis solution did not exceed 87 μM. Tables S1 and S2 provide a summary of the characteristics of the solutions used in each sunlight exposure experiment.

Quenching experiments

Quenching experiments were conducted on 10 July 2017 for diBrE2 at pH 7 in solutions containing SRHA (5 mg L^{-1}) and individual quenching compounds. Bulk quenching solutions were prepared by dissolving pure diBrE2 in ultrapure water (72 h), adding solid SRHA, and allowing the mixture to equilibrate for at least 60 minutes. Quenching compounds were added to aliquots of the bulk SRHA solution to achieve a concentration of 3 mM, and the pH of each was adjusted to 7. Sorbic acid and histidine were added as pure solids; isopropyl alcohol (IPA) was added as a pure liquid. Deoxygenated samples were prepared by sparging with N_2 gas for 6 minutes in individual quartz tubes (6 mL) then immediately capped with silicon stoppers. Control tubes containing only ultrapure water, diBrE2, and quenchers (sorbic acid, histidine, and IPA) were exposed to sunlight on 25 July 2018.

Sunlight exposure and controls

Quartz round-bottom tubes (11 × 13 × 100 mm; GE momentus semiconductor grade) were filled with 6 mL of a common, homogenized, and pH adjusted solution, and then sealed with silicon stoppers. Estrogen and actinometer solutions were prepared, exposed to sunlight,

and analyzed individually. Equivalent sets of quartz tubes used as dark controls were covered in foil and treated in the same manner as tubes exposed to light. All samples were secured onto a custom-built black ultra high molecular weight polyethylene rack, which held the tubes at an angle of 30º from horizontal (Figure S1). Dark controls were separated from all other tubes when possible in order to minimize uneven exposures due to reflections off the foil covering. When it was not possible to place light and dark tubes on separate racks, black plastic dividers were placed between the dark and light samples. The solution temperature in tubes under light-exposed and foil-covered conditions was measured during summertime exposure experiments and did not exceed 39.7 °C and 42.0 °C, respectively.

Samples were exposed to ambient sunlight on the roof of the Collins Science Center at Willamette University (44.937143° N; -123.032004° W). All samples faced south to ensure even, maximal light exposure. Sampling time points were chosen based on the expected solar irradiance and degradation rates of each analyte. At each time point, entire tubes were removed from their holding racks, covered in foil, and analyzed by HPLC-UV within 72 hours.

Analysis via HPLC-UV

Once sample tubes were removed from their racks, 1 mL of solution was transferred to an amber vial for subsequent analysis by HPLC-UV (Agilent 1260 HPLC-DAD). Separation was achieved using an Agilent Poroshell 120 EC-C18 column (50 × 4.6 mm; 2.7 μm) or a Phenomenex Kinetex EVO C18 column (150 × 4.6 mm; 5 μm), a flow rate of 1 mL min^{-1}, and a 10 minute gradient elution program using 5% methanol in water (A) and 100% methanol (B) as the mobile phases. The gradient program began with a ramp from 60 - 85% B over 0.1 minutes, followed by a hold at 85% B for 0.5 minutes, and finally a ramp to 95% B over 3.4 minutes. The column was then washed at 100% B for 1.7 minutes and re-equilibrated at 60% B for 3.2 minutes. Estrogens were quantified by peak area at 280 nm or 290 nm (Figure S2) and normalized to samples not exposed to light (*e.g.*, t_0 samples or dark controls). The actinometers, PNA and PNAP, were monitored at 320 nm and 280 nm, respectively.

Molar Absorptivity Spectra

Molar absorptivity data were acquired on an Agilent Cary 300 UV-Vis dual-beam spectrophotometer using matched quartz cuvettes (Starna Cells; 1 cm pathlength). Wavelength

calibration was performed using the holmium oxide standard. Molar absorptivity values were determined for solutions of each analyte in 50:50 MeOH/H_2O (75 - 431 μM), and pH was adjusted using HCl or NaOH (Table S3). These solutions were prepared at higher concentrations than the photolysis solutions to improve signal strength at wavelengths > 290 nm. Triplicate absorbance spectra for each sample were collected from 200 - 800 nm, corrected using the average absorbance spectra of replicate (n ≥ 3) blanks (50:50 MeOH/H_2O), and converted to molar absorptivity values at each wavelength via the Beer-Lambert Law.

Rate Constant Calculations

Estrogen photolysis was modeled using first order kinetics according to Equation 1,

$$\ln\left(\frac{A_{e,t}}{A_{e,0}}\right) = -k_{obs}t \quad (1)$$

where $A_{e,0}$ and $A_{e,t}$ represent HPLC-UV estrogen peak area at time zero and time t, respectively, and k_{obs} represents the observed photolysis rate constant. Actinometer normalized photolysis rate constants (k_e/k_a) were then determined by plotting linearized relative estrogen peak areas against corresponding actinometer values, using Equation 2,[44]

$$\ln\left(\frac{A_{e,t}}{A_{e,0}}\right) = \left(\frac{k_e}{k_a}\right)\ln\left(\frac{A_{a,t}}{A_{a,0}}\right) \quad (2)$$

where $A_{a,0}$ and $A_{a,t}$ represent actinometer (PNAP or PNA) HPLC-UV peak areas at time zero and time t, respectively. Since estrogen concentrations in dark control tubes did not change appreciably over the course of a 5-week exposure (Figure S3), observed degradation in tubes exposed to sunlight was assumed to reflect photolysis alone. Reported rate constants have not been corrected for tube lens effects, which can result in rates that are 1.5 - 2.2× slower in flat natural waters than in experimental tubes.[45, 46]

Quantum Yield Calculations

Quantum yields were calculated using Equation 3,[44]

$$\Phi_{de} = \left(\frac{k_e}{k_a}\right)\frac{\Sigma_\lambda(\varepsilon_{\lambda,a}L_\lambda)}{\Sigma_\lambda(\varepsilon_{\lambda,e}L_\lambda)}\ \Phi_{da} \quad (3)$$

where Φ_{de} is the direct photolysis quantum yield of the estrogen, Φ_{da} is the direct photolysis quantum yield of the actinometer, and $\Sigma_\lambda(\varepsilon_{\lambda,a}L_\lambda)$ and $\Sigma_\lambda(\varepsilon_{\lambda,e}L_\lambda)$ are the sum across all relevant wavelengths of the product of solar irradiance and molar absorptivity for the actinometer and

estrogen, respectively. Solar irradiance was calculated using the global horizontal irradiance output from the program SMARTS (v2.9.6) for the date, time, and location (44.937143° N; 123.032004° W) of each experiment.[47] The quantum yields of both 10 μM PNAP and 10 μM PNA in the presence of pyridine have been characterized[45] and recently updated[48] as equations 4 and 5, respectively.

