

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/266616907>

# On the Use of Hydroxyl Radical Kinetics to Assess the Number- Average Molecular Weight of Dissolved Organic Matter

ARTICLE *in* ENVIRONMENTAL SCIENCE & TECHNOLOGY · SEPTEMBER 2014

Impact Factor: 5.33 · DOI: 10.1021/es5021873

---

CITATIONS

5

---

READS

74

## 3 AUTHORS:



[Elena Appiani](#)

ETH Zurich

2 PUBLICATIONS 7 CITATIONS

SEE PROFILE



[Sarah E. Page](#)

Arcadis

9 PUBLICATIONS 225 CITATIONS

SEE PROFILE



[Kristopher McNeill](#)

ETH Zurich

126 PUBLICATIONS 3,437 CITATIONS

SEE PROFILE

# On the Use of Hydroxyl Radical Kinetics to Assess the Number-Average Molecular Weight of Dissolved Organic Matter

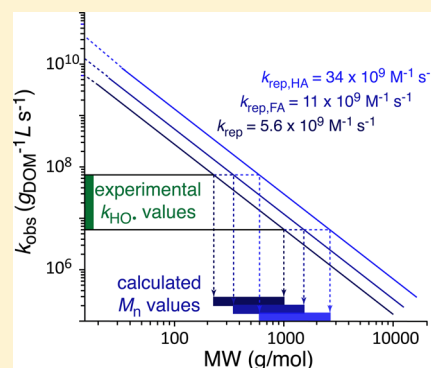
Elena Appiani,<sup>†</sup> Sarah E. Page,<sup>\*,‡</sup> and Kristopher McNeill<sup>\*,†</sup>

<sup>†</sup>Department of Environmental Systems Science, ETH Zurich, Universitätsstrasse 16, 8092 Zurich, Switzerland

<sup>‡</sup>Department of Earth and Environmental Sciences, University of Michigan, 1100 N. University Avenue, Ann Arbor, Michigan 48109, United States

## Supporting Information

**ABSTRACT:** Dissolved organic matter (DOM) is involved in numerous environmental processes, and its molecular size is important in many of these processes, such as DOM bioavailability, DOM sorptive capacity, and the formation of disinfection byproducts during water treatment. The size and size distribution of the molecules composing DOM remains an open question. In this contribution, an indirect method to assess the average size of DOM is described, which is based on the reaction of hydroxyl radical ( $\text{HO}^\bullet$ ) quenching by DOM.  $\text{HO}^\bullet$  is often assumed to be relatively unselective, reacting with nearly all organic molecules with similar rate constants. Literature values for  $\text{HO}^\bullet$  reaction with organic molecules were surveyed to assess the unselectivity of DOM and to determine a representative quenching rate constant ( $k_{\text{rep}} = 5.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ). This value was used to assess the average molecular weight of various humic and fulvic acid isolates as model DOM, using literature  $\text{HO}^\bullet$  quenching constants,  $k_{\text{C,DOM}}$ . The results obtained by this method were compared with previous estimates of average molecular weight. The average molecular weight ( $M_n$ ) values obtained with this approach are lower than the  $M_n$  measured by other techniques such as size exclusion chromatography (SEC), vapor pressure osmometry (VPO), and flow field fractionation (FFF). This suggests that DOM is an especially good quencher for  $\text{HO}^\bullet$ , reacting at rates close to the diffusion-control limit. It was further observed that humic acids generally react faster than fulvic acids. The high reactivity of humic acids toward  $\text{HO}^\bullet$  is in line with the antioxidant properties of DOM. The benefit of this method is that it provides a firm upper bound on the average molecular weight of DOM, based on the kinetic limits of the  $\text{HO}^\bullet$  reaction. The results indicate low average molecular weight values, which is most consistent with the recent understanding of DOM. A possible DOM size distribution is discussed to reconcile the small nature of DOM with the large-molecule behavior observed in other studies.



## INTRODUCTION

Dissolved organic matter (DOM) is ubiquitous and is directly involved in numerous important processes in environmental aquatic chemistry. For instance, DOM has been shown to play roles in redox reactions,<sup>1</sup> fate and transport of pollutants,<sup>2</sup> nutrient cycling in aqueous systems,<sup>3</sup> and microbial respiration.<sup>4</sup> Decades of research has led to the currently accepted model that describes DOM as being composed of a complex mixture of molecules of different size, structures, and chemical properties dynamically associated and stabilized by noncovalent interactions.<sup>5,6</sup> However, DOM molecular size and size distribution are a subject of ongoing research.

The average molecular weight and size of DOM is often mentioned as an important factor influencing its bioavailability.<sup>7</sup> DOM molecular size affects the sorptive capacity and strength of binding with organic compounds.<sup>8,9</sup> There is also evidence that DOM can differently affect the sorption capacity of the materials it is bound to, such as carbon nanotubes, depending on its molecular weight.<sup>10</sup> Similarly, formation of water disinfection byproducts has been shown to depend on DOM size.<sup>11</sup>

The definition of size for mixtures is not trivial. This is well-known in polymer chemistry where two different molecular weight averages, the number-average ( $M_n$ ) and the weighted average ( $M_w$ ) are widely employed depending on the property under investigation and the analytical technique in use. The number-average,  $M_n$ , is the most intuitive descriptor, being the simple arithmetic average molecular mass of all of the components with each component weighted equally. However, many detection methods are weighted toward larger molecules and thus do not give  $M_n$  directly. One of the best examples is dynamic light scattering (DLS) analysis, which preferentially detects larger molecules in a mixture, given the 6<sup>th</sup> power dependence of scattering efficiency on radius.<sup>12,13</sup> Measurements based on colligative properties, such as ebulliometry, which measures boiling point depression, are notable exceptions that count each molecule equally.<sup>14</sup>

Received: May 3, 2014

Revised: September 10, 2014

Accepted: September 15, 2014

One possible method to measure the range of  $M_n$  is based on the quenching of hydroxyl radical ( $\text{HO}^\bullet$ ) by DOM, and this is the focus of the present work.  $\text{HO}^\bullet$  is often discussed as having very low selectivity, reacting with nearly all organic molecules at near-diffusion-controlled rates. This notion has been taken advantage of in the protein footprinting technique, which uses the high reactivity and low selectivity of  $\text{HO}^\bullet$  to determine solvent-exposed amino acids in folded proteins.<sup>15</sup> If one makes the assumption that  $\text{HO}^\bullet$  reacts with all organic molecules unselectively, then the number of molecules in solution can be enumerated by assessing how well  $\text{HO}^\bullet$  is quenched in that solution. Knowing the number of molecules in solution and their average molecular formula, in turn, would allow one to estimate the number-average molecular weight ( $M_n$ ) of the solutes.

The present work contains two components. First, a large collection of previously measured  $\text{HO}^\bullet$  rate constants was examined to assess the unselective reactivity assumption and to determine a representative rate constant to use for  $\text{HO}^\bullet$  reaction with organic molecules. Second, published rate constants for  $\text{HO}^\bullet$  quenching by humic substance isolates were used in combination with the representative rate constant to estimate  $M_n$  of each isolate. In addition, we compared our results with previous estimates of DOM average molecular weight and discuss a molecular weight distribution model that reconciles the various results.

