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# Hydroxyl radical production from irradiated Arctic dissolved organic matter

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**Abstract.** The hydroxyl radical (OH·) plays an important role in the environmental chemistry and biogeochemistry of surface waters. OH· acts as a strong oxidant within the irradiated water column, and affects the bioavailability, cycling, and mineralization of dissolved organic matter (DOM), the speciation and redox state of important trace metals e.g., iron and copper, and the fate of persistent organic pollutants (POPs). The generation of this species from irradiated DOM may be especially important in Arctic surface waters during the boreal summer, which contains high levels of DOM and experiences continual solar irradiance. Here, we investigate the OH· produced from laser irradiated Arctic DOM isolated from Toolik Lake, AK (68°38′ N, 149°43′ W). We measured the wavelength dependence of OH· production for aqueous solutions of DOM and report that the greatest OH· production occurs at wavelengths less than 360 nm. OH· production rates ranged from 1.7 $(\pm 0.1)\times 10^{-7}$ M $h^{-1}$ to 6.4 $(\pm 0.2)\times 10^{-7}$ M $h^{-1}$, with the rate depending on both irradiation wavelength and to a lesser degree the method used to isolate the DOM matrix. These findings lead to a better understanding of the potentially important photo-oxidation processes that may impact DOM cycling in the Arctic.

## Introduction

Dissolved organic matter (DOM) plays an important role in the biogeochemistry of Arctic ecosystems. DOM is an important carbon source for microbial communities (Voelker et al. 1997; Tranvik and Kokalj 1998; Moran et al., 1999; Foreman and Covert 2003) and plays a critical role in the cycling of global carbon (Amon and Benner 1994; Hedges et al. 1997; Dittmar and Kattner 2003). Organic matter concentrations in Arctic rivers are generally among the highest reported in the world's rivers, with dissolved organic carbon (DOC) levels between 230 and 1000 $\mu$M C (e.g., Lara et al. 1998; Opsahl et al. 1999; Kohler et al. 2003), which typically exceed particulate organic carbon levels (Lobbes et al. 2000). Unfortunately, processes that influence the cycling of DOM in the Arctic remain unclear, despite its importance to the global carbon cycle (Dittmar and Kattner 2003).

The effects of solar radiation on DOM cycling have received considerable attention in recent years because photo-oxidation processes have been shown to alter the bioavailability of DOM. Studies by a number of investigators have shown that sunlight plays an important role in the cycling of dissolved organic matter in both oceans (Amon and Benner 1994; Obernosterer and Benner 2004) and continental surface waters (Moran and Zepp 1997, 2000; Kaiser et al. 2003; Kaiser and Sulzberger 2004). Photochemical degradation of marine DOM gives rise to a number of products including labile low molecular weight compounds, $CO_2$ and CO (Moran and Zepp 1997; Gao and Zepp 1998; Opsahl and Benner 1998; Mopper and Kieber 2000; Moran and Covert 2003) as well as recalcitrant substances (Tranvik and Kokalj 1998; Kaiser and Sulzberger 2004). Conversely, the type of photolyzed organic matter formed is partially dependent upon the composition of the original DOM (Bertilsson and Tranvik 2000; Kaiser and Sulzberger 2004).

The effects of solar radiation on DOM cycling have been shown to vary in different environments and these differences have been attributed to DOM source and its diagenetic state. Photo-oxidation processes may play a particularly important role in Arctic DOM cycling due to the continual solar irradiance during boreal summer, and the existence of relatively shallow and unshaded lakes and streams. Additionally, the Arctic is now known to be an ecosystem sensitive to climate change. Current estimates indicate that up to 60% of soil organic matter resides in taiga and tundra soils (Dixon et al. 1994) found in Arctic drainage basins, and thawing of permafrost due to global warming could significantly elevate the amount of terrigenous DOM discharged into the Arctic Ocean (Opsahl et al. 1999). Given the effects of climate change coupled with seasonal thinning of the ozone layer in the Arctic (Rex et al. 1997), sunlight may play an increasingly important role in the cycling of carbon in this environment.