$$\Phi_{PNAP} = 7.4 \times 10^{-3}[\mathrm{PYR}] + 1.1 \times 10^{-5} \quad (4)$$

$$\Phi_{PNA} = 0.29[\mathrm{PYR}] + 2.9 \times 10^{-4} \quad (5)$$

In this equation, Φ_{PNAP} and Φ_{PNA} represent the quantum yield of PNAP and PNA, respectively, and [PYR] represents the concentration of pyridine in the actinometer solution. Reported uncertainties in quantum yield were determined by propagating errors associated with linear regression, molar absorptivity errors (triplicate samples, uncertainty in analyte concentration, and uncertainty in cuvette path length), and uncertainty in the concentration of pyridine.

Connecting Rate Constants and Quantum Yields

Quantum yields and rates are linked via equation 6,[44]

$$k_{\mathrm{dE}} = \Phi_{\mathrm{de}} \Sigma_{\lambda}(\varepsilon_{\lambda,\mathrm{e}} \mathrm{L}_{\lambda}) \quad (6)$$

where k_{dE} is the system-specific direct photolysis rate constant, Φ_{de} is the direct photolysis quantum yield of the estrogen, $\varepsilon_{\lambda,\mathrm{e}}$ is the molar absorptivity of the estrogen at wavelength λ, and L_{λ} is the solar irradiance at wavelength λ.

Results and Discussion

Molar Absorptivity Spectra

Molar absorptivity spectra for E2, monoBrE2, diBrE2, and diClE2 at pH 5.6 (Figure 2) and pH 12 - 13 (Figure S4) demonstrate how halogen identity and quantity affect absorbance characteristics. Spectra for diBrE2 at pH 4, 5.6, and 12 (Figure 3) specifically highlight differences related to protonation state (phenol vs. phenolate). As the degree of halogenation increased, spectra exhibited a red shift in λ_{max} and generally more intense molar absorptivity values at wavelengths above 300 nm (Figure 2; Table S3). The net effect was an increase in the overlap between the absorbance spectra and the solar irradiance spectrum with increasing halogenation of E2.

The observed shift in molar absorptivity to longer wavelengths with halogenation is related to the halogen's ability to participate in the conjugation of the phenol chromophore. A similar trend has been observed for other halogenated phenolic compounds.[35, 49] The large overlap at pH 5.6 between the dihalogenated estrogen absorbance spectra and the solar irradiance spectrum (Figure 2; Figure 3) may result from the fact that some small portion (1.2 - 1.5 %) of diBrE2 (pK_a 7.50) and diClE2 (pK_a 7.43)[50] exist in solution as the phenolate form, which exhibits a much higher intensity and red shifted local λ_{max} compared to the phenol form (Figure S4; Table S3). In contrast, at pH 5.6, monoBrE2 (pK_a 8.99)[50] and E2 (pK_a 10.71)[51] are likely to be present almost entirely (> 99.96 %) as the phenol form (Figure 2). Thus, halogenated estrogens absorb more solar radiation than free estrogens, a finding that has important implications for photolysis rates.

Direct Photolysis Rates

The direct photolysis of 17β-estradiol and its halogenated derivatives occurred significantly faster as the extent of halogenation increased (Figure 4; Figure S5). This trend is consistent with the observation that halogenated estrogens absorb more sunlight (Figure 2; Figure S4) and that monohalogenated derivatives have larger direct photolysis quantum yields than free estrogens and dihalogenated forms (see discussion below). Actinometer normalized rate constants for the phenol forms (pH 4 - 5.6; Table 1) suggest that under equal irradiance conditions, direct photolysis rates increase in the order: E2 < monoBrE2 < diClE2 < diBrE2. During the month of February (2015; 2016), direct photolysis at pH 5.6 was slowest for E2 ($t_{1/2}$ = 37 ± 6 d), followed by diClE2 ($t_{1/2}$ = 19.6 ± 1.0 d), monoBrE2 ($t_{1/2}$ = 13.9 ± 1.0 d), and diBrE2 ($t_{1/2}$ = 5.18 ± 0.21 d) (Figure 4). In this case, the fact that monoBrE2 degraded faster than diClE2 is related to higher intensity sunlight irradiance (k_{PNAP} = 0.004 h^{-1}) during the February 2015 exposure (E2; monoBrE2) compared to the February 2016 exposure (k_{PNAP} = 0.002 h^{-1}; diClE2; diBrE2).

The phenolate forms of estrogens are considerably more likely to degrade by direct photolysis than the corresponding phenol forms. The fact that phenolate estrogens (pH 12 - 13) degraded more rapidly in July 2018 ($t_{1/2}$ = 3.2 - 79.8 min; Figure S5) than phenol estrogens (pH 5.6) did in February 2015/2016 ($t_{1/2}$ = 5.18 - 37 d; Figure 4) is partially due to seasonal differences in solar irradiance. But, as large actinometer normalized rate constants suggest (Table

1), phenolate forms are also inherently more photo-labile.

The direct photolysis half-life of E2 determined in the current study ($t_{1/2}$ = 37 d; pH 5.6) corresponds to the phenol form of E2 and is consistent with similar experiments conducted under natural sunlight ($t_{1/2}$ ~ 60 d)[38] but 7 - 270× longer than those that used simulated sunlight.[36, 37, 41, 42, 52-54] This difference may be attributed to a combination of relatively low irradiance conditions (overcast wintertime skies) in Oregon during our February experiments, as well as the artificially high intensity light often produced by solar simulators, photoreactors, and UV light sources in others' studies.[39, 55]

Additional direct photolysis experiments using monoBrE2 and diBrE2 at pH 5.6 and conducted under natural sunlight during September 2015, October 2015, and March 2016 support the overall trends described above and, perhaps not surprisingly, suggest that the photolysis rates of halogenated estrogens are sensitive to seasonal differences in solar irradiation (Figure S6). Indeed, the fastest direct photolysis rate for diBrE2 at pH 5.6 ($t_{1/2}$ = 0.82 ± 0.08 d) was observed during a clear sky September 2015 exposure.

