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**ARTICLE** *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · AUGUST 2004

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# Photochemical Fate of Sulfa Drugs in the Aquatic Environment: Sulfa Drugs Containing Five-Membered Heterocyclic Groups

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The photochemical fate of five sulfa drugs with varying five-membered heterocyclic substituents (sulfamethoxazole, sulfisoxazole, sulfamethizole, sulfathiazole, and sulfamoxole) was investigated in aqueous solution. The rate of direct photolysis of these compounds is dependent upon the identity of the heterocyclic R group as well as the pH of the solution. Matrix deconvolution methods were employed to determine the absorption spectrum and photolysis rate of each protonation state (cationic, neutral, and anionic). From these data, quantum yields for direct photodegradation were calculated for each protonation state of the sulfa drugs. The quantum yields calculated range from <0.005 for the neutral state of sulfamethizole to 0.7 ± 0.3 for the protonated state of sulfisoxazole. The protonation state that is most photoreactive varies among the sulfa drugs and cannot be attributed to the rate of photon absorption. Products arising from the direct photolysis of the sulfa drugs were also investigated. For all the compounds, sulfanilic acid was observed as a common product. The singlet oxygen quenching rates of the sulfa drugs were determined by laser flash photolysis and range from $(2 \pm 1) \times 10^4$ $M^{-1}$ $s^{-1}$ for sulfamethoxazole to $(3.0 \pm 0.7) \times 10^8$ $M^{-1}$ $s^{-1}$ for sulfamoxole. Reaction of the sulfa drugs with hydroxyl radical is not modulated by the R group, and the rate constants are all near the bimolecular diffusion-controlled limit of $10^{10}$ $M^{-1}$ $s^{-1}$. The photodegradation of the sulfa drugs in natural water samples of Lake Josephine (St. Paul, MN) and Lake Superior was attributed solely to direct photolysis. This study indicates that these similarly structured antibiotics will be subject to a wide range of photodegradation rates with sulfathiazole degrading relatively quickly, sulfisoxazole and sulfamethizole degrading moderately, and sulfamethoxazole degrading much more slowly.

## Introduction

Pharmaceuticals and personal care products (PPCPs) have received increased attention as aquatic contaminants (*1*−*6*), with their presence in the environment confirmed by studies conducted in Europe (*2*, *6*−*11*), Brazil (*12*), and the United States (*13*, *14*). One class of compounds repeatedly found at concentrations ranging from 0.13 to 1.9 μg/L (*6*, *13*, *15*, *16*) is a group of antibiotics known as the sulfa drugs or sulfonamides, which are used in aquaculture (*11*), as agricultural herbicides (*16*, *17*), as a preventative measure for veterinary purposes (*15*, *16*), and in the treatment of respiratory and urinary tract infections in humans (*10*). The general structure of the sulfa drugs is shown in Table 1. Members of this class differ in the N-bound substituent of the sulfonamide linkage.

Although the low concentrations of the sulfa drugs found in aquatic environments do not exceed any current water standards, one of the concerns related to PPCPs, and antibiotics in particular, is that their biological activity will lead to adverse effects on aquatic ecosystems (*2*, *6*, *10*, *11*, *13*, *16*). Specifically, bacterial resistance is believed to be of particular concern at the constant, low concentrations that are found for pharmaceuticals (*18*−*22*). Thus, the effect of these compounds on the environment may be irreversible and exerted at the concentration levels detected (*3*). The ecological impact of PPCPs is presently uncertain, in part because the environmental persistence of these compounds is unknown.

Photochemical degradation may be a central factor in determining the environmental fate of PPCPs (*23*−*26*). Specifically, pharmaceuticals may be degraded through direct photodegradation or through sensitized photoprocesses such as reaction with transient excited species such as singlet oxygen ($^1O_2$, $O_2$ ($^1\Delta_g$)), hydroxyl radical (•OH), and other reactive species formed in sunlit natural waters (*27*−*32*). Also, because the sulfa drugs are designed to have a p$K_a$ of physiological relevance (around 7; *33*), the protonation state of these compounds is expected to influence their environmental photochemical behavior.

We report the photochemical behavior of a class of sulfa drugs in aqueous solution. Specifically direct photolysis rates and bimolecular rate constants for reactions with the reactive oxygen species $^1O_2$ and •OH are reported. An important aspect of this study is the deconvolution of the total absorbance and direct photolysis degradation rates into their components and the calculation of the quantum yield for each protonation state of the sulfa drugs. The photodegradation of the compounds in selected natural water samples was studied through comparisons to photolysis in DI $H_2O$ to assess the importance of direct versus indirect photolysis processes for the sulfa drugs. Finally, some of the major photolysis products were identified, and the amount of sulfanilic acid formed was quantified.

## Experimental Section

**Chemicals.** Chemicals, suppliers, and purities are listed in the Supporting Information.

**p$K_a$ Measurements.** UV−vis absorbance spectra were measured on a Jasco V-530 spectrophotometer. The absorbance at a single wavelength of the UV−vis absorbance spectra of 100 μM solutions of sulfamethoxazole (**1**), sulfisoxazole (**2**), sulfamethizole (**3**), and sulfathiazole (**4**) in buffered $H_2O$ solutions of varied ionic strength were plotted versus pH of the solution, and the resultant data curves were fit using a nonlinear regression (Scientist for Windows v. 2.01, Micromath).

**Direct Photolysis Experiments.** Direct photolysis experiments were conducted under natural sunlight on May 13, June 5, and June 9, 2003, in Minneapolis, MN (45° latitude). Solutions of **1**−**4** (100 μM) in various pH buffers of approximately 0.01 M ionic strength were photolyzed in

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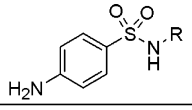
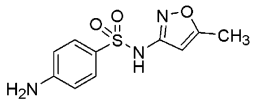
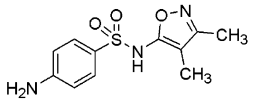
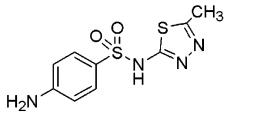
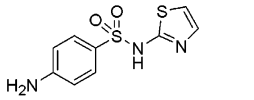
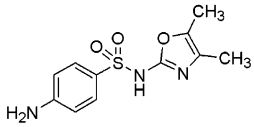
10.1021/es0353053 CCC: $27.50 
Published on Web 06/17/2004