Although photo-oxidation processes are known to be important to DOM cycling, the mechanistic pathways are currently poorly understood. One important transient formed by the irradiation of DOM is the hydroxyl radical (OH·). This radical is a highly reactive chemical transient and can participate in a number of environmentally important reactions including the transformation of persistent organic pollutants (Miller and Chin 2002; Grannas et al. in review), the cycling of metals (Voelker and Sulzberger 1996; Voelker et al. 1997) and DOM photo-bleaching processes (Moran and Zepp 1997; Miller et al. 2002; Goldstone et al. 2002). Because OH· is a strong oxidizer, it can degrade organic materials that would otherwise be recalcitrant to biological, chemical, or direct photochemical decomposition. Evidence has emerged from the Antarctic, which demonstrates that the annual thinning of the ozone layer can enhance OH· generation from DOM (Qian et al. 2001). This phenomenon may also occur in the Arctic during ozone thinning events (Rex et al. 1997).

The photochemical production of the hydroxyl radical by DOM can occur either through photo-Fenton pathways (Zepp et al. 1992; Southworth and Voelker 2003; White et al. 2003) or by direct photolysis of DOM itself (Mopper and Zhou 1990; Vaughan and Blough 1998). Because of the ubiquitous nature of DOM in the environment, the photochemical production of OH· from DOM can potentially have a large impact on the local chemistry of irradiated surface waters. To date there has been no systematic study of OH· production by Arctic DOM. Moreover, given the increase in UV-A and UV-B radiation due to recent severe ozone thinning episodes in the Arctic (Randall et al. 2005) the role of wavelength on OH· generation by DOM should also be investigated. Because large amounts of DOM is released into surface waters during the Arctic summer, we believe that photolysis and OH· production may play an important role in the carbon cycle in this system.

In this paper, we studied the OH· generated by irradiated DOM isolated from a well-studied lake in the Alaskan Arctic (Toolik Lake) as a function of wavelengths present in UV-A and B radiation. DOM isolates were used for this study because the optical densities of the natural water samples were too low for use in our various laser light sources (an absorbance of 1.0 is needed). Further, we took this opportunity to investigate how DOM isolated by three common processes at the sample site (tangential flow ultrafiltration (TFUF), XAD chromatography and C-18 solid phase extraction) influences the photoproduction of OH·. Additionally, the isolated DOM fractions were characterized by fluorescence and carbon-13 nuclear magnetic resonance spectroscopy ($^{13}$C-NMR) in an effort to elucidate structural differences that may explain differences in the OH· production rates from the various DOM fractions.

## Materials and methods

### *Isolation of DOM from Arctic surface waters*

DOM was isolated from Toolik Lake, Alaska (68°38′ N, 149°43′ W, elevation 760 m) by ultrafiltration, C-18 solid phase extraction and XAD-8 solid phase extraction. Ultrafiltration was conducted using the methods described in Everett et al. (1999). Briefly, filtered and acidified water samples were passed through a tangential-flow UF system (Amicon CH2M) equipped with a 1000 Dalton membrane until the DOM was concentrated roughly ten times. The retentate was treated with a strong cation exchanger (Biorad GX-50) to remove paramagnetic metal ions, and freeze-dried. C-18 extraction was done with cartridges (Varian 10 g Mega BondElut) using the methods described in Schwede-Thomas et al. (2005) and Kaiser (2003). Approximately 20 l of pH 3 acidified water was passed through each C-18 column to remove impurities prior to use. Samples were acidified to pH $< 3$ and pumped through the stationary phase until the UV absorbance at 280 nm of the eluent was approximately half that of the sample. The DOM was eluted off the column with methanol followed by rotary evaporation to remove the solvent. The remaining liquid was reconstituted, treated with Biorad GX-50 resin to remove paramagnetics and freeze-dried. XAD-8 isolation of the Toolik water was conducted concurrently. Details regarding this technique are presented elsewhere

(Aiken et al. 1992), and involves passing Balston filtered (0.5 $\mu$m) Toolik Lake water (pH = 2) across a 2 l glass column packed with cleaned XAD-8 resin. The sorbed DOM is subsequently eluted with a NaOH solution (pH = 11), treated with Biorad GX-50 resin, and freeze-dried.