Direct Photolysis Quantum Yields

Direct photolysis quantum yields were calculated for E2 and its halogenated derivatives at pH 5.6 and pH 12 - 13, and also for diBrE2 at pH 4 and 7 (Table 1). The quantum yield for the phenol form of E2 ($\Phi = (6.9 \pm 1.5) \times 10^{-3}$ mol Einstein^{-1}; Feb 2015; pH 5.6) is at the low end of the range (3.3×10^{-3} to 0.1 mol Einstein^{-1}) reported in the literature for wavelengths > 290 nm,[40-42] but ours is also the only E2 quantum yield determined under natural solar irradiance. Compared to E2, the quantum yield for the phenol form of monoBrE2 was 3.1× higher, while diBrE2 and diClE2 were smaller by 32% and 15%, respectively (pH 5.6; Table 1). There is evidence in the chlorophenol literature that mono-halogenated phenol derivatives have higher quantum yields than their dihalogenated counterparts.[56, 57] The higher quantum yield of monoBrE2 is part of the reason its direct photolysis degradation rate at pH 5.6 is similar to diClE2 despite much weaker absorption at wavelengths > 300 nm.

Though quantum yields determined in different seasons did not exhibit much variability, large pH effects were observed for all estrogens. Quantum yields determined for the phenolate forms (pH 12 - 13) of E2, monoBrE2, diBrE2, and diClE2 were larger than the phenol forms by 13.9×, 14.5×, 16.1×, and 15.0×, respectively (Table 1). In the literature, similar pH dependence

was observed for chlorophenols,[58] the pharmaceutical paroxetine,[59] as well as hydroxylated polybrominated diphenyl ethers[60] and chlorinated triclosan derivatives.[35] The size of phenolate quantum yields for the halogenated derivatives relative to E2 showed similar trends compared to the phenol quantum yields. The phenolate quantum yield for monoBrE2 was 3.2× higher, while diBrE2 and diClE2 were smaller by 31% and 8%, respectively (pH 12 - 13; Table 1). With quantum yields, molar absorptivity spectra, and relative abundance for both phenol and phenolate forms, it is possible to predict quantum yields (Figure 5) and direct photolysis rates as a function of pH.

Role of pH in the Direct and Indirect Photolysis of diBrE2

In light of evidence that phenolate estrogens absorb more solar radiation and have larger quantum yields than their phenol counterparts, we studied the direct and indirect photolysis of diBrE2 at pH 4 and 7 under mid-day summer sun. Higher pH values are known to increase the photolysis rate of chlorophenols,[56] triclosan and its chlorinated derivatives,[35, 61] and certain hydroxylated polybrominated diphenyl ethers[60] under UV light. At pH 4, diBrE2 exists almost entirely as the phenol form (f_{HA} = 0.9997), while the phenolate form represents nearly 25% (f_{A^-} = 0.2403) of the diBrE2 present at pH 7. Thus, we hypothesized that phenolate abundance would be an important factor determining estrogen photolysis rates in many aquatic systems.

We found that diBrE2 degrades on timescales of days ($t_{1/2}$ ~ 3 d) at pH 4 (Figure 6), which is consistent with the small relative abundance of phenolate and the low molar absorptivity and quantum yield of the more abundant phenol form. In contrast, photolysis at pH 7 occurred on minute timescales ($t_{1/2}$ ~ 45 min), a finding that underscores the importance of photolysis as a removal process for halogenated estrogens in natural waters and wastewater exposed to sunlight at circumneutral pH.

Although Suwannee River Humic Acid (SRHA; 5 mg L^{-1}) had a relatively minor effect on observed diBrE2 photolysis rates, the role of SRHA changed with pH. At pH 4, diBrE2 photolysis rate constants were 31% faster (p = 0.0364) in the presence of SRHA (Figure 6), suggesting a role for photochemically produced reactive intermediates (PPRIs). At pH 7, however, SRHA had an inhibitory effect on diBrE2 photolysis during three separate experiments (5 Jun, 19 Jun, 10 Jul) conducted in the summer of 2017 (Figure 7). The inhibitory effect (6 - 56 %) was statistically significant (p_{Jun5} = 0.000269; p_{Jun19} = 0.0362; p_{Jul10} = 0.000108; α = 0.05) in

the presence of 5 mg L^{-1} SRHA at pH 7. The size of inhibition for diBrE2 is similar to a range of phenols, including 17α-ethynylestradiol (EE2) in 2.5 mg L^{-1} Suwannee River Fulvic Acid.[62] Since light screening in 5 mg L^{-1} SRHA ($S_{290\text{-}800nm}$ = 0.9885) was minimal and PPRIs would increase degradation rates, the observed inhibitory effect at pH 7 suggests that SRHA may act as an antioxidant by quenching the excited state diBrE2 intermediate formed after photo excitation.[62-68] A previous study of androgen photolysis found that the inhibitory effect of dissolved organic matter (DOM) was due mostly to screening rather than physical quenching.[69] The fact that net inhibition was only observed at pH 7 suggests that the phenolate form of diBrE2 may be more prone to inhibition by SRHA, as was the case for the sulfonamide antibiotic, sulfadiazine.[70]

The results of our work support the view that, as a source of DOM, SRHA is a relatively weak sensitizer[71, 72] but a relatively strong antioxidant/quencher.[68] Though the mechanisms underpinning these characteristics require further study, the data presented here suggest that lower pH values promote PPRI formation while circumneutral pH enhances the antioxidant properties of SRHA.