**TABLE 1. General Structure of a Sulfa Drug and Structure of Five Sulfa Drugs Containing a Five-Membered Heterocyclic R Substituent and the Corresponding Measured p$K_a$ Values**

**General Structure**



| | Compound | Structure | p$K_{a,1}$ [a] | p$K_{a,2}$ [a] |
|---|---|---|---|---|
| **1** | Sulfamethoxazole |  | 1.6 ± 0.2 | 5.7 ± 0.2 |
| **2** | Sulfisoxazole |  | 1.5 ± 0.3 | 5.00 ± 0.07 |
| **3** | Sulfamethizole |  | 2.1 ± 0.2 | 5.3 ± 0.2 |
| **4** | Sulfathiazole |  | 2.2 ± 0.1 | 7.2 ± 0.4 |
| **5** | Sulfamoxole |  | nd [b] | 7.4 [c] |

[a] Errors represent the 95% confidence levels and were calculated using Scientist for Windows (v. 2.01). [b] nd, not determined. [c] Ref *43*.

uncapped quartz test tubes (o.d. = 1.3 cm, i.d. = 1.1 cm, $V$ = 10 mL) alongside solutions of *p*-nitroanisole/pyridine actinometer solutions of similar ionic strength prepared as described elsewhere (*34*) with a pyridine concentration of 500 $\mu$M. The test tubes were held approximately 45° from normal. Aliquots (500 $\mu$L) were removed at a range of time points and placed in amber vials until quantification by HPLC.

**Laser Flash Photolysis (LFP) Experiments.** The laser flash photolysis apparatus used in these experiments is of similar design to those employed by others (*30*, *35*, *36*) and described in earlier work (*30*, *31*) (see Supporting Information for details).

**Steady-State Photolysis Experiments.** The steady-state photolysis experiments were conducted as described previously (*30*). (Details are in the Supporting Information.)

**Fenton Reaction.** Hydroxyl radical was generated through the use of Fenton's reagent (*37*−*39*) at pH 3 as described previously (*30*, *31*). (See Supporting Information for details.)

**Natural Water Photolysis Experiments.** Water samples from Lake Josephine (St. Paul, MN) and Lake Superior (Duluth, MN) were collected and filtered through 0.2 $\mu$m filters (Lake Josephine: DOC = 5.9 ± 0.7 mg/L, pH 8.4; Lake Superior: DOC = 2.5 ± 0.7 mg/L, pH 8.3; see Supporting Information for analysis details). Solutions of **1**−**3** (100 $\mu$M) in both natural waters, and **4** (100 $\mu$M) in Lake Josephine Water, were photolyzed on a turntable apparatus alongside solutions of **1**−**4** (100 $\mu$M) in buffered DI $H_2O$ at a similar pH to the natural water samples in uncapped quartz test tubes under four Pyrex-filtered 175 W medium-pressure Hg vapor lamps. Solutions of **4** in Lake Superior water and buffered DI $H_2O$ were photolyzed in uncapped quartz test tubes under natural sunlight in Minneapolis, MN (45° latitude) on September 1, 2003, held approximately 45° from normal. Aliquots (500 $\mu$L) were removed, stored, and analyzed as described above.

**HPLC Analysis.** HPLC chromatograms were obtained on an 1100 series Hewlett-Packard HPLC equipped with a UV absorbance detector. Furfuryl alcohol (FFA) and *p*-nitroanisole (PNA) were analyzed on a Phenomenex Spherisorb 3 ODS-2, 250 × 4.6 mm, 3 $\mu$m particle size column with a mobile phase of 70:30 acetonitrile (ACN):pH 5 ammonium acetate ($NH_4OAc$) buffered $H_2O$ mixture, flow rate of 1.0 mL/min, 10 $\mu$L injection volume, a 219 nm detection wavelength for FFA, and a 313 nm detection wavelength for PNA. Acetophenone, all sulfa drugs, and suspected products were analyzed on a Restek Ultra IBD, 150 × 4.6 mm, 5 $\mu$m particle size column. The mobile phase was a 20:80 ACN:pH 5 $NH_4$-OAc buffered $H_2O$ mixture with a flow rate of 1.0 mL/min, injection volume of 10 $\mu$L, and a 254 nm detection wavelength.

**Mass Spectrometry.** Mass spectra were obtained on a ThermoFinnigan LCQ Advantage ion trap MS/MS equipped with an orthogonal ESI and APCI source operated under Xcalibur software. Solutions of authentic standards of **1**, **3**, and the amino-R substituents of both compounds (for **1**, 3-amino-5-methylisoxazole; for **3**, 2-amino-5-methyl-1,3,4-thiadiazole) were directly infused into the inlet of the mass spectrometer. Photolyzed solutions of **1** and **3** in volatile buffers (100 $\mu$M **1** or **3** in pH 5 ammonium acetate or pH 8 ammonium carbonate buffered $H_2O$) were also directly infused into the mass spectrometer. The spectra were obtained under positive ionization mode.

## Results

**p$K_a$ Measurements.** The sulfa drugs listed in Table 1 are able to undergo two acid−base processes (Scheme 1). Thus, it was expected that the photochemical behavior of each sulfa drug would be dependent upon its speciation. To quantify the amount of each protonation state present at a given pH,