### *Laser irradiation of DOM*

Irradiation of reconstituted DOM solutions by monochromatic lasers serves as a well controlled method to probe the photochemical generation of OH· radicals in solution. Irradiations conducted at 308 nm were performed using a Lambda Physik-LPX 100 XeCl eximer laser firing at 10 Hz with energies of 20–25 mJ/cm$^2$ (per pulse). A Lambda Physik Scanmate dye laser (pumped by the eximer laser described above) was used to irradiate samples at 330 nm and was operated at 50 Hz with energies of 1.2–1.5 mJ cm$^{-2}$ (per pulse). Samples irradiated at 355 nm were obtained using a Spectra-Physics Quanta Ray YAG laser firing at 10 Hz with energies of 7.0–8.0 mJ cm$^{-2}$ (per pulse).

Typical UV-B (290–320 nm) ambient radiation energies at Toolik Lake, Alaska range from about 0.03 mJ cm$^{-2}$ s$^{-1}$ at midnight to 1.5 mJ cm$^{-2}$ s$^{-1}$ at noon (measured using a photometer during the June 2003 and June 2004 field seasons). Average *total* solar irradiance at noon in June was found to be about 35 mJ cm$^{-2}$ s$^{-1}$. Using spectral data obtained by LTER monitoring stations at Toolik, it was possible to calculate from the area under the measured solar spectrum the proportion of total energy at discrete wavelength intervals. Using the observed noontime ambient radiation energies, we calculated the number of laser pulses needed to irradiate the sample (with an irradiated laser area of 2 cm$^2$ in a quartz cuvette of 1 cm pathlength) with an equivalent dose of energy comparable to a sample exposed to ambient Toolik Lake, Alaska sunlight. For a wavelength interval of 305–310 nm (represented by the 308 nm laser irradiation experiments), Toolik noontime radiation corresponded to approximately 0.35 mJ cm$^{-2}$ s$^{-1}$ (or 21 mJ cm$^{-2}$ for a 1 min irradiation period). This corresponded to about 2 pulses of the 308 nm laser. Likewise, 360 pulses were used to mimic a 1 h Toolik Lake ambient irradiation time, 4320 pulses were used to mimic a 12 h Toolik ambient irradiation time, etc. Similar calculations were performed for the 330 nm and 355 nm laser irradiation experiments. Thus, each irradiation experiment represents a typical dose of monochromatic radiation that would be received had the sample been irradiated with natural sunlight at the intensity typically observed at Toolik Lake, Alaska.

### *Quantification of OH· production*

The production of OH· was quantified by measuring the amount of formaldehyde (HCHO) formed by reaction with excess methanol (50 mM) present in the irradiated solution. Following laser irradiation, the amount of HCHO produced (proportional to OH·) was determined using 2,4-dinitrophenylhydrazine (DNPH) derivatization of HCHO (to the corresponding hydrazone) with analysis by HPLC with UV detection (Mopper and Zhou 1990; Sirju and Shepson 1995). Solutions of DOM in water were prepared so that the initial absorbance of each sample at 308 nm was approximately 1.0, pH was adjusted to 3.0, and methanol was added just prior to irradiation (final methanol concentration in the solution was 50 mM). Immediately following laser irradiation of the solution, DNPH solution was added to the aqueous solution and acidified by adding several drops of concentrated sulfuric acid. The DNPH solution was prepared by diluting 25 ml of a saturated solution of three-times recrystallized DNPH in spectroscopic grade acetonitrile to 500 ml with acetonitrile.

The HCHO hydrazone was then separated from the excess DNPH via high performance liquid chromatography followed by UV absorbance detection (at 360 nm). Separation was achieved using a C-18 column with a guard column. The water–acetonitrile mobile phase composition was 60% water/40% acetonitrile and the method was run isocratically at a mobile phase flow rate of 1 ml $\text{min}^{-1}$. DNPH typically eluted around 4 min and HCHO-hydrazone eluted around 6.6 min.