PPRIs and the Indirect Photolysis diBrE2

Quenching experiments were conducted to determine the extent to which hydroxyl radical (•OH), singlet oxygen ($^1O_2$), and excited triplet state dissolved organic matter (^{3}DOM*) influence the indirect photolysis of diBrE2 under environmentally relevant conditions (5 mg L^{-1} SRHA; pH 7; natural solar irradiance). While each of these PPRIs has demonstrated reactivity with compounds containing similar phenolic chromophores,[43, 73, 74] our data point to ^{3}DOM* as the most important PPRI for diBrE2 degradation by indirect photolysis. This conclusion is based on the observation that removing O_2 from solution increased the observed photolysis rate ($k_{SRHA+N2}/k_{SRHA}$ = 1.44) while the addition of sorbic acid, a ^{3}DOM* quencher,[75] decreased the rate ($k_{SRHA+SorbicAcid}/k_{SRHA}$ = 0.76) (Figure 8). Faster degradation of diBrE2 in deoxygenated solutions implicates ^{3}DOM* because the lack of $^1O_2$ would result in higher steady state ^{3}DOM* concentrations.[63, 76, 77] This interpretation is consistent with previous work showing an inverse relationship between O_2 concentration and initial photolysis rates of 2,4,6-trimethylphenol in solutions containing DOM.[78] Interestingly, sorbic acid also decreased photolysis rates ($k_{Direct+SorbicAcid}/k_{Direct}$ = 0.70) in control experiments conducted in ultrapure water (Figure S7),

which suggests that the direct photolysis of diBrE2 proceeds through a triplet intermediate,[79] and that most of the observed inhibitory effect of sorbic acid in the presence of SRHA is related to triplet quenching within the direct photolysis pathway.

The results of our quenching experiments show that •OH, carbonate radical (CO_3•$^-$), peroxy radical (ROO•), superoxide (O_2•$^-$), and $^1O_2$ are not dominant players in the indirect photolysis of diBrE2. Faster degradation in the presence of isopropyl alcohol (a radical quencher) (Figure 8) rules out •OH, CO_3•$^-$, and ROO•,[80, 81] and lends support to the idea that IPA can enhance photolysis under certain conditions.[80] Faster degradation in deoxygenated solutions and those containing histidine (a $^1O_2$ quencher) (Figure 8) rules out O_2•$^-$ and $^1O_2$.[82] The reason that histidine increases the photolysis rate of diBrE2 is not immediately clear, though one possible explanation is that by quenching $^1O_2$, histidine indirectly weakens SRHA's ability to inhibit the direct photolysis of diBrE2.

Previous studies support the reactivity of ^{3}DOM* with free estrogens (*e.g.*, EE2)[34] and other phenolic compounds.[63] Overall, the results of our quenching experiments imply that radicals and singlet oxygen are less important reactive intermediates than ^{3}DOM* during the indirect photolysis of diBrE2, and that at pH 7 SRHA has a net inhibitory effect toward degradation.

Conclusion

We have shown that estrogen photolysis rates are faster for halogenated derivatives and at higher pH. These trends are well described by the absorbance characteristics, quantum efficiencies, and relative abundance of the phenolate and phenol forms of estrogen. Direct processes dominated the photolysis of diBrE2, which occurs on sub-hour timescales at pH 7. Suwannee River Humic Acid played a relatively minor role in diBrE2 photolysis, enhancing rates at pH 4 but acting as an inhibitor at pH 7. Taken together, these findings suggest that photolysis may be a key removal process for phenolic contaminants, including estrogens, which can be halogenated within chlorine-based wastewater disinfection basins then rapidly photolyzed when exposed to natural sunlight in the disinfection basin itself or in receiving waters. If dehalogenation is a primary photodegradation pathway for halogenated phenols as some literature suggests,[49, 83] then it will be important to identify conditions that maximize halogenated estrogen degradation rates while minimizing free estrogen formation.

Conflicts of Interest

There are no conflicts to declare.

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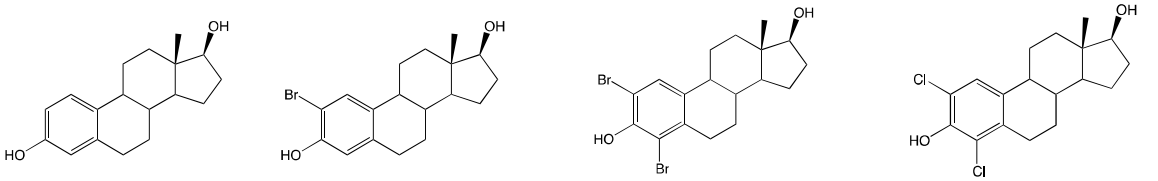
Figure 1. Structures and pK_a values[50, 51] of 17β-estradiol and several halogenated derivatives.



	17β-estradiol (E2)	2-bromo-17β-estradiol (monoBrE2)	2,4-dibromo-17β-estradiol (diBrE2)	2,4-dichloro-17β-estradiol (diClE2)
pKa:	10.71	8.99	7.50	7.43

Figure 2. Molar absorptivity spectra of E2 and three of its halogenated derivatives (colored lines) at pH 5.6 acquired in matched quartz cuvettes in 50:50 methanol/water. Overlap with the solar irradiance spectrum (black line; SMARTS v2.9.6; 12:00 PM; 12 September 2015; 44.937143° N; 123.032004° W) increases with halogenation at pH 5.6.