## ■ APPROACH AND METHODS

**Estimation of a General  $\text{HO}^\bullet$  Quenching Rate Constant.** To use  $\text{HO}^\bullet$  quenching to determine the number of molecules in solution, the first goal was to determine a suitable quenching rate constant that could be used to describe the reaction of  $\text{HO}^\bullet$  with a DOM mixture. The concept of a general rate constant for  $\text{HO}^\bullet$  is not new and has been recently reported in a DOM study by Arakaki et al.<sup>16</sup> In that study, a general scavenging rate constant for reaction of  $\text{HO}^\bullet$  was estimated using the same approach of compiling a training set.<sup>16</sup>

Bimolecular rate constants of  $\text{HO}^\bullet$  reacting with numerous compounds are available because of the importance of  $\text{HO}^\bullet$  in fields such as biology, environmental sciences, and medicine. We compiled published rates of  $\text{HO}^\bullet$  reaction with organic compounds.<sup>17–22</sup> Only organic molecules of  $\text{C}_x\text{H}_y\text{N}_z\text{O}_w$  composition were taken into account because the elemental analysis of DOM reveals that C, H, N, and O account for more than 98% of the mass of International Humic Substances Society (IHSS) isolates.<sup>23</sup> We selected a large range of molecular sizes because DOM is heterogeneous and both small organic molecules such as methanol<sup>24</sup> and large protein-like biomolecules have been found in DOM.<sup>25</sup> We also selected a large range of functional groups to reflect the structural diversity of DOM. The selected interval ranges from 1 C and 32 g  $\text{mol}^{-1}$  for methanol up to 156 C and 2018 g  $\text{mol}^{-1}$  for a fullerene- $\text{C}_{60}$ - $\gamma$ -cyclodextrin complex.

A rate constant of  $5.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  was chosen as a representative rate constant ( $k_{\text{rep}}$ ) for DOM based on the distribution of rate constants found in this data set (see the Results and Discussion section for more on the selection of this rate constant).

**Estimation of Number-Average Molecular Weight ( $M_n$ ).** The  $M_n$  value of a DOM sample was estimated using four different  $k$  values (Table 1).

**Table 1. Description of the  $k$  Values Used in the Estimation of the  $M_n$  of DOM Isolates**

rate	unit	description
$k_q$	$\text{M}^{-1} \text{ s}^{-1}$	bimolecular rate constant values for $\text{HO}^\bullet$ quenching by known molecules
$k_{\text{rep}}$	$\text{M}^{-1} \text{ s}^{-1}$	representative bimolecular rate constant for $\text{HO}^\bullet$ quenching by organic molecules
$k_{\text{C,DOM}}$	$\text{L mol}_\text{C}^{-1} \text{ s}^{-1}$	bimolecular rate constant values for $\text{HO}^\bullet$ quenching by DOM on a per molar-carbon basis
$k_{\text{DOM}}$	$\text{L g}_{\text{DOM}}^{-1} \text{ s}^{-1}$	bimolecular rate constant values for $\text{HO}^\bullet$ quenching by DOM on a per mass basis

First, for known molecules, the  $\text{HO}^\bullet$  quenching bimolecular rate constants are reported as  $k_q$  in units of per molar per second ( $\text{M}^{-1} \text{ s}^{-1}$ ). Second, as mentioned in the previous section and discussed in the Results and Discussion section below, these  $k_q$  values were used to determine a representative bimolecular rate constant for  $\text{HO}^\bullet$  reaction with organic molecules,  $k_{\text{rep}} = 5.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ . Third, for DOM, which is composed of an unknown mixture of molecules, the rate constants for  $\text{HO}^\bullet$  quenching are commonly reported as  $k_{\text{C,DOM}}$ , in units of liters per mole carbon per second ( $\text{L mol}_\text{C}^{-1} \text{ s}^{-1}$ ). Fourth, for this paper, we have converted  $k_{\text{C,DOM}}$  to a per-mass rate constant of quenching ( $k_{\text{DOM}}$ ), with units of liters per gram carbon per second ( $\text{L g}_{\text{DOM}}^{-1} \text{ s}^{-1}$ ) (eq 2, described below).

The number-average molecular weight of a DOM sample was determined from the DOM-specific  $\text{HO}^\bullet$  quenching rate constant ( $k_{\text{DOM}}$ ,  $\text{L g}_{\text{DOM}}^{-1} \text{ s}^{-1}$ ) and the general  $\text{HO}^\bullet$  rate constant ( $k_{\text{rep}}$ ,  $\text{M}^{-1} \text{ s}^{-1}$ ). The DOM-specific rate constants have been determined for various DOM isolates.<sup>26–34</sup> In this study, we selected rate constants of quenching of  $\text{HO}^\bullet$  by isolated DOM material, choosing only the studies that used IHSS standard isolates<sup>26–34</sup> and nonstandard materials where the elemental composition of the DOM was reported, since this information is a prerequisite for the calculation (eq 2), although it is not always reported in  $\text{HO}^\bullet$  quenching studies.

The average molecular weight of DOM ( $M_n$ ) was obtained by dividing the representative rate constant ( $k_{\text{rep}}$ ) by the DOM-specific  $\text{HO}^\bullet$  quenching rate constant ( $k_{\text{DOM}}$ ) (eq 1).

$$M_n \left[ \frac{\text{g}_{\text{DOM}}}{\text{mol}_{\text{DOM}}} \right] = \frac{k_{\text{rep}}}{k_{\text{DOM}}} \left[ \frac{\text{mol}_{\text{DOM}}^{-1} \text{L s}^{-1}}{\text{g}_{\text{DOM}}^{-1} \text{L s}^{-1}} \right] \quad (1)$$

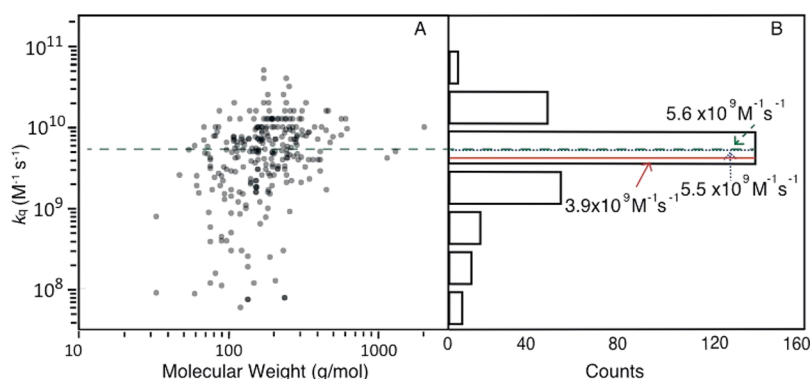
As the rate constant of  $\text{HO}^\bullet$  quenching by DOM is usually reported on a carbon basis ( $k_{\text{C,DOM}}$ ), the literature data was converted to the per-mass-based rate constant ( $k_{\text{DOM}}$ ) using the carbon atomic mass ( $\text{MW}_\text{C}$ ) and the carbon fraction in DOM ( $f_\text{C}$ ) according to eq 2

$$k_{\text{DOM}} \left[ \frac{\text{L}}{\text{g}_{\text{DOM}} \text{ s}} \right] = k_{\text{C,DOM}} \left[ \frac{\text{L}}{\text{mol}_\text{C} \text{ s}} \right] \frac{1}{\text{MW}_\text{C}} \left[ \frac{\text{mol}_\text{C}}{\text{g}_\text{C}} \right] f_\text{C} \left[ \frac{\text{g}_\text{C}}{\text{g}_{\text{DOM}}} \right] \quad (2)$$

The ratio of  $k_{\text{rep}}$  and  $k_{\text{C,DOM}}$  is the average number of carbons per DOM molecule (eq 3).