Calibration of the HPLC involved preparation of pure HCHO-hydrazone. This was done by mixing a solution of recrystallized DNPH in sulfuric acid with a solution of HCHO in ethanol (Shriner et al. 1980). The crystals were then vacuum filtered, washed with ethanol and dried in an oven at ~60 °C. The calibration curve resulted in a sensitivity of $2.2 \times 10^{10}$ area counts per mol/l (HCHO-hydrazone) injected and $r^2 = 0.99$.

### *Characterization of DOM via $^{13}C$ NMR spectroscopy*

Nuclear magnetic resonance spectroscopy provides qualitative and quantitative information regarding the chemical environments of certain nuclei within molecules and elucidates the important functional groups and their relative abundances present in DOM. Solid state $^{13}$C-NMR spectra were obtained using the ramp cross polarization magic angle spinning (CPMAS) pulse program and two pulse modulated decoupling on a Bruker DSX 300 NMR spectrometer, operating at a frequency of 75.48 MHz for $^{13}$C (Dria et al. 2002). We determined the relative abundances of the major DOM structural components using chemical shift assignments of 0–60 for alkyl groups, 60–110 for O-alkyls, and 110–160 ppm for aromatics (Hatcher and Wilson 1991; Nelson et al. 1993; Knicker et al. 1996).

### *Characterization of DOM via fluorescence spectroscopy*

Fluorescence spectroscopy has been used to characterize the fluorescent properties of DOM for several decades. The technique yields important information on the chemical nature of bulk DOM as a function of its fluorescence intensity and functional groups. Here, excitation emission matrices (EEMs) were constructed by sequential and simultaneous measurement of excitation wavelength, emission wavelength and fluorescence intensity. Excitation emission matrices of 40 mg/l (as total mass DOM *not* DOC) solutions (pH 3) were obtained using a Cary Eclipse fluorescence spectrometer (Varian Instruments), with the following instrument parameters: bandpass of 5 nm, scan rate of 120 nm/min, and PMT voltage of 600 V. In addition, we conducted fluorescence ratio analysis (McKnight et al. 2001) to determine the origin of the Toolik Lake DOM using an excitation wavelength of 370 nm and measuring the peak intensities at 450 and 500 nm.

## Results

We observed the photoproduction of the hydroxyl radical in all our irradiated DOM isolates as a function of wavelength and equivalent Toolik Lake 'irradiation time' (Figure 1). As defined previously the 'equivalent Toolik Lake irradiation time' was determined by examining the typical wavelength distribution of incoming sunlight at a latitude of 68°38′ N and scaling that to the measured power output of the laser at a given wavelength.

The maximum OH· production rate was found to occur at 308 nm irradiation and decreased at the higher wavelengths as illustrated by its action spectrum (Table 1 and Figure 2). The OH· production rates at 308 and 355 nm were found to be independent of the two chromatographic DOM isolation methods i.e., C-18 and XAD-8 employed, as each sample had statistically similar production rates. Conversely, the TFUF isolated DOM showed statistically slightly less OH· production, particularly at the 12 h irradiation equivalents. Over the time frame of the experiments, photobleaching of the DOM occurred based upon loss of absorbance at 308 nm (Figure 3) as illustrated by the change in absorbance of the ultrafiltered DOM sample at 308 nm after varying irradiation times and its corresponding decrease in OH· produced over the same time interval in the sample.

Solid-state NMR studies of the isolated DOM reveal that the TFUF isolated sample is different from both C-18 and XAD-8 only in the aliphatic and carbohydrate regions (0–60 and 60–90 ppm respectively). Both the XAD and C-18 samples have relatively larger proportions of aliphatic moieties relative to the TFUF isolate (Table 2). Conversely, the amount of carbohydrates in the TFUF sample is significantly higher in the TFUF sample is significantly higher and corroborates observations made by others (Aluwihare and Repeta 1999; Repeta et al. 2002).