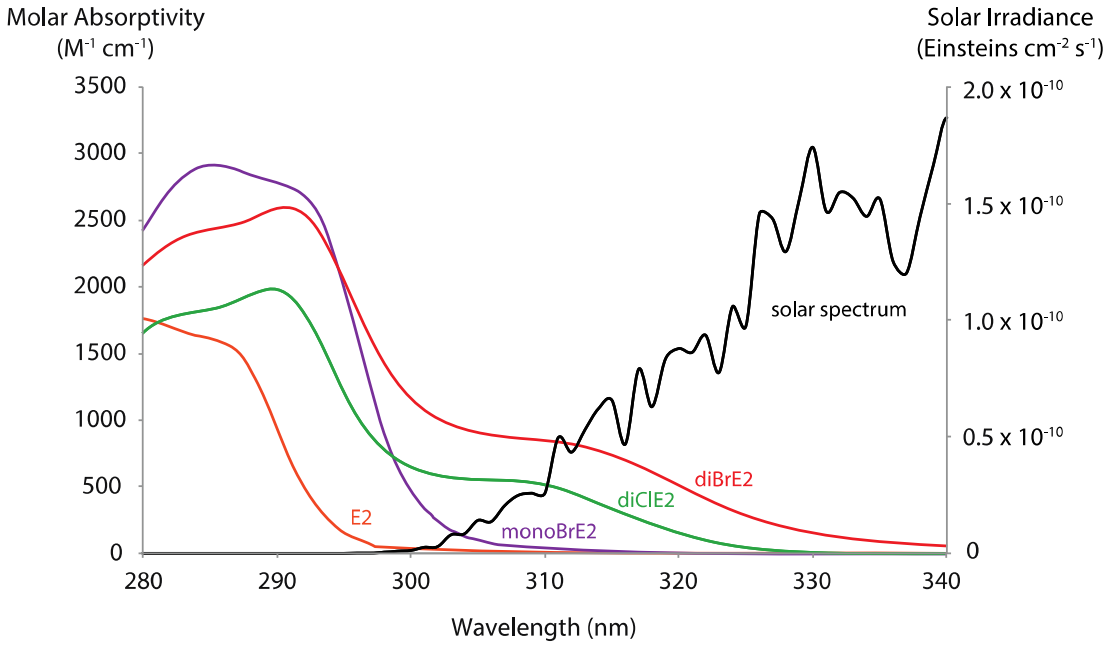


Figure 3. The overlap between the solar irradiance spectrum (gray line; SMARTS v2.9.6; 12:00 PM; 12 September, 2015; 44.937143° N; 123.032004° W) and the molar absorptivity spectra of diBrE2 (pK_a 7.50) increases as solution pH increases. Error bars represent ±1 standard deviation, propagated from replicate (n = 9) blank corrected absorbance spectra in 50:50 methanol/water.

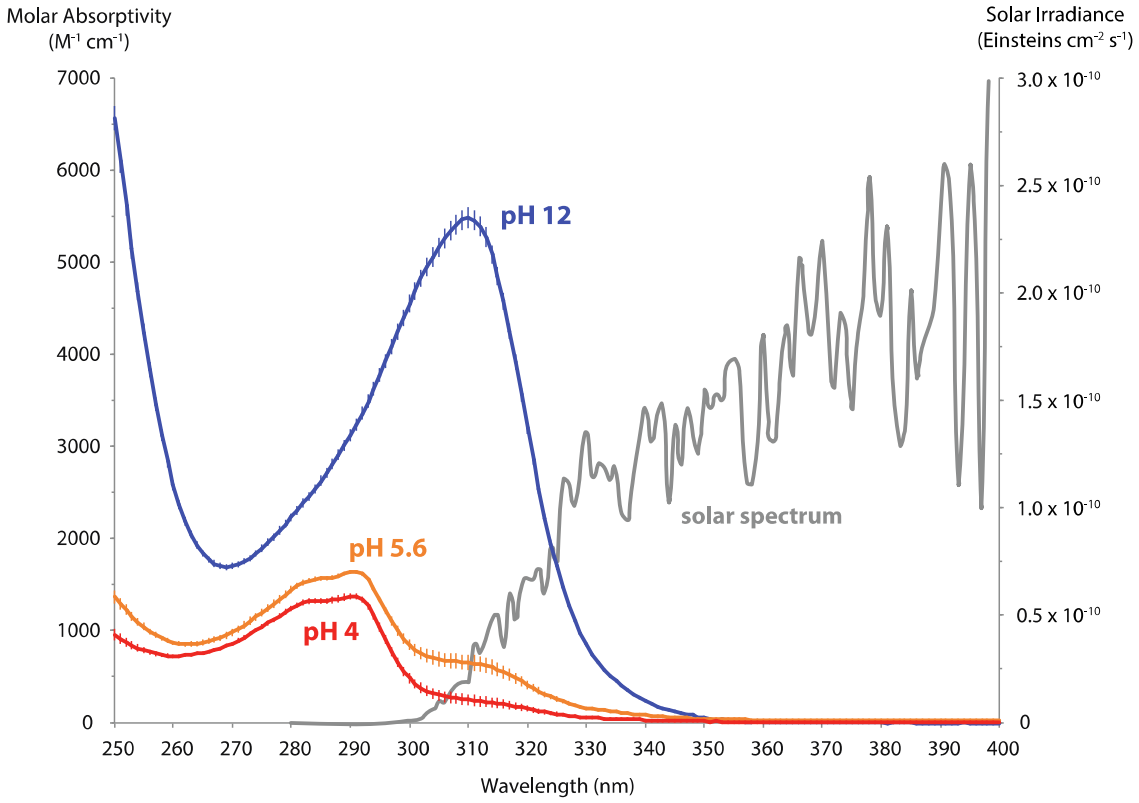


Figure 4. Halogenated E2 derivatives degrade faster than E2 by direct photolysis at pH 5.6 (February of 2015 and 2016). Observed half-lives ($t_{1/2}$) are longest for E2 (37 ± 6 d), intermediate for diClE2 (19.6 ± 1.0 d) and monoBrE2 (13.9 ± 1.0 d), and shortest for diBrE2 (5.18 ± 0.21 d).

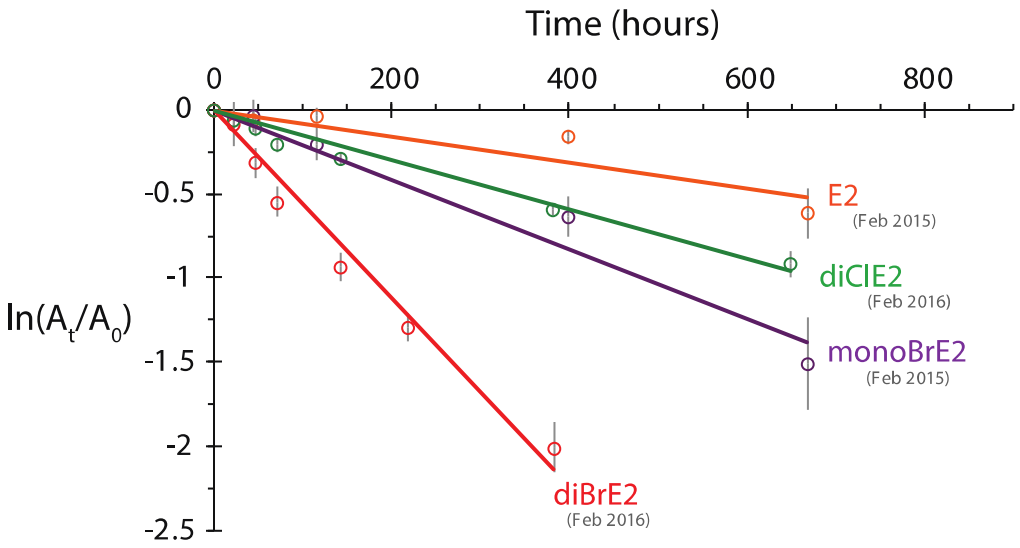


Figure 5. Direct photolysis quantum yields of diBrE2 increase with pH. Error bars represent propagated uncertainty for the entire quantum yield calculation and are smaller than most symbols. The dashed blue line is the predicted quantum yield of diBrE2 (pK_a = 7.50), calculated as a weighted average of the phenol (HA; pH 4) and phenolate (A^-; pH 12) quantum yields according to the relative abundance of HA and A^- at each pH.