$$\frac{k_{\text{rep}}}{k_{\text{C,DOM}}} \left[ \frac{\text{mol}_{\text{DOM}}^{-1} \text{L s}^{-1}}{\text{mol}_\text{C}^{-1} \text{L s}^{-1}} \right] = \left[ \frac{\text{mol}_\text{C}}{\text{mol}_{\text{DOM}}} \right] \quad (3)$$

Notice that with the method described above it is not possible to distinguish between aggregates and small molecules. This is important because many authors have suggested that DOM self-aggregates.<sup>5</sup> Such aggregates would appear as “large



**Figure 1.** Distribution of HO• quenching rate constants for organic molecules with formula  $C_cH_hN_nO_o$ . (A) The dots correspond to published HO• quenching rate constants<sup>17–22,35–39</sup> versus the molecular weight of the quenchers. Both axes are reported on a logarithmic scale. (B) A histogram of the rate constants from A. The colored lines in the histogram are possible representative HO• quenching bimolecular rate constants ( $k_{rep}$ ) selected using different criteria: red line as the average of all values ( $3.9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ); blue dotted line, average of all values excluding molecules less than 150 g/mol ( $5.5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ); green dashed line, the mode of the distribution ( $5.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ). In this study, we choose to use the value represented with the green dashed line,  $5.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ .

molecules” to this method, and the true  $M_n$  considering the building blocks of these aggregates as separate molecules would be smaller than those found by this method.

## RESULTS AND DISCUSSION

**Determination of the General HO• Quenching Rate Constant.** To determine a representative rate constant for quenching of HO• by natural organic matter, we examined previously measured rate constants for a wide variety of organic molecules. Examining the rate constants<sup>17–22,35–39</sup> (Figure 1), we observed that the experimental data were distributed between  $6 \times 10^7$  and  $5 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ . The histogram (Figure 1B) shows that most of the rate constants cluster in the middle of this range, with 75% of the values being between  $3 \times 10^9$  and  $10 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ . The distribution is also asymmetric, tailing toward lower rate constants, which might be expected given the fact that the diffusion-controlled limit for a bimolecular reaction in water is on the order of  $10^{10} \text{ M}^{-1} \text{ s}^{-1}$ . The very highest values in this compilation, which approach  $5 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ , are essentially at the diffusion-controlled limit.

The data presented in Figure 1 cover a wide range of rate constants and argue against the assumption of HO• being unselective in its reactivity. The outliers indicate that there are some structures that lead to exceptionally fast (e.g., electron-rich phenols) and exceptionally slow reactions with HO• (e.g., electron-poor molecules lacking aromatic substructures) (Table S1, Supporting Information).

Happily, for considering the reactivity of DOM, the distribution of rate constants narrows considerably for larger molecules (>150 g/mol): 90% of the rate constants in this group are in the  $1\text{--}30 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  range. This is possibly due to a balancing effect, in which larger molecules have both fast-reacting and slow-reacting substructures within the same molecule. It is also important to note that for the method to work, one needs only the average rate constant for the entire DOM mixture. If the high and low outliers balance each other, then an accurate  $M_n$  will be calculated. If the training set (i.e., data in Figure 1) is sufficiently similar to DOM, then it should not matter that the individual components have rate constants that span a wide range; the average should be close to what we have chosen. If, however, DOM contains a significant pool of either fast-reacting or slow-reacting molecules that shifts the average quenching rate constant away from our estimate of  $k_{rep}$ ,

then inaccurate  $M_n$  values will be obtained. This possibility is discussed in more detail in the next section.

A general rate constant for HO• reaction with DOM was estimated in different ways: (1) Assuming that the suite of molecules taken into account is representative of DOM and averaging the rate constants of all the molecules considered above. This approach resulted in a general HO• quenching rate constant of  $3.9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  (red line Figure 1B). (2) Averaging all the rate constants of quenching by molecules with a molecular weight higher than 150 g/mol, because it is generally believed that low molecular weight material (<150 g/mol) is not the major constituent of DOM. This approach resulted in a general rate constant of  $5.5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  (blue dotted line in Figure 1B). (3) Selecting the mode of the distribution. This approach resulted in a value of  $5.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  (green dashed line Figure 1B). The HO• quenching rate constant obtained with the three approaches are comparable. We choose to use the mode approach in the subsequent calculations, giving  $5.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  as the general HO• quenching rate constant, in part because we felt that the training set was overrepresented in slow-reacting N-rich molecules. Note that choosing a lower rate constant would result in lower calculated  $M_n$  values (see eq 1). The general rate constant previously reported by Arakaki et al,  $6.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ , is the average of the rate constants reported in their training set, and it is in good agreement with our estimate.<sup>16</sup> Arakaki et al. analyzed a very large data set (1400 individual molecules) that did not exclude any organic compounds. We attribute the difference between Arakaki’s  $k_{rep}$  and ours to our exclusion of fast-reacting S- and P-containing compounds in our dataset.

### Determination of DOM Size by HO• Reaction.

Numerous groups have determined rate constants for the quenching of HO• by DOM in the last 15 years.<sup>26–34</sup> By far, the best studied of these is Suwannee River Fulvic Acid I (SRFA I),<sup>26–33</sup> for which at least ten different rate constant determinations by different methods have been reported (Table 2). It is instructive that even for SRFA I, the best studied isolate, the  $k_{C,DOM}$  value is not known to a high level of precision. This is important because the near factor-of-four spread between the lowest and highest  $k_{C,DOM}$  values leads directly to a near factor-of-four range in the calculated  $M_n$  values for SRFA I. The accuracy of  $M_n$  values obtained from

**Table 2. Hypothetical Number Average Molecular Weight ( $M_n$ ) Values and Average Number of Carbon Atoms Per DOM Unit Calculated for International Humic Substance Society (IHSS) DOM Isolates Based on Uniform Reactivity Toward  $\text{HO}^\bullet$  ( $k_{\text{rep}} = 5.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ )<sup>a</sup>**

organic matter source	%C	$k_{\text{C,DOM}} (10^8 \text{ M}^{-1} \text{ s}^{-1})$	$\text{HO}^\bullet$ production method	$\text{HO}^\bullet$ detection method	reference	average number of C per unit DOM	$M_n$ ( $\text{g mol}^{-1}$ )
Suwanee River Fulvic Acid I	52.44	1.4	nitrate photolysis	nitrobenzene	26	40	915
		1.4 ( $\pm 0.16$ )	radiolysis	transient absorption	28	40	915
		1.6 ( $\pm 0.04$ )	radiolysis	$\text{SCN}^-$ competition	28	35	800
		1.6	radiolysis	$\text{SCN}^-$ competition	27	35	800
		1.9 ( $\pm 0.07$ )	radiolysis	transient absorption	28	29	674
		2.1 ( $\pm 0.18$ )	radiolysis	transient absorption	30	27	610
		3.2	radiolysis	$^{14}\text{C}$ -formate	31	17	400
		3.7	ozonation	p-Cl benzoic acid	29	15	346
		3.7	nitrate photolysis	1-Cl-butane	32	15	346
		5.3 ( $\pm 0.1$ )	fenton reaction	$^{14}\text{C}$ -formate	33	11	241
average ( $n = 10$ )		2.6 ( $\pm 1.3$ )				26 ( $\pm 11$ )	610 ( $\pm 250$ )
Suwanee River Fulvic Acid II	52.34	1.3 ( $\pm 0.1$ )	radiolysis	terephthalate	34	43	988
		2.4 ( $\pm 0.5$ )	photolysis	terephthalate	34	23	535
		1.9 ( $\pm 0.8$ )				33 ( $\pm 14$ )	760 ( $\pm 320$ )
Suwanee River Humic Acid I	52.55	2.3	radiolysis	$^{14}\text{C}$ -formate	31	24	556
		6.8	nitrate photolysis	1-Cl-butane	32	8	188
		8.1	ozonation	p-Cl benzoic acid	29	7	158
		5.7 ( $\pm 3.0$ )				13 ( $\pm 10$ )	300 ( $\pm 220$ )
Suwanee River Humic Acid II	52.63	10.36 ( $\pm 0.02$ )	radiolysis	transient absorption	30	5	123
Pony Lake Fulvic Acid	52.47	2.4 ( $\pm 0.1$ )	radiolysis	terephthalate	34	23	534
		4.4 ( $\pm 0.6$ )	photolysis	terephthalate	34	13	291
		6.9 ( $\pm 0.8$ )	radiolysis	transient absorption	30	8	186
		4.6 ( $\pm 2.3$ )				15 ( $\pm 7.6$ )	340 ( $\pm 120$ )
Elliot Soil Humic Acid	58.13	1.2 ( $\pm 0.2$ )	radiolysis	transient absorption	30	47	963
Leonardite Humic Acid	63.81	6.5 ( $\pm 0.3$ )	radiolysis	transient absorption	30	9	162