DOM's chromophoric moieties are presumably aromatic in nature and they may be responsible for the formation of OH· when irradiated e.g., quinones (see Vaughan and Blough 1998). In this study, the aromatic carbons were most abundant for the XAD isolate (Table 2), which also had the statistically

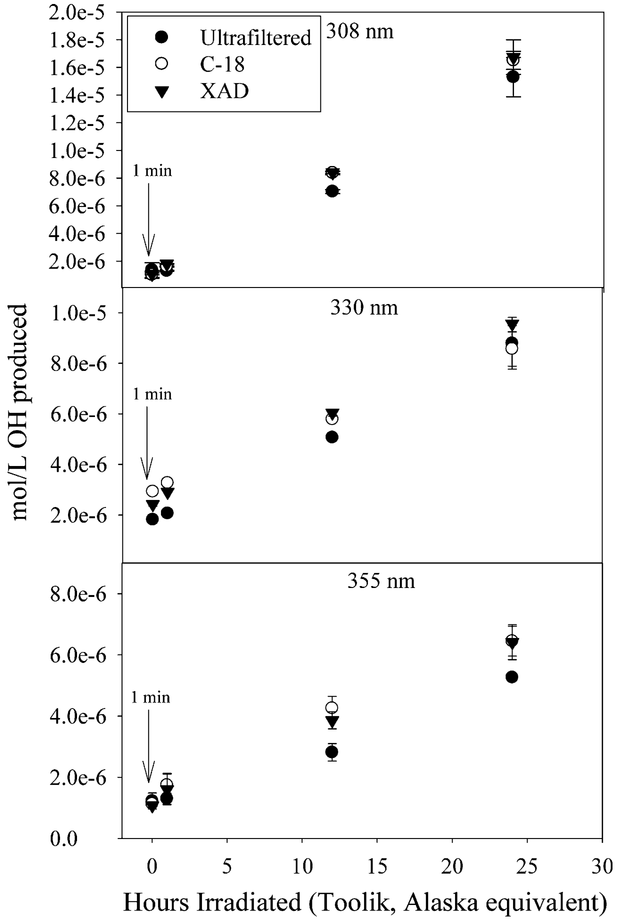

*Figure 1.* Production of OH·from isolated Toolik Lake, Alaska DOM irradiated at 308 nm (top panel), 330 nm (middle panel) and 355 nm (bottom panel). Filled circles represent DOM isolated by ultrafiltration, open circles represent DOM isolated by C-18 solid phase extraction, and filled triangles represent DOM isolated by XAD-8 chromatography. Error bars represent 1s for $n = 3$ (where error bars are not observed, the error is smaller than the size of the point at this scale).

*Table 1.* OH· production rates with associated error from irradiated Toolik Lake DOM.

| Wavelength (nm) | OH· production rate (M h$^{-1}$) XAD | OH· production rate (M h$^{-1}$) C-18 | OH· production rate (M h$^{-1}$) TFUF |
|---|---|---|---|
| 308 | 6.4 (±0.2)×10$^{-7}$ | 6.4 (±0.1)×10$^{-7}$ | 5.9 (±0.3)×10$^{-7}$ |
| 330 | 2.9 (±0.1)×10$^{-7}$ | 2.3 (±0.1)×10$^{-7}$ | 2.9 (±0.2)×10$^{-7}$ |
| 355 | 2.0 (±0.1)×10$^{-7}$ | 2.2 (±0.2)×10$^{-7}$ | 1.7 (±0.1)×10$^{-7}$ |

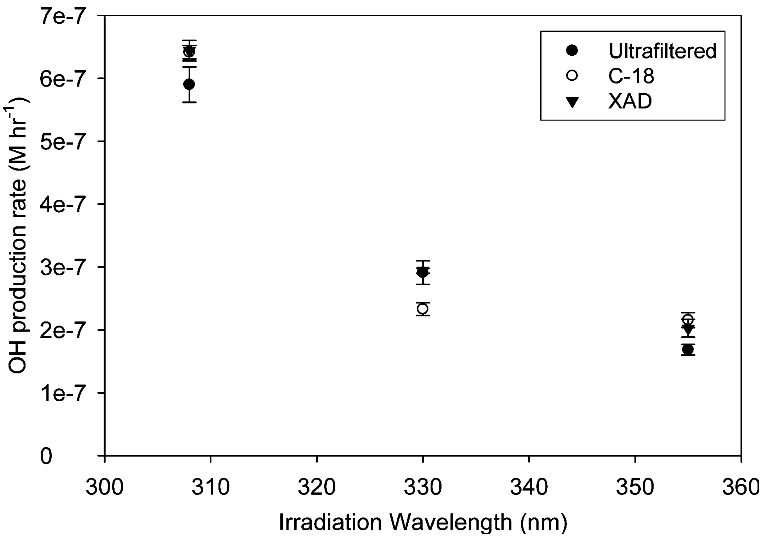

*Figure 2.* OH· production rates as a function of irradiation wavelength and DOM isolation method. Error bars represent 1s for $n = 3$ (where error bars are not observed, the error is smaller than the size of the point at this scale).