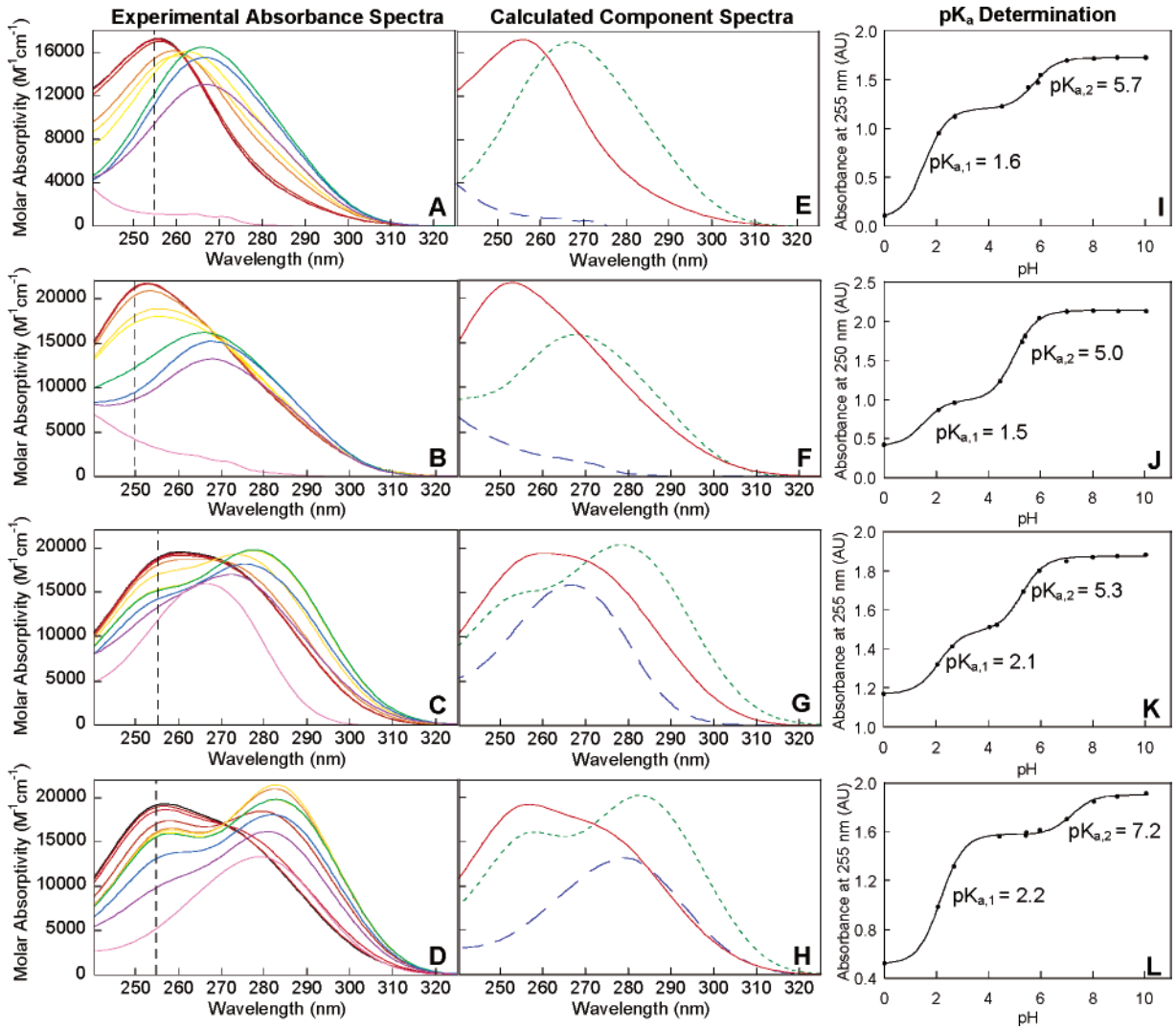
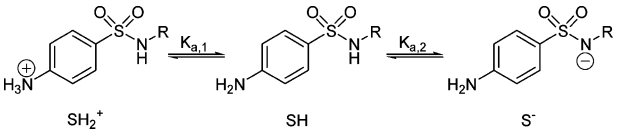



**FIGURE 1. Absorbance spectra of 100 $\mu$M solutions of sulfamethoxazole (1, A), sulfisoxazole (2, B), sulfamethizole (3, C), and sulfathiazole (4, D) in various pH buffers (red = high pH, purple = low pH). Panels E−H show the molar absorptivity of the components of each compound (1, E; 2, F; 3, G; 4, H; red — = anionic component, green - - = neutral component, blue — — = cationic component) calculated using matrix deconvolution outlined in eqs 3−5. Panels A−D were used to determine panels I−L and show the corresponding absorbance intensity at a single wavelength (wavelength used is indicated in panels A−D with a dashed line) versus pH (1, I; 2, J; 3, K; 4, L). The solid line represents a fit of the data to eq 1 to obtain the p$K_a$ values.**

**SCHEME 1. Protonation States of the Sulfa Drugs[a]**




[a] $SH_2^+$ = cationic form, SH = neutral form, $S^-$ = anionic form.

the p$K_a$ values were determined through a spectrophotometric titration. The absorbance and pH of the solutions were used to determine the p$K_a$ values by fitting the data to eq 1:

$$(\chi_{SH_2^+})(\epsilon_{SH_2^+,\lambda}) + (\chi_{SH})(\epsilon_{SH,\lambda}) + (\chi_{S^-})(\epsilon_{S^-,\lambda}) = \epsilon_{tot} \quad (1)$$

where $\chi_{SH_2^+}$, $\chi_{SH}$, and $\chi_{S^-}$ are the fraction of the fully protonated ($SH_2^+$), neutral (SH), and anionic forms ($S^-$), respectively, and $\epsilon$ is the molar absorptivity at a specific wavelength (*40*). Absorbance spectra obtained for **1**−**4** at various pH values are shown in Figure 1. The absorbance at a single wavelength was plotted versus the pH of the solution (Figure 1), and the solid line represents a fit to eq 1, with the inflection points in the curve representing the concentration-based p$K_a$ values for the compounds. These values listed in Table 1 are not corrected for ionic strength of the solutions and agree within error with values reported elsewhere (*41*−*44*).

**Nonphotochemical Degradation.** Sulfamoxole (**5**) was found to degrade in aqueous solutions in the absence of light, with half of the substrate remaining after approximately 4 h in a pH 5 solution. The non-photochemical decomposition observed was less pronounced in pH 10 buffered solutions. All other sulfa drugs examined were found to be stable at all pH values.

**Direct Photolysis.** The direct photodegradation rate constants measured for **1**−**4** in a variety of buffered $H_2O$ solutions under natural sunlight are listed in Table 2. Compound **5** was omitted from these studies, as it is unstable in aqueous solutions.