<sup>a</sup>The  $\text{HO}^\bullet$  production and detection methods are also listed for each entry.

$\text{HO}^\bullet$  quenching kinetics is limited to the accuracy of  $k_{\text{C,DOM}}$  values. Nevertheless, even with large uncertainties, the  $M_n$  estimates by this approach remain useful.

Using the rate constants of  $\text{HO}^\bullet$  quenching by DOM in Tables 1 and 2, the representative  $\text{HO}^\bullet$  quenching rate constant ( $k_{\text{rep}} = 5.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ), and eqs 1–3, we calculated  $M_n$  and the average number of carbon atoms per DOM molecule, as summarized in Table 2 for IHSS isolates ( $\sim 100$ – $1000$  Da and  $5$ – $47$  C) and Table 3 for other isolates ( $276$ – $490$  Da and  $12$ – $21$  C).

Analyzing the results obtained with this method, we observe two unexpected results. First, in general, the  $M_n$  values obtained using this method are quite low (Tables 2 and 3) compared to the values published in the literature obtained by various analytical techniques (Supporting Information, Tables S2–S3). Second, the humic acid samples appear to have lower  $M_n$  values than fulvic acid samples, which does not reflect the trends reported in previous studies (Supporting Information, Table S2).

With regard to the first observation, one must consider what changes would yield a higher  $M_n$  value. The number-average molecular weight,  $M_n$ , is determined by  $k_{\text{rep}}$  and  $k_{\text{C,DOM}}$  (eq 1), thus inaccuracies in those two values will affect the quality of  $M_n$ . An overestimation of  $k_{\text{C,DOM}}$  will result in an underestimation of  $M_n$  by the same factor, because  $M_n$  and  $k_{\text{rep}}$  are inversely proportional (eq 1). On the other hand, increasing  $k_{\text{rep}}$  will increase  $M_n$  by the same factor (eq 1). Inaccuracies in  $k_{\text{rep}}$  and  $k_{\text{C,DOM}}$  may be contributing to the lower than expected values of  $M_n$  reported in Table 2. There are two possibilities: Either  $k_{\text{rep}}$  is underestimated or  $k_{\text{C,DOM}}$  is overestimated. If the true value of  $k_{\text{rep}}$  is higher than our estimate, it could not be much higher, because our estimate of  $5.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  is already close to the diffusion-controlled limit. However, analyzing Table S1 (Supporting Information), we notice that the exceptionally fast quenching reaction rate constants are reported for electron-rich phenols, a moiety known to be a DOM constituent. This suggests that the rate constant chosen might underestimate the overall reactivity of DOM, because it



**Table 3. Hypothetical Number Average Molecular Weight ( $M_n$ ) Values and Average Number of Carbon Atoms Per DOM Unit for Various Isolates<sup>a,b</sup> Calculated Using the Assumption of a Uniform Reactivity Toward  $\text{HO}^\bullet$ <sup>c</sup>**

organic matter source	%C	$k_{\text{C,DOM}} (10^6 \text{ M}^{-1} \text{ s}^{-1})$	$M_n$ g/mol for $k_{\text{rep}} 5.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$	$M_n$ g/mol for $k_{\text{rep}} 1.1 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$	$M_n$ from VPO <sup>41</sup>	aromatic <sup>29</sup> (%C)
groundwater sample, MN	52.7	2.6	490	980	NA	11.9
Lake Michigan, IL	48.5	3	463	926	NA	14
Shingobee River, MN (1992)	51.1	3.3	399	798	NA	20.4
Lake Shingobee, MN	51.1	3.4	387	774	NA	18.5
Lake Fryxell, Antarctica	55	3.5	408	816	NA	13.1
Missouri River, IA	55.4	3.5	405	810	640	20.4
Williams Lake, MN	51.8	3.5	371	742	NA	10.4
Yakima River at Kiona, WA	56.1	3.6	334	668	NA	25.3
Calif. State Project water, CA	48.1	4	350	700	NA	25
Yakima River at CleElum, WA	57.2	4	294	588	NA	26.6
Ohio River, OH	55.5	4.1	296	592	606 <sup>d</sup>	24.3
Coal Creek, CO	52.8	4.3	295	590	743	27.4
Ogeechee River, GA	54	4.5	276	552	714	24.8
Shingobee River, MN (1993)	50.6	4.5	294	588	NA	24.5

<sup>a</sup>All the materials come from the same study (Westerhoff et al.<sup>29</sup>) and were isolated with the same method. <sup>b</sup> $\text{HO}^\bullet$  production method is ozonation, with p-Cl benzoic acid as  $\text{HO}^\bullet$  probe. <sup>c</sup> $M_n$  is estimated using two different  $k_{\text{rep}}$  values. Quenching rate constant ( $k_{\text{C,DOM}}$ ), percent carbon (%C) values,  $M_n$  from VPO measurements and aromaticity are also listed. <sup>d</sup>Average of spring and fall samples.

does not take into account these highly reactive species. If we double  $k_{\text{rep}}$  to  $1.1 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ , for example, the  $M_n$  range would change from  $\sim 120$ – $1000 \text{ Da}$  (Table 2) to  $\sim 220$ – $1800 \text{ Da}$ . Thus, focusing only on the fulvic acid results, the  $M_n$  calculated with the higher  $k_{\text{rep}}$  (PLFA 680 g/mol, SRFA I 1220 g/mol, and SRFA II 1520 g/mol) would match the values reported by other techniques such as VPO, FFF, and SEC (Supporting Information, Table S2). If an incorrectly chosen  $k_{\text{rep}}$  value is the reason for the low  $M_n$  values, this suggests that the fulvic acid ensembles react with  $\text{HO}^\bullet$  about 2 times faster than the mixture of molecules chosen as the training set (i.e.,  $k_{\text{rep,fulvic}} \sim 1.1 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ ).