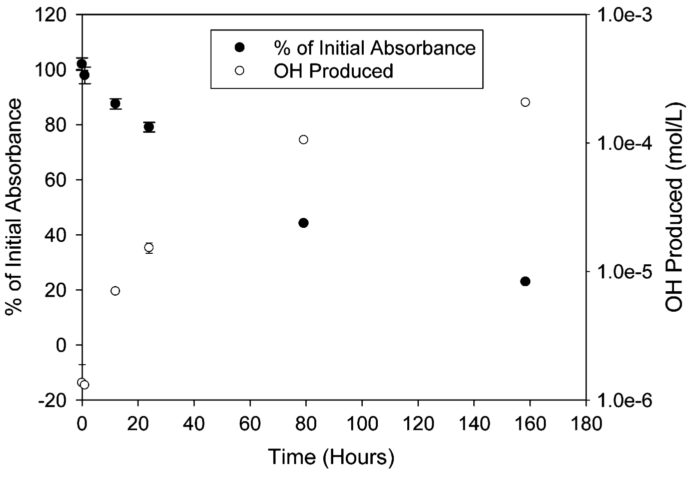

*Figure 3.* OH· production from irradiated Toolik Lake DOM (isolated via ultrafiltration) and the absorbance of the sample as a function of irradiation time (equivalent to hours of ambient Toolik Lake sunlight exposure), illustrating the effects of photobleaching. Error bars represent 1 s for $n = 3$ (where error bars are not observed, the error is smaller than the size of the point at this scale).

highest OH· production rates irrespective of wavelength (Table 1). It also had the lowest carbohydrate to aromatic ratio (Carb/Ar), which demonstrates its low affinity for isolating hydrophilic neutrals. Thus, the propensity for the

XAD method to concentrate photoreactive moieties may explain why this material has the highest OH· production rate.

An analysis of the fluorescence excitation–emission matrices (EEMs) from the three isolates reveals subtle differences among the samples (Figures 4 and 5). All three DOM isolates show two distinct excitation/emission (Ex/Em)

*Table 2.* $^{13}$C nuclear magnetic resonance characterization data.

| Toolik isolate | % Aliphatic | % Carbohydrate | % Aromatic | Carb/Ar ratio | Al/Ar ratio |
|---|---|---|---|---|---|
| C-18 | 45.8 | 19.6 | 13.6 | 1.44 | 2.54 |
| XAD-8 | 43.7 | 14.3 | 17.7 | 0.80 | 1.94 |
| TFUF | 32.8 | 25.8 | 14.4 | 1.79 | 1.72 |