Due to the systematic changes of the direct photolysis rate constant with pH, the quantum yield ($\Phi$) of direct photodegradation for the three protonation states ($SH_2^+$, SH, and $S^-$) was determined. The quantum yields were calculated according to eq 2:

**TABLE 2. Direct Photolysis Rate Constants for Sulfamethoxazole (1), Sulfisoxazole (2), Sulfamethizole (3), and Sulfathiazole (4) in Various Buffered $H_2O$ Solutions Measured under Natural Sunlight[a]**

| compd | pH | $k_{direct}/10^{-5}$ ($s^{-1}$)[b] | protonation state | $\sum\epsilon_{295-340\ nm}L_\lambda$ (mE $cm^{-3}$ $M^{-1}$ $d^{-1}$ $nm^{-1}$)[c] | $k_{direct}$ calcd/$10^{-5}$ ($s^{-1}$)[d] | $\Phi$[d] |
|---|---|---|---|---|---|---|
| **1** | 2.6 | 5.1 ± 0.9 | $SH_2^+$ | 0 | ≤0.3 | 0 |
| | 4.1 | 6 ± 1 | | | | |
| | 5.3 | 5.1 ± 0.8 | SH | 7.8 | 6 ± 1 | 0.50 ± 0.09 |
| | 6.9 | 1.3 ± 0.3 | $S^-$ | 4.6 | 0.8 ± 0.2 | 0.09 ± 0.01 |
| | 10.8 | 0.6 ± 0.1 | | | | |
| **2** | 2.5 | 7 ± 2 | $SH_2^+$ | 7.0 | 11 ± 5 | 0.7 ± 0.3 |
| | 4.1 | 6 ± 1 | | | | |
| | 5.2 | 4 ± 1 | SH | 27.9 | 7 ± 1 | 0.17 ± 0.03 |
| | 6.8 | 2.5 ± 0.5 | $S^-$ | 19.9 | 2.1 ± 0.5 | 0.07 ± 0.02 |
| | 10.7 | 1.7 ± 0.4 | | | | |
| **3** | 2.4 | ≤0.3 | $SH_2^+$ | 8.7 | ≤0.3 | ≤0.01 |
| | 3.2 | ≤0.3 | | | | |
| | 4.0 | ≤0.3 | SH | 51.6 | ≤0.3 | ≤0.005 |
| | 5.2 | 0.5 ± 0.1 | | | | |
| | 6.7 | 1.3 ± 0.3 | $S^-$ | 15.9 | 1.3 ± 0.3 | 0.05 ± 0.01 |
| | 8.6 | 1.3 ± 0.2 | | | | |
| **4** | 2.5 | 2.3 ± 0.4 | $SH_2^+$ | 21.1 | 0.6 ± 0.6 | 0.02 ± 0.02 |
| | 3.9 | 2.3 ± 0.4 | | | | |
| | 4.9 | 2.5 ± 0.5 | SH | 66.9 | 3.1 ± 0.6 | 0.07 ± 0.03 |
| | 6.3 | 5.7 ± 0.8 | $S^-$ | 27.2 | 14 ± 1 | 0.40 ± 0.04 |
| | 8.4 | 13 ± 1 | | | | |

[a] Spectral overlap integral, natural sunlight direct photolysis rate constant, and direct photolysis quantum yield data calculated for the three components of these sulfa drugs using matrix deconvolution of measured data. [b] Errors represent the 95% confidence levels. [c] $L_\lambda$ values obtained from Leifer (*34*) averaged for 45° N noon, mid-spring sunlight; mE = millieinstein. [d] Error values were estimated through a sensitivity analysis described in the Supporting Information.

$$\Phi_s = \frac{k_s}{k_a}\frac{\sum L_\lambda \epsilon_\lambda{}^a}{\sum L_\lambda \epsilon_\lambda{}^s}\Phi_a \qquad (2)$$

where s is the substrate, a is the actinometer, $k$ is the direct photolysis degradation rate constant, $L_\lambda$ values (solar irradiance at a specific wavelength) for 45° latitude were taken as the average of the 40° and 50° latitude values for mid-spring (*34*), $\epsilon_\lambda$ values are the molar absorptivities of the substrate or actinometer, and $\Phi$ are the quantum yields (*34*). The component values ($\epsilon_{SH_2^+,\lambda}$, $\epsilon_{SH,\lambda}$, $\epsilon_{S^-,\lambda}$) were obtained by solving eq 1 using Microsoft Excel. An expansion of this equation into matrix form used to solve for the component values is shown in eq 3:

$$\begin{matrix}\text{pH 10}\\ \text{pH 2}\end{matrix}\begin{pmatrix}\chi_{SH_2^+} & \chi_{SH} & \chi_{S^-}\\ \vdots & \vdots & \vdots\\ \vdots & \vdots & \vdots\end{pmatrix}\begin{pmatrix}\epsilon_{SH_2^+,\lambda}\\ \epsilon_{SH,\lambda}\\ \epsilon_{S^-,\lambda}\end{pmatrix} = \begin{matrix}\text{pH 10}\\ \text{pH 2}\end{matrix}\begin{pmatrix}\epsilon_{tot,\lambda}\\ \vdots\\ \vdots\end{pmatrix} \qquad (3)$$

Equation 3 is of the form $\mathbf{Aa} = \mathbf{b}$, where the values of the speciation matrix ($\mathbf{A}$) and the $\epsilon_{tot,\lambda}$ values of matrix $\mathbf{b}$ are known. The component extinction coefficients ($\epsilon_{SH_2^+,\lambda}$, $\epsilon_{SH,\lambda}$, and $\epsilon_{S^-,\lambda}$) were determined using the least-squares method by employing the pseudo-inverse of $\mathbf{A}$, $(\mathbf{A}^T\mathbf{A})^{-1}\mathbf{A}^T$, as shown in eq 4:

$$\mathbf{a} = (\mathbf{A}^T\mathbf{A})^{-1}\mathbf{A}^T\mathbf{b} \qquad (4)$$

The speciation matrix ($\mathbf{A}$) was calculated using the measured p$K_a$ values, while the absorbance spectra measured for solutions of **1**−**4** at a variety of pH values were used for matrix $\mathbf{b}$. The calculated component absorbance spectra obtained for the three components of **1**−**4** are shown in Figure 1. The component direct photodegradation rate constants were calculated in an analogous fashion, by expanding eq 5 into matrix form and solving for the component rate constant matrix:

$$(\chi_{SH_2^+})(k_{SH_2^+}) + (\chi_{SH})(k_{SH}) + (\chi_{S^-})(k_{S^-}) = k_{tot} \qquad (5)$$

The measured direct photolysis rate constants and the calculated component direct photolysis rate constants are listed in Table 2. With the direct photolysis rate constants and the absorbance spectra for each component of the sulfa drugs, the quantum yield of direct photodegradation for each component was determined using eq 2 and correcting for internal screening (Table 2).

**Singlet Oxygen ($^1O_2$): Laser Flash Photolysis.** Substrates may physically quench $^1O_2$ as well as undergo chemical reaction with $^1O_2$. The interaction of the sulfa drugs with $^1O_2$ therefore was studied in two stages to determine rate constants for both processes. First, laser flash photolysis (LFP) was employed to determine the total quenching rate constant ($k_{tot}$), the sum of both physical quenching and chemical reaction of a substrate with $^1O_2$. Subsequently, steady-state photolysis was used to determine the reaction rate constant ($k_{rxn}$).