The second possibility is that the  $k_{\text{C,DOM}}$  values are artificially high. It is important to note that  $k_{\text{C,DOM}}$  data reported in Table 2 are from different studies, performed in different laboratories, and using different techniques. There is no clear trend in the scavenging rate constants with  $\text{HO}^\bullet$  generation methods, which is possibly complicated by variations in both  $\text{HO}^\bullet$  generation techniques and  $\text{HO}^\bullet$  detection methods. For example, in the case of SRFA I,  $\text{HO}^\bullet$  generation by both nitrate photolysis and ozonation results in  $\text{HO}^\bullet$  scavenging rates that vary by a factor of 2 in different studies (Table 2). There is previous evidence that benzoic acid, a representative aromatic probe for  $\text{HO}^\bullet$ , responds differently to standard  $\text{HO}^\bullet$  production methods,<sup>40</sup> which may be a contributing factor to the variability of  $\text{HO}^\bullet$  scavenging rates observed in these studies. The unknown nature of this variability does make comparison of results between laboratories and different techniques challenging, but the data in Table 2, when taken together, should reflect the order of magnitude of the  $M_n$  of the isolates. The focus of this work is to explore what  $\text{HO}^\bullet$  quenching kinetics say about the  $M_n$  of DOM rather than assessing the quality of the data reported in these studies. Thus, all of the compiled  $\text{HO}^\bullet$  scavenging data (Table 2) have been treated equally. When examining the data as a whole, there is no indication that these previous studies have produced  $k_{\text{C,DOM}}$  values that are artificially high.

The second unexpected result, that humic acids appear smaller than fulvic acids, is also connected to both the choice of  $k_{\text{rep}}$  and the heterogeneity of the source of the data reported in

Table 2. In particular, in essentially every study of humic and fulvic acids, it has been shown that humic acids have a larger average molecular size than fulvic acids (Supporting Information, Table S2). Looking more closely at the data in Table 2, there are four studies that examined the  $\text{HO}^\bullet$  quenching of both SRFA and SRHA, all using different methods.<sup>29–32</sup> In three of the four cases, the  $k_{\text{C,DOM}}$  values for SRHA were higher than those for SRFA (by a factor of 2.2 on average). If we take as a given that SRHA has a higher average  $M_n$  than SRFA, according to eq 1, the only way  $k_{\text{C,DOM}}$  can be higher for SRHA than SRFA is if the value for  $k_{\text{rep}}$  is also higher for SRHA than SRFA, but by an even greater factor. Assuming, the  $M_n$  value for SRHA is about 50% larger than for SRFA (see Table S2, Supporting Information),  $k_{\text{rep, humic}}$  would be about 3.3 times  $k_{\text{rep, fulvic}}$  or approximately  $3.4 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ . This is consistent with the fact that SRHA and SRFA have different chemical compositions, with SRHA having higher aromatic and phenolic content. Table S1 (Supporting Information) shows that electron-rich aromatic compounds such as phenols can have an exceptionally high reactivity toward  $\text{HO}^\bullet$ .

Westerhoff et al. have collected a dataset (Table 3) that supports the suggestion that the  $k_{\text{rep}}$  value of  $5.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  is too low.<sup>29</sup> In the Westerhoff et al. study,  $\text{HO}^\bullet$  quenching rate constants were obtained for a range of organic matter isolates from a variety of natural water sources. The isolation procedure (XAD8 sorption at pH 2 with elution at pH 13) presumably resulted in a mixture of humic and fulvic acids, but the characterization data suggests that these isolates are dominated by fulvic acid character. For four of the source waters,  $M_n$  values from VPO data are also available.<sup>41</sup> Table 3 lists the  $M_n$  values from  $\text{HO}^\bullet$  quenching calculated using a  $k_{\text{rep}}$  value of  $5.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  as well as a 2-times higher value of  $1.1 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ , as suggested by the fulvic acid data discussed above. The  $M_n$  values calculated using the low  $k_{\text{rep}}$  value are very low and not in agreement with the VPO data. On the other hand, the  $M_n$  values calculated using the higher  $k_{\text{rep}}$  value are in good agreement with the VPO data.

The data in Table 3 also reinforce the postulate that the  $\text{HO}^\bullet$  quenching rate constant varies with the composition of the organic matter sample, with higher aromatic and phenolic

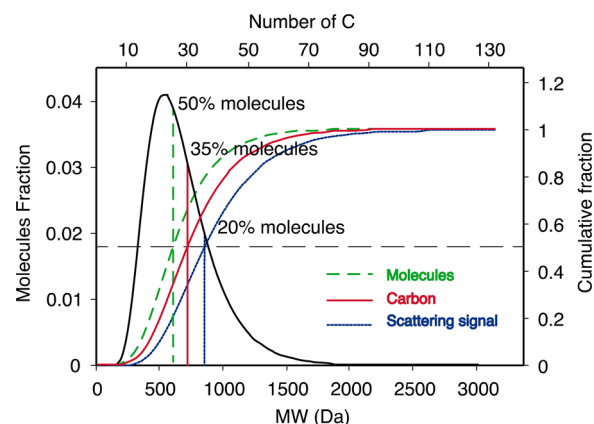
content giving rise to higher rate constants. The aromatic carbon content was estimated for the samples examined by Westerhoff et al. (Table 3) and a correlation between  $k_{\text{C,DOM}}$  and % aromatic C content is observed ( $r^2 = 0.60$ ; Figure S1, Supporting Information). From eq 1, there are only two ways for more aromatic-rich samples to have higher per-mole-carbon rate constants of  $\text{HO}^\bullet$  quenching. Either the more aromatic-rich samples have lower average molecular weights or they have higher intrinsic reactivity toward  $\text{HO}^\bullet$ . We believe the latter explanation is more compelling, especially given that aromatic compounds are known to react with  $\text{HO}^\bullet$  with especially high rate constants.<sup>42</sup>

**Assessment of Using  $\text{HO}^\bullet$  Quenching to Determine Molecular Weight.** The current study is focused on two main questions: First, is  $\text{HO}^\bullet$  unselective enough to assess a representative rate of quenching for a mixture? Second, can  $\text{HO}^\bullet$  quenching kinetics be used to estimate DOM  $M_n$ ? With regard to the first question, characterizing  $\text{HO}^\bullet$  as unselective is not valid given that the pool of  $k_q$  values analyzed in this study spans nearly 3 orders of magnitude ( $6 \times 10^7$ – $4.8 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ , Figure 1 and Table S1 (Supporting Information)). Because DOM is a mixture, we tested the idea of using a rate constant representative of the averaged rate constant for all DOM components. This rate constant was obtained by analyzing a training set of literature  $\text{HO}^\bullet$  quenching rate constants ( $k_q$ ) (Figure 1) from molecules of CHNO composition. We observed that the  $k_{\text{rep}}$  value obtained from this artificial mixture was likely too low to represent DOM and that the actual rate constants were probably 2 times higher for fulvic acids. Furthermore, humic acids were concluded to react even faster than fulvic acids (ca. 3 times faster). These observations suggest that fulvic acids and humic acids are much more reactive toward  $\text{HO}^\bullet$  than “average” organic molecules, in line with previous observations of DOM acting as an antioxidant.<sup>43</sup>

With regard to the second question, the inability to determine a general average quenching rate constant for DOM means that  $M_n$  values cannot be obtained precisely from  $\text{HO}^\bullet$  kinetics. Nevertheless, because DOM apparently reacts with  $\text{HO}^\bullet$  at close to diffusion-controlled rates, we can estimate a firm upper bound to DOM  $M_n$ . If we were to estimate the highest possible  $M_n$ , the prerequisite to the estimation are the lowest reported carbon content (48%, Table 3), the lowest reported  $k_{\text{C,DOM}}$  ( $1.2 \times 10^8 \text{ M}_\text{C}^{-1} \text{ s}^{-1}$ ) and the extremely high average quenching rate constant estimated for humic acids ( $3.4 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ ). Using these parameters, we obtain 7000 Da as the upper limit for the DOM  $M_n$  value. Notice that the 7000 Da upper limit is calculated assuming that all the parameters synergistically contribute to the high molecular weight, namely the material must have a high intrinsic reactivity toward  $\text{HO}^\bullet$  (i.e., high aromatic content), the %C must be lower than average, and the reported  $k_{\text{C,DOM}}$  must be below average. This coincidence has not been observed in the selection of studies reported (Tables 2, 3, and S4 (Supporting Information)).