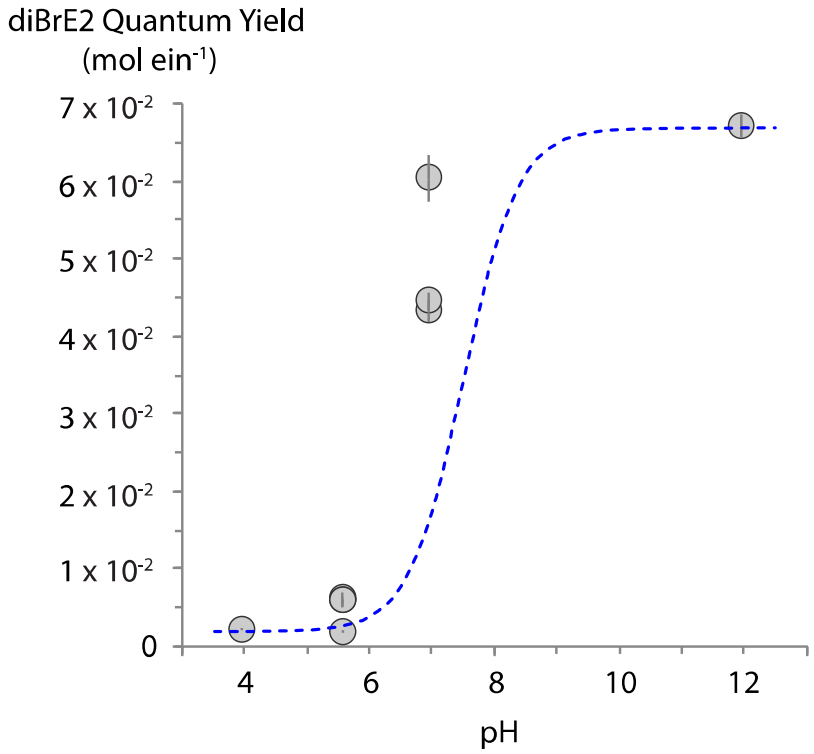


Figure 6. Observed degradation of diBrE2 at pH 4 in ultrapure water ("Direct"; open symbols; dashed lines) and 5 mg L^{-1} Suwannee River Humic Acid ("Indirect"; filled symbols; solid lines) during June-July 2016. Error bars represent ± 1 standard deviation for triplicate samples. Degradation at pH 7 is shown for context.