Using LFP as described previously (*30*), the $^1O_2$ phosphorescent decay at a range of substrate concentrations was monitored, and a Stern−Volmer plot of the observed rate constant for singlet oxygen decay ($k_{obs}$) versus substrate concentration yielded a slope equivalent to $k_{tot}$. $D_2O$ was employed as the solvent in the LFP experiments due to the substantially longer lifetimes of $^1O_2$ it affords relative to $H_2O$, thus simplifying the detection of the $^1O_2$ phosphorescence signal (*45*−*47*) (see Supporting Information for representative $^1O_2$ phosphorescence decay transients and all Stern−Volmer plots). The $k_{tot}$ values range from $(2 \pm 1) \times 10^4$ $M^{-1}$ $s^{-1}$ for the neutral form of **1** to $(3.0 \pm 0.7) \times 10^8$ $M^{-1}$ $s^{-1}$ for the anionic form of **5**. The $k_{tot}$ values for all five sulfa drugs are listed in Table 3. Compounds **2** and **4** have two values reported, pD 9.5 and 11.1, as $k_{tot}$ is pH-dependent.

**Singlet Oxygen ($^1O_2$): Steady-State Photolysis.** Steady-state photolysis was used to determine $k_{rxn}$ for those sulfa drugs displaying high $k_{tot}$ values ($>10^7$ $M^{-1}$ $s^{-1}$; **2** and **4**; **5** was excluded from these studies as it degrades in the absence of light). The loss of substrate upon reaction with $^1O_2$ was

**TABLE 3. Bimolecular Rate Constants for the Interaction between Sulfamethoxazole (1), Sulfisoxazole (2), Sulfamethizole (3), Sulfathiazole (4), and Sulfamoxole (5) and the Reactive Oxygen Species $^1O_2$ and $^{\bullet}OH$**

| compd | $^1O_2$ $k_{tot}$ ($M^{-1}s^{-1}$)[a] | $^1O_2$ $k_{rxn}$ ($M^{-1}s^{-1}$)[a,f] | $^{\bullet}OH$ $k_{rxn}$ ($M^{-1}s^{-1}$)[a,h] |
|---|---|---|---|
| **1** | $(2 \pm 1) \times 10^4$ [b] | nd[g] | $(5.8 \pm 0.2) \times 10^9$ |
| **2** | $(6.5 \pm 0.6) \times 10^7$ [c] | $(5.5 \pm 0.4) \times 10^7$ | $(6.6 \pm 0.2) \times 10^9$ |
| | $(1.0 \pm 0.3) \times 10^8$ [e] | | |
| **3** | $(3.6 \pm 0.8) \times 10^6$ [d] | nd | $(4.9 \pm 0.1) \times 10^9$ |
| **4** | $(5.6 \pm 0.7) \times 10^7$ [c] | $(6.9 \pm 0.3) \times 10^7$ | $(7.1 \pm 0.2) \times 10^9$ |
| | $(1.0 \pm 0.2) \times 10^8$ [e] | | |
| **5** | $(3.0 \pm 0.7) \times 10^8$ [c] | nd | nd |

[a] All errors represent the 95% confidence level. [b] Measured in acetone due to solubility. [c] Rate constant for the anionic form measured by LFP at a pD of 9.5 in $D_2O$. [d] Rate constant for a combination of the anionic and neutral form measured by LFP at a pD of 8.7 in $D_2O$. [e] Rate constant for the anionic form measured by LFP at a pD of 11.1 in $D_2O$. [f] Rate constant for the anionic form measured by steady-state photolysis in pH 10.2 carbonate buffered $H_2O$. [g] nd, not determined. [h] Measured with Fenton's reagent at pH 3 and the rate constants are for the neutral form of the sulfa drugs.

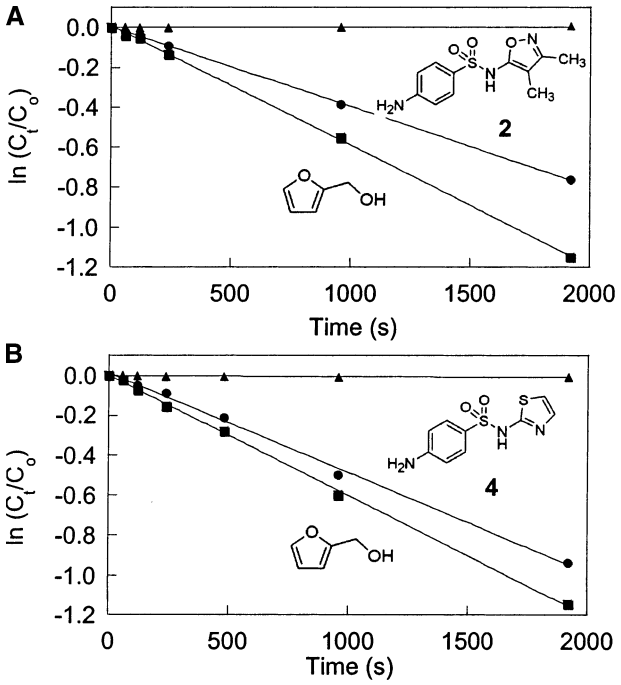



**FIGURE 2. Steady-state photolysis decay curves for the reaction of sulfisoxazole (2; A, ● = $5 \times 10^{-5}$ M), sulfathiazole (4; B, ● = $5 \times 10^{-5}$ M), and FFA (A and B, ■ = $1 \times 10^{-4}$ M) with singlet oxygen in pH 10 carbonate buffered $H_2O$ solutions with Rose Bengal sensitzer (40 $\mu$M) irradiated under a 175 W Hg vapor lamp. Samples of 2 (A, ▲ = $1 \times 10^{-4}$ M) and 4 (B, ▲ = $1 \times 10^{-4}$ M) without sensitizer are shown to indicate that no direct photolysis is contributing to the loss of substrate in these experiments.**

simultaneously monitored alongside the steady-state reaction of a compound with a known $k_{rxn}$ such as FFA ($(8.3 \pm 0.1) \times 10^7$ $M^{-1}s^{-1}$ in $D_2O$; *30*) as described previously (*30*). The $k_{rxn}$ for the substrate of interest was determined from the ratio of the observed rate constants as shown in eq 6:

$$k_{rxn,S} = \frac{k_{obs,S}}{k_{obs,FFA}} k_{rxn,FFA} \quad (6)$$

Figure 2 shows the degradation curves for **2** and **4** along with FFA under steady-state photolysis conditions. The $k_{rxn}$ values obtained for the anionic form of these compounds are given in Table 3. The conditions employed for steady-state photolysis were optimized to promote reaction with $^1O_2$ and used shorter exposure times and less intense light conditions than the direct photolysis experiments. Consequently, no degradation of substrate was observed without sensitizer present, and the observed loss of substrate was not due to direct photodegradation (Figure 2). Also, upon addition of sodium azide, a known $^1O_2$ quencher (*48*, *49*), or the use of $D_2O$ as a solvent in the place of $H_2O$ (*45*−*47*), the rate of degradation was suppressed or enhanced accordingly (see Supporting Information for azide suppression and $D_2O$ enhancement of degradation).