Similarly, this study provides guidelines to predict whether measured  $k_{\text{DOM}}$  values are plausible. If we set a reasonable lower limit for FA and HA  $M_n$  (600 and 900 Da) and we assume our estimates of  $k_{\text{rep, fulvic}}$  and  $k_{\text{rep, humic}}$  are accurate ( $1.1 \times 10^{10}$  and  $3.4 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ ), we calculate the maximum  $k_{\text{C,DOM}}$  values to be approximately  $4 \times 10^8$  and  $9 \times 10^8 \text{ M}_\text{C}^{-1} \text{ s}^{-1}$ , which correspond to  $k_{\text{DOM}}$  values of approximately  $4 \times 10^7$  and  $7 \times 10^7 \text{ L g}_{\text{DOM}}^{-1} \text{ s}^{-1}$ . Thus, measured values of  $k_{\text{C,DOM}}$  exceeding this limit are either incorrect, or indicate the presence of additional  $\text{HO}^\bullet$  quenchers in the system.

**Distribution of DOM Sizes and Its Effect on  $M_n$  Determination.** The disparate values for the size of DOM isolates, obtained from different experiments, is initially puzzling. For example, DOM is well-known to sorb relatively large organic molecules (e.g., benzo[a]pyrene (20 C atoms)<sup>44</sup> and DDT (14 C atoms)<sup>45</sup>), suggesting that DOM is much larger. On the other hand,  $M_n$  values determined by VPO, and now by  $\text{HO}^\bullet$  quenching, indicate average molecular weights below 1000 Da. In addition, recent ultrahigh resolution mass spectrometry studies demonstrate the presence of numerous low molecular weight species in DOM.<sup>46,47</sup> These apparently discordant results can be reconciled or rationalized by invoking a broad molecular weight distribution. To illustrate this, consider the simple log-normal distribution model illustrated in Figure 2. There is precedent for using a log-normal



**Figure 2.** One possible log-normal distribution of size of DOM versus molecules fraction. The black function represents the log-normal distribution as percentage of molecules, obtained by eq 4 setting  $\mu$  (center of the distribution) equal to 610 Da and  $\sigma$  to 0.316. The green dashed, red full, and blue dotted curves represent the cumulative fraction of molecules, carbon, and scattering response, respectively. The dashed green function ( $g(x)$ ) is the cumulative molecules fraction distribution and is described as  $g(x) = \sum_{i=0}^x f(i)$ , where  $f(x)$  is the log-normal distribution described above. The red full function ( $h(x)$ ) is the cumulative carbon fraction distribution, and is described as  $h(x) = \frac{\sum_{i=0}^x f(i)C_i}{\sum_{i=0}^{\text{max}} f(i)C_i}$ , where  $f(x)$  is the log-normal distribution described above and  $C_i$  is the number of carbons contained in the molecule of molecular weight  $x$ . The dotted blue function ( $z(x)$ ) is the cumulative scattering contribution distribution, and is described as  $z(x) = \frac{\sum_{i=0}^x f(i)r_i^6}{\sum_{i=0}^{\text{max}} f(i)r_i^6}$ , where  $f(x)$  is the log-normal distribution described above and  $r$  is the radius of the molecule of molecular weight  $x$  estimated assuming a density of DOM of 1.05. The dashed green, full red, and dotted blue vertical line on the distribution, mark the fraction of small molecules needed to cover 50% of the molecules, of the carbon, and of the scattering response, respectively.

distribution to describe the range of DOM molecular weights and to rationalize the size distribution of fulvic acids, because chromatograms obtained by high performance size exclusion chromatography (HP-SEC) analysis fit this kind of distribution.<sup>48</sup> Such a distribution can be defined by eq 4.<sup>48</sup>

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{\left(\frac{1}{2\sigma^2}(\log(x)-\mu)^2\right)} \quad (4)$$

The distribution is characterized by two coefficients, the mean value ( $\mu$ ), which is the center of the distribution, and the

standard deviation ( $\sigma$ ), which represents the width of the distribution. In the distribution in Figure 2, we set  $\mu$  to 610 Da according to the average molecular weight calculated using the SRFA I data in Table 2, and  $\sigma$  to 0.316, according to the value reported by Cabannis et al. for SRFA I.<sup>48</sup> In that study, the authors estimated the width of the distribution measuring the standard deviation of size exclusion chromatography results. This value of  $\sigma$ , although arbitrary, does give good agreement with a prior study that showed 35% of SRFA passing through a nominal 500 Da filter.<sup>46</sup> In this distribution, although half of the molecules have a mass of >700 Da (Figure 2, green dashed line), half of the total carbon is contained in molecules >800 Da (>34 C per molecule, 35% of the molecules, Figure 2, red line). Similarly, half of the scattering contribution would be given by molecules >900 Da (>38 C per molecule, 20% of the molecules, Figure 2, blue dotted line). This illustrates further that methods weighted by, for example, the carbon content of a molecule or its scattering properties, will yield discordant answers, and may explain why the same materials (e.g., SRFA I) have been assigned a wide range of average molecular weights.

Notice that this is only one possible distribution that could reconcile the apparent large and small aspects of DOM. Real distributions might be expected to be more complicated than a simple monomodal distribution, as the sources of DOM are believed to be composed of large components, such as biomacromolecules and biopolymers, and low molecular weight components, such as secondary metabolites and membrane lipids.

## ENVIRONMENTAL IMPLICATIONS

The  $\text{HO}^\bullet$  quenching rate constant of a DOM sample ( $k_{\text{DOM}}$ ) is the ratio of the average rate constant for the molecules composing the DOM ( $k_{\text{rep,DOM}}$ ) and the number-average molecular weight of the molecules composing the sample ( $M_n$ ). In order to infer the value of one of the members of the ratio, information on the other are essential. In this study, we calculated the  $M_n$  for DOM assuming its reactivity to be represented by the average reactivity of model organic molecules with  $\text{HO}^\bullet$ . The  $M_n$  values estimated with this method were found to be too low compared to previously reported  $M_n$  values. This is an indication that the  $k_{\text{rep}}$  value used was too low, meaning that DOM reactivity is at the higher end of the distribution of measured rate constants. Using reported values of DOM  $M_n$ , we estimated  $k_{\text{rep}}$  for fulvic and humic acids. The calculated value showed that DOM is highly reactive toward  $\text{HO}^\bullet$  and that specifically Suwanee River humic acid is approximately three times more reactive than Suwanee River fulvic acid. The high  $\text{HO}^\bullet$  scavenging ability of these materials is a further demonstration of the antioxidant properties of DOM.<sup>43</sup>

In a system where  $\text{HO}^\bullet$  quenching rate constants are observed to vary over a series of samples (e.g., across a spatial gradient), it may be tempting to ascribe these changes to different  $M_n$  values in the samples. For example,  $\text{HO}^\bullet$  quenching rate constants have been seen to vary in in pristine Arctic surface waters,<sup>49</sup> and it may be reasonable to expect changes in the  $M_n$  values. Dong et al.<sup>50</sup> analyzed the reactivity of different size fraction (<1 kDa to <10 kDa) of effluent organic matter with  $\text{HO}^\bullet$ . If each fraction has the same reactivity, then the  $M_n$  value would have to decrease by a factor of approximately 20, going from <1 kDa fraction to the <10 kDa fraction, which fits perfectly with the rates measured by the authors.<sup>50</sup> However, one cannot definitively state that the

average molecular weight has changed between the samples without knowing the  $\text{HO}^\bullet$  reactivity of the material in each sample. The present study suggests that it is so far not possible to know a priori the  $\text{HO}^\bullet$  quenching rate constant.