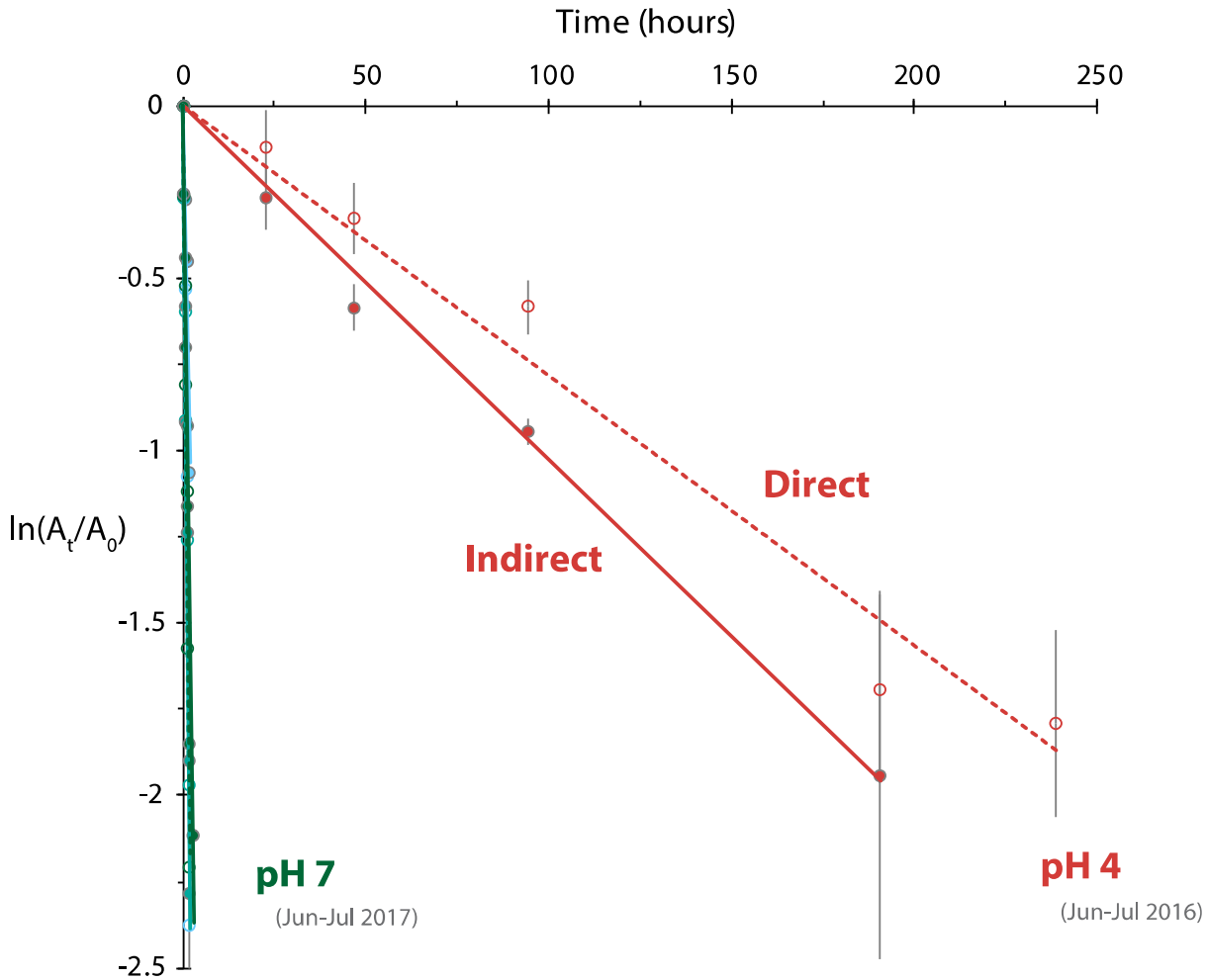


Figure 7. Observed degradation of diBrE2 at pH 7 in ultrapure water ("Direct"; open symbols; dashed lines) and 5 mg L^{-1} Suwannee River Humic Acid ("Indirect"; filled symbols; solid lines) on 5 June 2017, 19 June 2017, and 10 July 2017. Error bars for the 19 June 2017 experiment represent ± 1 standard deviation for triplicate samples. Degradation at pH 4 is shown for context.

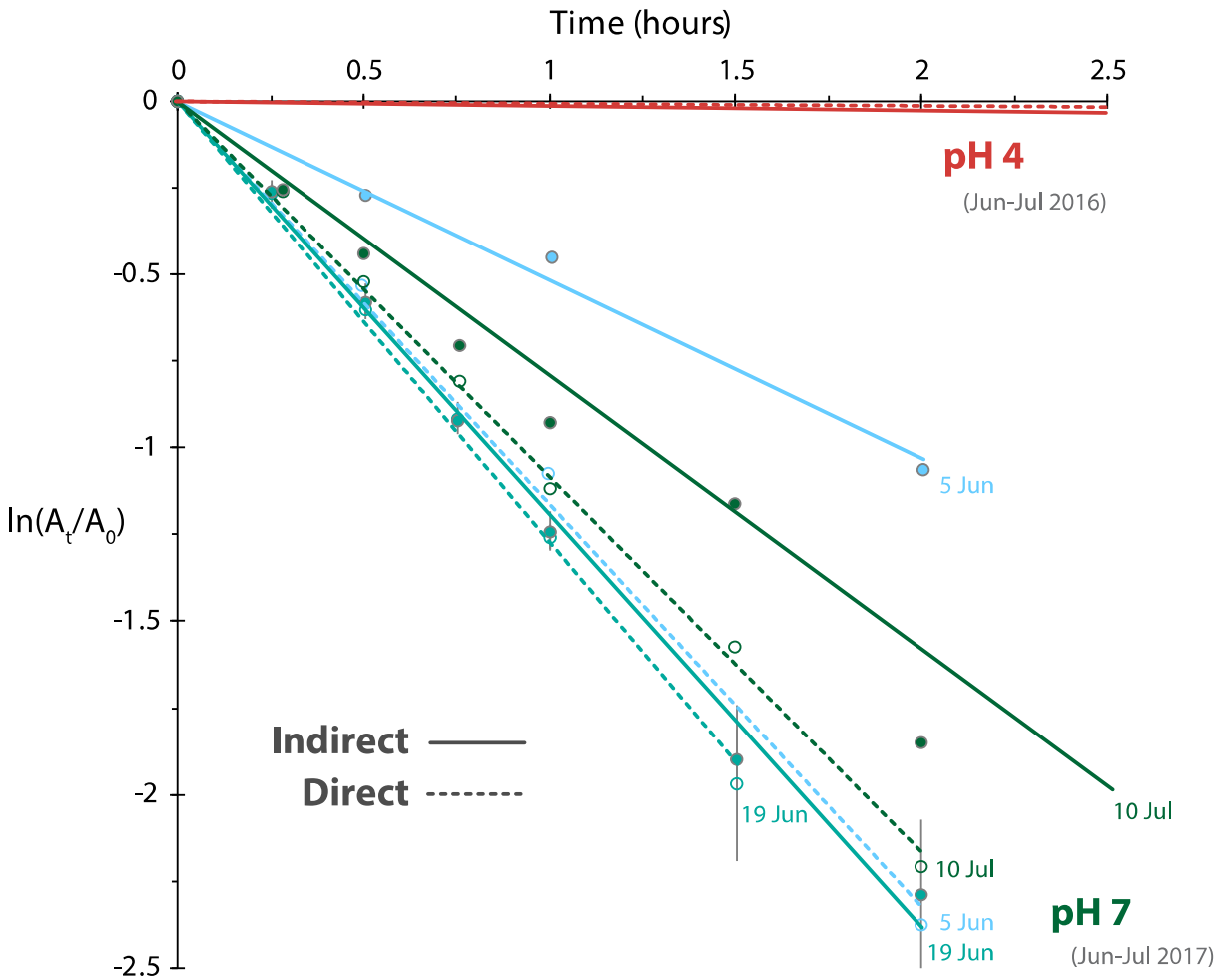


Figure 8. Observed degradation of diBrE2 at pH 7 in 5 mg L^{-1} Suwannee River Humic Acid ("Indirect"; filled symbols; solid lines) on 10 July 2017 is influenced by deoxygenation via N_2 sparging and the addition of the quenchers histidine, isopropyl alcohol (IPA), and sorbic acid. Degradation of diBrE2 in ultrapure water ("Direct"; open symbols; dashed line) at pH 7 is shown for context.