**Hydroxyl Radical ($^{\bullet}OH$).** The bimolecular reaction rate constant for the reaction between the neutral form of the sulfa drugs and hydroxyl radical was quantified through the use of Fenton's reagent and a reference compound of known reaction rate constant (acetophenone, $k^R_{\cdot OH} = 5.9 \times 10^9$ $M^{-1}$ $s^{-1}$; *50*). The sulfa drugs' hydroxyl radical reaction rate constants ($k^S_{\cdot OH}$) were ascertained through the use of a competition kinetic scheme, detailed in eq 7:

$$\ln\left(\frac{[S]_t}{[S]_o}\right) = \frac{k^S_{\cdot OH}}{k^R_{\cdot OH}} \ln\left(\frac{[R]_t}{[R]_o}\right) \quad (7)$$

where S is the substrate and R is the reference compound. By plotting the natural logarithm of the substrate decay versus the natural logarithm of the reference compound decay, a plot with a slope equal to the ratio of the hydroxyl radical reaction rate constants can be produced and the reaction rate constant for the substrate can be attained (see Supporting Information for plots of $\ln([S]_t/[S]_o)$ vs $\ln([R]_t/[R]_o)$ for each of the sulfa drugs (excluding **5**)). The hydroxyl radical reaction rate constants, which range from $(4.9 \pm 0.1) \times 10^9$ $M^{-1}$ $s^{-1}$ to $(7.1 \pm 0.2) \times 10^9$ $M^{-1}$ $s^{-1}$, are listed in Table 3.

**Natural Water Photolysis.** Photolysis of the sulfa drugs in natural water samples was conducted concomitantly with photolysis in DI $H_2O$. Natural water samples were used from Lake Josephine and Lake Superior. The photodegradation rates of each of the sulfa drugs in both natural water samples matched the photodegradation rates observed within the DI $H_2O$ samples (see Supporting Information).

**Product Determination.** Several product peaks are observed in the HPLC chromatograms of the sulfa drug photolysate solutions. The HPLC retention time for an authentic standard of sulfanilic acid matches that of a prominent product peak that grows in at 2.2 min over the course of the photolyses of **1**−**4** (see Supporting Information for HPLC chromatograms). In addition, a much smaller product peak in each photolysate solution overlaps with an authentic standard of sulfanilamide. Compounds **2**−**4** photolysate chromatograms have a peak that is coincident with a standard of each respective amino-R substituent (for **2**, 5-amino-3,4-dimethylisoxazole; for **3**, 2-amino-5-methyl-1,3,4-thiadiazole; for **4**, 2-aminothiazole). No HPLC peaks coincident with aniline were observed. Mass spectra of photolysate solutions at pH 5 and pH 8 of **1** and **3** show signals indicating the presence of the amino-R substituent (for **1**, 3-amino-5-methylisoxazole or 2-amino-5-methyloxazole after isomerization of **1** (*59*), $m/z = 99$; for **3**, 2-amino-5-methyl-1,3,4-thiadiazole, $m/z = 116$). These signals are not present in the mass spectra of solutions that have not been photolyzed. The mass spectra of the photolysate solutions of the other sulfa drugs and of standards of sulfanilamide and sulfanilic acid showed no observable signals.

## Discussion

**p$K_a$ Measurements and pH-Dependent Absorbance Properties.** With natural water systems generally ranging in pH

from 6 to 9, the p$K_{a2}$ values determined for **1**−**4** are relevant to the speciation of the compounds found in aquatic environments. Because speciation of the compounds also has an effect on the optical properties and reactivity of the compounds, these p$K_a$ values are critical in elucidating their photochemical behavior. The range of p$K_a$ values (2 pH units) within the structurally similar class of sulfa drugs is significant, as one would expect them to have similar p$K_a$ values. The percent of anionic form ($S^-$) present at pH 7 ranges from 99.0% of **2** to 28.5% of **5**. In contrast, the p$K_{a1}$ values are not relevant to natural water systems, as above a pH of 5 < 0.1% of the cationic form ($SH_2^+$) of any of the sulfa drugs is present.

The pH of the water sample alters the protonation state of the sulfa drugs as well as the absorbance spectrum. As seen in Figure 1, the position of maximum absorbance shifts with pH for **1** and **2**, and the shape of the spectrum changes for **3** and **4** with changing pH. These spectral changes largely occur at wavelengths below 300 nm, which is not relevant to absorbance of sunlight irradiation; however, **1**, **3**, and **4** do show changes in the magnitude of absorption above 300 nm, which affects their rate of photon absorption.

**Direct Photolysis.** The direct photolysis rate constants (Table 2) were found to be highly pH-dependent, and the trend is not consistent among the class of sulfa drugs. Due to this pH dependence, the quantum yield for each of the forms of each sulfa drug was calculated (Table 2). A correlation between a higher direct photolysis rate constant and an increased absorption of sunlight is only seen for **1**. Sulfamethoxazole (**1**) degrades most rapidly in the neutral state, with a direct photolysis rate constant of $(6 \pm 1) \times 10^{-5}$ $s^{-1}$. The anionic component, most prevalent at environmentally relevant pH values, however, degrades much slower with a direct photolysis rate constant of $(0.8 \pm 0.2) \times 10^{-5}$ $s^{-1}$. This parallels the absorption of each component, but is much more pronounced. These findings are in good agreement with those of Moore and Zhou (*51*), who found that the direct photolysis of **1** was a function of pH, with elevated decay rates occurring at pH values below p$K_{a2}$. The single wavelength quantum yields measured in acidic and basic pH values by Moore and Zhou ($\Phi_{\lambda = 268\ nm} = 0.47 \pm 0.05$ at pH 3 and $\Phi_{\lambda = 257\ nm} = 0.084 \pm 0.016$ at pH 9; *51*) are also in agreement with the quantum yields measured under more environmentally relevant irradiation wavelengths reported here for the neutral and anionic components.