Despite the fact that it is not possible to unequivocally determine the size of DOM using  $\text{HO}^\bullet$  quenching rate constants, awareness of the directly proportional relationship between the two values allows one to put limits on reasonable values of both quenching rate constants for  $\text{HO}^\bullet$ , and DOM size. We estimated that the highest plausible quenching rate constant for fulvic acids and humic acids to be approximately  $4 \times 10^8$  and  $9 \times 10^8 \text{ M}_C^{-1} \text{ s}^{-1}$ , which correspond to values of approximately  $4 \times 10^7$  and  $7 \times 10^7 \text{ L g}_{\text{DOM}}^{-1} \text{ s}^{-1}$ . For higher quenching rate constant values, the calculated  $M_n$  for DOM would fall below 600 and 900 Da for fulvic acids and humic acids, respectively, even assuming an especially high reactivity. Similarly, the study provides a firm upper limit to the  $M_n$  for DOM. Assuming both an especially high intrinsic reactivity of DOM toward  $\text{HO}^\bullet$  and an especially slow measured rate of quenching, the maximum  $M_n$  value that can be estimated is 7000 Da.

## ASSOCIATED CONTENT

### Supporting Information

Additional tables showing molecules with  $\text{HO}^\bullet$  rate of quenching out of the range  $1\text{--}10 \times 10^9$ , molecular weight of DOM isolates from IHSS measured with different techniques, description of techniques used to characterize the DOM size and range measured, chemical composition of DOM isolates from IHSS, and figure showing correlation between  $k_{\text{C,DOM}}$  and % Aromatic C. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## AUTHOR INFORMATION

### Corresponding Authors

\*K. McNeill. Tel: +41 44 632 47 55. Fax: +41 44 632 14 38. E-mail: [kris.mcneill@env.ethz.ch](mailto:kris.mcneill@env.ethz.ch).

\*S. E. Page. Tel: 1-734-763-6859. Fax: 1-734-764-1435. E-mail: [sepage@umich.edu](mailto:sepage@umich.edu).

### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

We thank three anonymous reviewers for helpful comments that substantially improved the paper. We also gratefully acknowledge funding from the Swiss National Science Foundation (Grant no. CRSI22\_127568).

## REFERENCES

- (1) Aeschbacher, M.; Sander, M.; Schwarzenbach, R. P. Novel electrochemical approach to assess the redox properties of humic substances. *Environ. Sci. Technol.* **2009**, *44* (1), 87–93.
- (2) McCarthy, J. F.; Roberson, L. E.; Burrus, L. W. Association of benzo(a)pyrene with dissolved organic matter: Prediction of  $k_{\text{DOM}}$  from structural and chemical properties of the organic matter. *Chemosphere* **1989**, *19* (12), 1911–1920.
- (3) Thomas, J. D. The role of dissolved organic matter, particularly free amino acids and humic substances, in freshwater ecosystems. *Freshwater Biol.* **1997**, *38* (1), 1–36.
- (4) Stelzer, R. S.; Heffernan, J.; Likens, G. E. The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. *Freshwater Biol.* **2003**, *48* (11), 1925–1937.