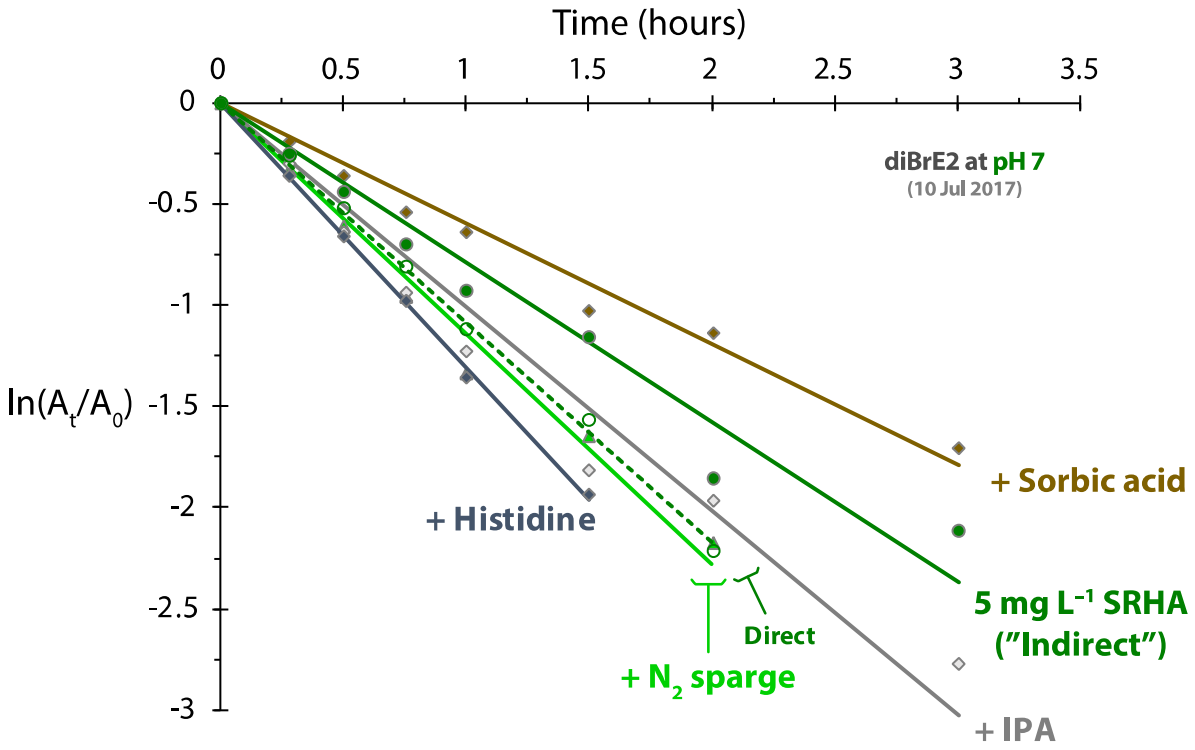


Table 1. Observed rate constants, actinometer normalized rate constants, and quantum yield values obtained from each photodegradation experiment.

Estrogen	Date	Observed Rate Constant (k_{obs}) (h^{-1})	Actinometer Normalized Rate Constant (k_e/k_a)	Quantum Yield (mol Ein^{-1})	pH
E2	13 Feb - 13 Mar 2015	$(7.7 \pm 1.2) \times 10^{-4}$	0.19 ± 0.04	$(6.9 \pm 1.5) \times 10^{-3}$	5.6[a]
monoBrE2	13 Feb - 13 Mar 2015	$(2.08 \pm 0.15) \times 10^{-3}$	0.52 ± 0.06	$(1.9 \pm 0.3) \times 10^{-2}$	5.6[a]
monoBrE2	14 Mar - 19 Apr 2016	$(3.08 \pm 0.13) \times 10^{-3}$	0.657 ± 0.010	$(2.41 \pm 0.26) \times 10^{-2}$	5.6[a]
diClE2	6 Feb - 4 Mar 2016	$(1.47 \pm 0.08) \times 10^{-3}$	0.720 ± 0.027	$(5.9 \pm 0.4) \times 10^{-3}$	5.6[a]
diBrE2	8 - 17 Sep 2015	$(3.5 \pm 0.3) \times 10^{-2}$	2.9 ± 0.3	$(6.1 \pm 0.8) \times 10^{-3}$	5.6[a]
diBrE2	14 - 18 Oct 2015	$(1.06 \pm 0.08) \times 10^{-2}$	2.58 ± 0.15	$(5.6 \pm 0.5) \times 10^{-3}$	5.6[a]
diBrE2	6 Feb - 4 Mar 2016	$(5.58 \pm 0.23) \times 10^{-3}$	2.66 ± 0.04	$(5.8 \pm 0.5) \times 10^{-3}$	5.6[a]
diBrE2	14 Mar - 19 Apr 2016	$(2.85 \pm 0.23) \times 10^{-3}$	0.69 ± 0.04	$(1.45 \pm 0.15) \times 10^{-3}$	5.6[a]
diBrE2	20 Jun - 8 Jul 2016	$(7.8 \pm 0.4) \times 10^{-3}$	0.65 ± 0.03	$(1.79 \pm 0.13) \times 10^{-3}$	4.0
diBrE2 Indirect	20 Jun - 8 Jul 2016	$(1.016 \pm 0.024) \times 10^{-2}$	0.795 ± 0.010	-	4.0
diBrE2	5 Jun 2017	1.163 ± 0.028	0.863 ± 0.009	$(4.31 \pm 0.14) \times 10^{-2}$	7.0
diBrE2 Indirect	5 Jun 2017	0.515 ± 0.018	0.384 ± 0.009	-	7.0
diBrE2	19 Jun 2017	1.271 ± 0.023	1.21 ± 0.04	$(6.03 \pm 0.29) \times 10^{-2}$	7.0
diBrE2 Indirect	19 Jun 2017	1.192 ± 0.023	1.184 ± 0.027	-	7.0
diBrE2	10 Jul 2017	1.085 ± 0.013	0.888 ± 0.015	$(4.45 \pm 0.10) \times 10^{-2}$	7.0
diBrE2 Indirect	10 Jul 2017	0.79 ± 0.04	0.65 ± 0.03	-	7.0
E2	20 Jul 2018	0.521 ± 0.018	0.205 ± 0.008	$(9.7 \pm 0.4) \times 10^{-2}$	13.0
monoBrE2	20 Jul 2018	12.96 ± 0.10	4.84 ± 0.08	$(3.12 \pm 0.06) \times 10^{-1}$	12.0
diBrE2	20 Jul 2018	5.62 ± 0.06	2.14 ± 0.05	$(6.67 \pm 0.15) \times 10^{-2}$	12.0
diClE2	20 Jul 2018	4.09 ± 0.11	1.55 ± 0.06	$(8.9 \pm 0.3) \times 10^{-2}$	12.0

[a] pH of ultrapure deionized water was 5.6 ± 0.1

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Photochemical degradation of halogenated estrogens under natural solar irradiance

Reid P. Milstead, Keeton T. Nance, Kulananalu S. Tarnas, Kira E. Egelhofer, and David R. Griffith*

Department of Chemistry, Willamette University, Salem, OR, USA

* *Corresponding author*

Table of Contents Entry

Estrogen photolysis is strongly influenced by the extent of halogenation and pH.