A similar correlation between absorbance and elevated direct photodegradation rate constant is not observed for the remaining compounds. For example, the neutral component of **2** has the largest spectral overlap integral, and yet the cationic component degrades most rapidly, although the quantum yield and direct photodegradation rate constant calculated for this component have a high error margin due to the low number of data points below the p$K_{a1}$ of this compound. The anionic components of **3** and **4** exhibit the highest degradation rates. The largest absorbance, however, is in the neutral component.

In addition to the variability in reactivity among the components of a single drug, there is a wide divergence within the class of sulfa drugs. We expected that compounds of such similar structure would exhibit very similar direct photolysis behavior; however, this was not the case. Two of the sulfa drugs photodegraded most readily in acidic media (**1** and **2**), whereas two others photodegraded in basic (**3** and **4**), and one was degraded nonphotochemically in aqueous solutions at all pH values (**5**). The entire class of sulfa drugs investigated contains an identical backbone structure, only differing in the heterocyclic R substituent. Thus, the differences arising within the photochemical behavior must be governed by this heterocyclic R substituent.

The environmental half-lives resulting solely from direct photodegradation of the sulfa drugs in mid-spring at 45° latitude range from 36 h for **1** at pH 7 to 5 h for **4** at pH 7, calculated from the direct photolysis rate constant components and correcting for the lens effect (*52*, *53*). These values increase to 231 h for **1** and 31 h for **4** in pH 7 natural water at 45° latitude in mid-winter and decrease to 22 h for **1** and 3 h for **4** in pH 7 natural water at 45° latitude in mid-summer. At 30° latitude, the half-lives range from 17 h for **1** and 2.5 h for **4** in mid-summer to 61 h for **1** and 8 h for **4** in mid-winter pH 7 natural waters. These represent the minimum expected half-lives, as these were calculated based on continuous exposure and no light-screening by humic substances or scattering of light in natural waters.

**Singlet Oxygen ($^1O_2$).** The $^1O_2$ $k_{tot}$ values obtained from LFP are not uniform within the group of sulfa drugs examined, with values ranging over 4 orders of magnitude from $10^4$ to $10^8$ $M^{-1}$ $s^{-1}$. This is not surprising given the divergent structures of the sulfa drugs and that the likely site of reaction is the heterocyclic substituent. Both **4** and **5**, which contain similar oxazole and thiazole groups, were found to have high $k_{tot}$ values. This is in line with observations that the reaction of $^1O_2$ with oxazoles is quite rapid, ranging from $1.2 \times 10^7$ to $1.6 \times 10^8$ $M^{-1}$ $s^{-1}$ (*47*, *54*, *55*). Compound **3** contains a thiadiazole group that shows very little reactivity toward $^1O_2$ ($k_{tot} = 3.6 \times 10^6$ $M^{-1}$ $s^{-1}$).

The most interesting comparison is between **1** and **2**. Both contain isoxazole groups, one of which (**2**) has a high $k_{tot}$ ($10^8$ $M^{-1}$ $s^{-1}$ at basic pH), and the other has a low $k_{tot}$ ($2 \times 10^4$ $M^{-1}$ $s^{-1}$). We speculate that for these compounds the mode of reaction is a [2 + 2] cycloaddition and that the critical difference is that the electron-rich alkene is substituted by both oxygen and nitrogen $\pi$-donors in **2**. The low interaction between $^1O_2$ and **1** corroborates the findings of Zhou and Moore, who found that **1** is able to sensitize the formation of $^1O_2$ but is not itself susceptible to singlet oxygenation (*28*).

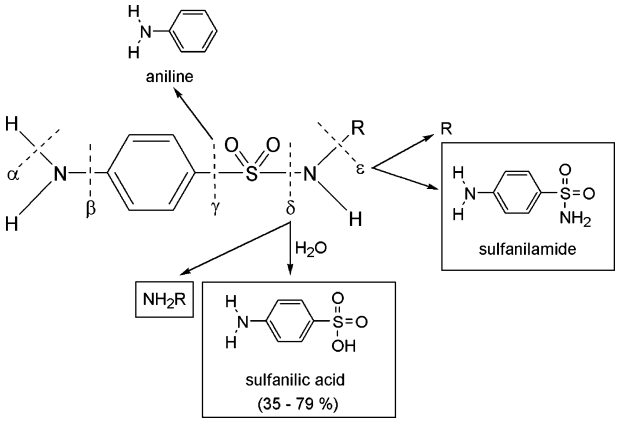
The $k_{tot}$ values for **2**, **4**, and **5** were found to be pH-dependent, with the anionic form displaying elevated quenching ability. Because $^1O_2$ is an electrophile (*56*), the increase in electron density at the site of reaction accordingly enhances the $k_{tot}$ values at the higher pH values.

The $k_{rxn}$ values obtained through steady-state photolysis for **2** and **4** are on the same order of magnitude as the $k_{tot}$ values. As a result, a majority of $k_{tot}$ is attributable to chemical reaction of the sulfa drugs with $^1O_2$, with a smaller component arising from physical quenching. Steady-state photolyses were conducted under significantly lower light intensities and for short time periods, with conditions optimized for singlet oxygen production and a lack of direct photolysis (Figure 2). The values for both $k_{tot}$ and $k_{rxn}$ for **4** (Table 3) are in very good agreement with those measured by Posadaz et al. (pH 6: $k_{tot} = 5.8 \times 10^7$ $M^{-1}$ $s^{-1}$, $k_{rxn} = 4.2 \times 10^7$ $M^{-1}$ $s^{-1}$; pH 12: $k_{tot} = 1.07 \times 10^8$ $M^{-1}$ $s^{-1}$, $k_{rxn} = 5.2 \times 10^7$ $M^{-1}$ $s^{-1}$; *57*).