- (5) Piccolo, A. The supramolecular structure of humic substances. *Soil Sci.* **2001**, *166* (11), 810–832.
- (6) S Sutton, R.; Sposito, G. Molecular structure in soil humic substances: The new view. *Environ. Sci. Technol.* **2005**, *39* (23), 9009–9015.
- (7) Amon, R. M.; Benner, R. Bacterial utilization of different size classes of dissolved organic matter. *Limnol. Oceanogr.* **1996**, *41* (1), 41–51.
- (8) Chen, G.; Lin, C.; Chen, L.; Yang, H. Effect of size-fractionation dissolved organic matter on the mobility of prometryne in soil. *Chemosphere* **2010**, *79* (11), 1046–1055.
- (9) Chin, Y.-P.; Aiken, G. R.; Danielsen, K. M. Binding of pyrene to aquatic and commercial humic substances: The role of molecular weight and aromaticity. *Environ. Sci. Technol.* **1997**, *31* (6), 1630–1635.
- (10) Sun, H.; Song, Q.; Luo, P.; Wu, W.; Wu, J. Sorption of phenanthrene on single-walled carbon nanotubes modified by DOM: Effects of DOM molecular weight and contact time. *Environ. Sci.: Processes Impacts* **2013**, *15* (1), 307–314.
- (11) Ma, D.; Gao, B.; Sun, S.; Wang, Y.; Yue, Q.; Li, Q. Effects of dissolved organic matter size fractions on trihalomethanes formation in MBR effluents during chlorine disinfection. *Bioresour. Technol.* **2013**, *136* (0), 535–541.
- (12) Pinheiro, J. P.; Mota, A. M.; d'Oliveira, J. M. R.; Martinho, J. M. G. Dynamic properties of humic matter by dynamic light scattering and voltammetry. *Anal. Chim. Acta* **1996**, *329* (1–2), 15–24.
- (13) Chu, B. *Laser Light Scattering Basic Principles and Practice*, 2nd ed.; Academic Press: San Diego, CA, 1991.
- (14) Świątosławski, W. *Ebulliometric Measurements*; Reinhold Publishing Corporation: New York, 1945.
- (15) Xu, G.; Chance, M. R. Hydroxyl radical-mediated modification of proteins as probes for structural proteomics. *Chem. Rev.* **2007**, *107* (8), 3514–3543.
- (16) Arakaki, T.; Anastasio, C.; Kuroki, Y.; Nakajima, H.; Okada, K.; Kotani, Y.; Handa, D.; Azechi, S.; Kimura, T.; Tsuchioka, A.; Miyagi, Y. A general scavenging rate constant for reaction of hydroxyl radical with organic carbon in atmospheric waters. *Environ. Sci. Technol.* **2013**, *47* (15), 8196–8203.
- (17) Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B. Critical-review of rate constants for reactions of hydrated electrons, hydrogen-atoms and hydroxyl radical ( $\cdot\text{OH}/\cdot\text{O}^-$ ) in aqueous-solution. *J. Phys. Chem. Ref. Data* **1988**, *17* (2), 513–886.
- (18) Bors, W.; Langebartels, C.; Michel, C.; Sandermann, H., Jr. Polyamines as radical scavengers and protectants against ozone damage. *Phytochemistry* **1989**, *28* (6), 1589–1595.
- (19) Ching, T.-L.; Haenen, G. R. M. M.; Bast, A. Cimetidine and other H<sub>2</sub> receptor antagonists as powerful hydroxyl radical scavengers. *Chem.-Biol. Interactions* **1993**, *86* (2), 119–127.
- (20) Helz, G. R.; Zepp, R. G.; Crosby, D. G. *Aquatic and Surface Photochemistry*; Lewis Publishers: Boca Raton, FL, 1994.
- (21) Pal, H.; Palit, D. K.; Mukherjee, T.; Mittal, J. P. Pulse radiolytic one-electron oxidation of some dihydroxy-substituted anthraquinones. *J. Chem. Soc. Faraday Trans.* **1992**, *88* (5), 681–687.
- (22) Oturan, M. A.; Pinson, J.; Bizot, J.; Deprez, D.; Terlain, B. Reaction of inflammation inhibitors with chemically and electrochemically generated hydroxyl radicals. *J. Electroanal. Chem.* **1992**, *334* (1–2), 103–109.
- (23) International Humics Substances Society website. <http://www.humicsubstances.org> (accessed July 2014).
- (24) Wilson, M. A.; Collin, P. J.; Malcolm, R. L.; Perdue, E. M.; Cresswell, P. Low molecular weight species in humic and fulvic fractions. *Org. Geochem.* **1988**, *12* (1), 7–12.
- (25) Schulze, W. Protein analysis in dissolved organic matter: What proteins from organic debris, soil leachate and surface water can tell us-a perspective. *Biogeosciences* **2005**, *2* (1), 75–86.
- (26) Zepp, R. G.; Faust, B. C.; Hoigne, J. Hydroxyl radical formation in aqueous reactions (pH 3–8) of iron(II) with hydrogen peroxide: The photo-Fenton reaction. *Environ. Sci. Technol.* **1992**, *26* (2), 313–319.
- (27) Rosario-Ortiz, F. L.; Mezyk, S. P.; Doud, D. F. R.; Snyder, S. A. Quantitative correlation of absolute hydroxyl radical rate constants with mon-isolated effluent organic matter bulk properties in water. *Environ. Sci. Technol.* **2008**, *42* (16), 5924–5930.
- (28) Westerhoff, P.; Mezyk, S. P.; Cooper, W. J.; Minakata, D. Electron pulse radiolysis determination of hydroxyl radical rate constants with Suwannee river fulvic acid and other dissolved organic matter isolates. *Environ. Sci. Technol.* **2007**, *41* (13), 4640–4646.
- (29) Westerhoff, P.; Aiken, G.; Amy, G.; Debroux, J. Relationships between the structure of natural organic matter and its reactivity towards molecular ozone and hydroxyl radicals. *Water Res.* **1999**, *33* (10), 2265–2276.
- (30) McKay, G.; Kleinman, J.; Johnston, K.; Dong, M.; Rosario-Ortiz, F.; Mezyk, S. Kinetics of the reaction between the hydroxyl radical and organic matter standards from the International Humic Substance Society. *J. Soils Sediments* **2013**, *14*, 1–7.
- (31) Goldstone, J. V.; Pullin, M. J.; Bertilsson, S.; Voelker, B. M. Reactions of hydroxyl radical with humic substances: Bleaching, mineralization, and production of bioavailable carbon substrates. *Environ. Sci. Technol.* **2002**, *36* (3), 364–372.
- (32) Brezonik, P. L.; Fulkerson-Brekken, J. Nitrate-induced photolysis in natural waters: controls on concentrations of hydroxyl radical photo-intermediates by natural scavenging agents. *Environ. Sci. Technol.* **1998**, *32* (19), 3004–3010.
- (33) Southworth, B. A.; Voelker, B. M. Hydroxyl radical production via the photo-Fenton reaction in the presence of fulvic acid. *Environ. Sci. Technol.* **2003**, *37* (6), 1130–1136.
- (34) Page, S. E.; Arnold, W. A.; McNeill, K. Terephthalate as a probe for photochemically generated hydroxyl radical. *J. Environ. Monit.* **2010**, *12* (9), 1658–1665.
- (35) Aruoma, O. I.; Loughton, M. J.; Halliwell, B. Carnosine, homocarnosine and anserine: Could they act as antioxidants in vivo? *Biochem. J.* **1989**, *264* (3), 863–869.
- (36) Heelis, P. F.; Parsons, B. J.; Phillips, G. O.; Swallow, A. J. One-electron oxidation of lumichromes: A flash photolysis and pulse radiolysis study. *J. Phys. Chem.* **1989**, *93* (10), 4017–4022.
- (37) Payá, M.; Halliwell, B.; Hoult, J. R. S. Interactions of a series of coumarins with reactive oxygen species: Scavenging of superoxide, hypochlorous acid and hydroxyl radicals. *Biochem. Pharmacol.* **1992**, *44* (2), 205–214.
- (38) Thornalley, P. J.; Vařák, M. Possible role for metallothionein in protection against radiation-induced oxidative stress. Kinetics and mechanism of its reaction with superoxide and hydroxyl radicals. *Biochim. Biophys. Acta* **1985**, *827* (1), 36–44.
- (39) Aguer, J. P.; Richard, C. Use of furoin as probe molecule for the involvement of singlet oxygen in aqueous phase photoinduced transformations. *Toxicol. Environ. Chem.* **1993**, *39* (3–4), 217–227.
- (40) Page, S. E.; Arnold, W. A.; McNeill, K. Assessing the contribution of free hydroxyl radical in organic matter-sensitized photohydroxylation reactions. *Environ. Sci. Technol.* **2011**, *45* (7), 2818–2825.
- (41) Aiken, G. R.; Malcolm, R. L. Molecular weight of aquatic fulvic acids by vapor pressure osmometry. *Geochim. Cosmochim. Acta* **1987**, *51* (8), 2177–2184.
- (42) Minakata, D.; Li, K.; Westerhoff, P.; Crittenden, J. Development of a group contribution method to predict aqueous phase hydroxyl radical ( $\text{HO}\cdot$ ) reaction rate constants. *Environ. Sci. Technol.* **2009**, *43* (16), 6220–6227.
- (43) Aeschbacher, M.; Graf, C.; Schwarzenbach, R. P.; Sander, M. Antioxidant properties of humic substances. *Environ. Sci. Technol.* **2012**, *46* (9), 4916–4925.
- (44) McCarthy, J. F.; Jimenez, B. D. Interactions between polycyclic aromatic hydrocarbons and dissolved humic material: Binding and dissociation. *Environ. Sci. Technol.* **1985**, *19* (11), 1072–1076.
- (45) Carter, C. W.; Suffet, I. H. Binding of DDT to dissolved humic materials. *Environ. Sci. Technol.* **1982**, *16* (11), 735–740.
- (46) Remucal, C. K.; Cory, R. M.; Sander, M.; McNeill, K. Low molecular weight components in an aquatic humic substance as

characterized by membrane dialysis and orbitrap mass spectrometry. *Environ. Sci. Technol.* **2012**, *46* (17), 9350–9359.

(47) Kramer, R. W.; Kujawinski, E. B.; Hatcher, P. G. Identification of black carbon derived structures in a volcanic ash soil humic acid by Fourier transform ion cyclotron resonance mass spectrometry. *Environ. Sci. Technol.* **2004**, *38* (12), 3387–3395.

(48) Cabaniss, S. E.; Zhou, Q.; Maurice, P. A.; Chin, Y.-P.; Aiken, G. R. A log-normal distribution model for the molecular weight of aquatic fulvic acids. *Environ. Sci. Technol.* **2000**, *34* (6), 1103–1109.

(49) Page, S. E.; Logan, J. R.; Cory, R. M.; McNeill, K. Evidence for dissolved organic matter as the primary source and sink of photochemically produced hydroxyl radical in arctic surface waters. *Environ. Sci.: Processes Impacts* **2014**, *16* (4), 807–822.

(50) Dong, M. M.; Mezyk, S. P.; Rosario-Ortiz, F. L. Reactivity of effluent organic matter (EfOM) with hydroxyl radical as a function of molecular weight. *Environ. Sci. Technol.* **2010**, *44* (15), 5714–5720.