Reaction of the sulfa drugs with $^1O_2$ in natural waters results in minimum half-lives of 2.8 h ($[^1O_2] = 10^{-12}$ M) to 2800 h ($[^1O_2] = 10^{-15}$ M) for **4** and 3.5 h ($[^1O_2] = 10^{-12}$ M) to 3500 h ($[^1O_2] = 10^{-15}$ M) for **2**. Thus, the role of $^1O_2$ reaction in the environmental fate of **2** and **4** is expected to be highly dependent upon the characteristics of the natural water. These half-lives correspond only to reaction with $^1O_2$ and do not account for any additional processes.

**Hydroxyl Radical (•OH).** The reaction of the sulfa drugs with •OH was not found to be dependent upon the identity of the heterocyclic R substituent. Rather, **1**−**4** were all found to react at near the bimolecular diffusion-controlled limit of $10^{10}$ $M^{-1}$ $s^{-1}$. This high reactivity is typical for the •OH reaction with most substrates, as it is an unselective, highly reactive species. Even though the reaction rate constants are near the diffusion-controlled limit, the environmental fate of the sulfa drugs is likely not controlled by reaction with •OH, except possibly in high nitrate-containing waters ($[^\bullet OH] = 10^{-15}$ M; *29*, *58*).

**SCHEME 2. Potential Direct Photolysis Cleavage Sites (Adapted from Refs *60* and *63*)[a]**




[a] Boxed products have been detected in the photolyses reported here.

**Natural Water Photolysis.** The photolysis rates of **1**−**4** in the two natural water samples, Lake Josephine (DOC = 5.9 mg/L) and Lake Superior (DOC = 2.5 mg/L), matched the photodegradation rates in DI $H_2O$ run concurrently, thus implicating direct photolysis as the dominant photochemical loss process in these natural waters. Although the solutions of the sulfa drugs are not optically dilute at 100 $\mu$M, these studies were used only to elucidate the photochemical loss process occurring within the natural water samples.

**Product Determination.** A number of studies investigating the photochemical behavior of sulfonamides have determined that degradation occurs through cleavage at various positions (*59*−*63*). Although many other reactions could occur, it seemed most likely that the products of direct photolysis of the sulfa drugs arise from similar cleavage as shown in Scheme 2 (*60*, *63*). Comparison of HPLC retention time and mass spectral data with authentic standards has shown both expected products arising from $\delta$-cleavage in photolysate solutions of **1**−**4** and appears to be the dominant cleavage pathway (Scheme 2). Cleavage at this position has been observed in each of the studies mentioned previously (*59*−*63*) and thus is not a surprising result here. One of these $\delta$-cleavage products, sulfanilic acid, accounts for 35% of the degraded parent compound of **1** and 66% of **2** in pH 2 photolyses, 70% of **3** and 79% of **4** in pH 10 photolyses. (See Supporting Information for plots of sulfanilic acid growth over time). The amino-R substituent arising from $\delta$-cleavage was previously detected as a product of the photolysis of **3** by Chiang et al. (*64*).

Prior studies on the photolysis of **4** have shown aniline (via $\gamma$-cleavage) as a photoproduct (*60*), and Zhou and Moore have identified five photoproducts arising from the photolysis of **1** including aniline (*59*). No peak matching an authentic standard of aniline, however, was detected in our photolyses. Cleavage at the $\epsilon$ position is also observed, as a small peak coincident with an authentic standard of sulfanilamide was detected in the photolysis solutions of **1**−**4**, although to an appreciably lesser extent than $\delta$-cleavage. The pH of the photolysis solutions appeared to affect the rate of product appearance but not their identity. Although the identification has been made only with HPLC retention overlap and mass spectral data, the observation of both products of $\delta$-cleavage and the results of previous studies on the photochemical cleavage of sulfa drugs supports this assignment.

**Environmental Significance.** Photochemical degradation is expected to have an impact on the environmental fate of the sulfa drugs. The importance will be largely dependent upon the pH of the natural water sample, latitude, and season. Degradation is expected to be solely due to direct photodegradation except in extremely DOC- or nitrate-enriched waters where the direct photolysis is hindered and large concentrations of reactive species are formed. (See Supporting Information for table of environmental half-lives due to various photochemical degradation processes in different natural waters.) Sulfathiazole (**4**) is expected to be readily photodegraded in most natural waters, as the anionic form is most susceptible to direct photodegradation and is the most abundant form of the drug in most natural waters. Sulfamethoxazole (**1**) is likely to be more persistent. The 1999−2000 USGS reconnaissance of U.S. streams found higher concentrations of **1** as compared to **3** and did not detect any **4** (*13*). This may be due to differing loads of the sulfa drugs into the environment or could be explained by their varying photodegradation rates.

The photodegradation may be impeded by sorption to particles within the waters, although the sulfa drugs have relatively low sorption coefficients (**4**; log $K_{ow}$ = 0.05; $K_{d,solid}$ = 4.9 L/kg; *65*) and therefore have little affinity for particles. Photolysis rates reported here were measured under continual irradiation in isolated systems and represent an upper limit, as the degradation of these compounds may also be hindered by limited depth of the photic zone and overcast conditions. Likewise, their removal may be accelerated by biological degradation.

The photochemical degradation of the sulfa drugs highlights that if one compound is more photolabile than others of similar structure, this drug may be used preferentially to those compounds that are found to be recalcitrant, provided the degradation products are benign and the compound maintains similar efficacy as the alternatives.

## Acknowledgments

We thank the University of Minnesota and the National Institutes for Water Resources/USGS National Water Quality Competitive Grants Program for support of this work. We thank Kathy Lee (USGS) for helpful discussions, Abdul Rafi Khwaja and Ann M. McNally (University of Minnesota) for DOC measurements, and Michael Ross (College of St. Benedict/St. John's University, St. Joseph, MN) for use of the mass spectrometer.

## Supporting Information Available

Chemical purity and supplier information; experimental details for laser flash photolysis, steady-state photolysis, and hydroxyl radical reaction; representative $^1O_2$ decay transients; Stern−Volmer plots for **1**−**5**; steady-state photolysis of **2** and **4** in the presence of $D_2O$ and sodium azide; competitive hydroxyl radical oxidation plots for **1**−**4** versus acetophenone; characterization of the natural water samples used in this work; photolysis of **1**−**4** in natural water samples; product analysis HPLC chromatograms; growth of sulfanilic acid over time; details of the error estimation for component data using sensitivity analysis; and table of environmental half-lives. This information is available free of charge via the Internet at http://www.pubs.acs.org.

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*Received for review November 24, 2003. Revised manuscript received April 19, 2004. Accepted May 6, 2004.*

ES0353053