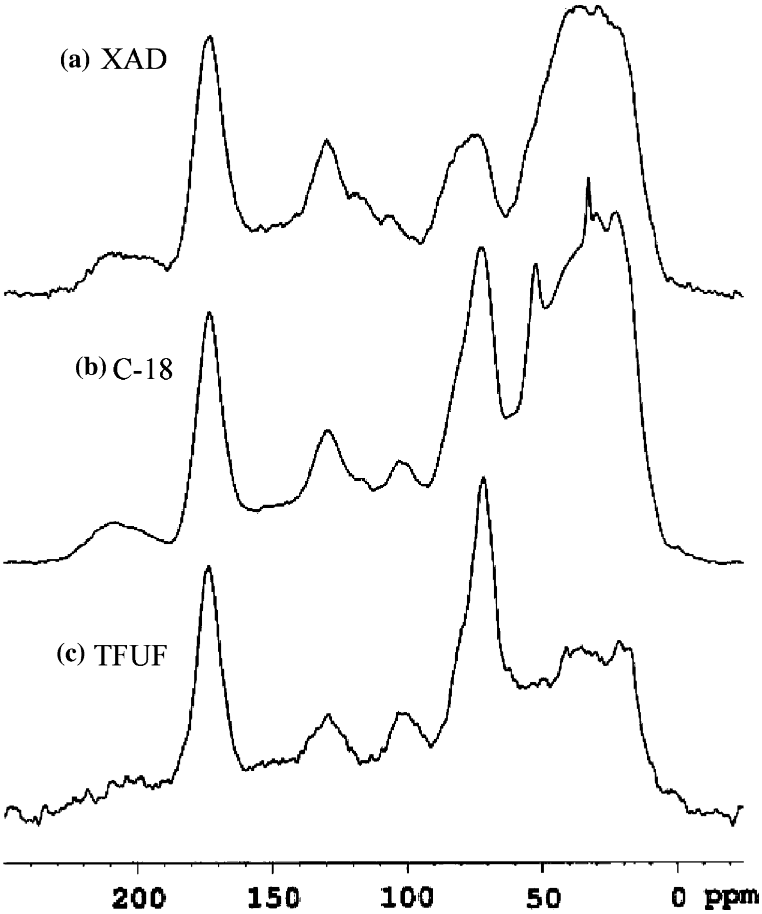

*Figure 4.* Solid-state $^{13}$C NMR of Toolik Lake DOM isolated by (a) XAD chromatography (b) C-18 chromatography (c) tangential flow ultrafiltration.

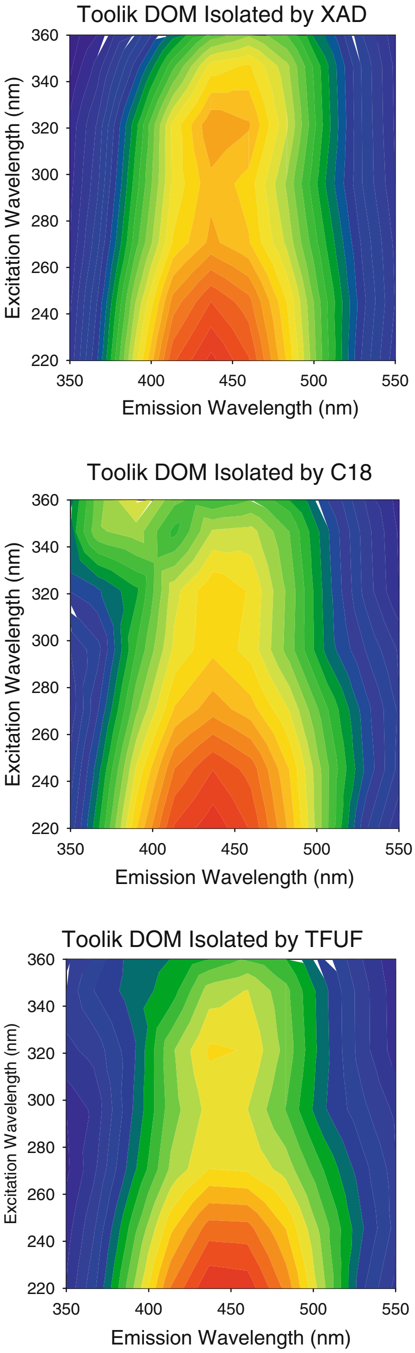

*Figure 5*. Fluorescence excitation emission spectra of Toolik Lake DOM isolated by XAD (top), C-18 (middle) and ultrafiltration (bottom).

maxima, which are similar to those reported by others for DOM isolated from lower latitude surface waters (Kowalczuk et al. 2003; Schwede-Thomas et al. 2005) and International Humic Substances Society fulvic acids (Alberts and Takacs 2004). We observed some differences between the location of these peaks for the chromatographically isolated materials as compared to the ultrafiltered DOM. The EEM spectra of the XAD isolated DOM samples reveal average Ex/Em maxima at 220/440 nm and 320/450 nm. The C-18 sample does not exhibit a well-defined second maxima (320/450 nm). Similarly, while the ultrafiltered DOM second excitation and emission maximums are present, it is less intense relative to the XAD sample. Based upon our action spectrum the highest production of OH· occurs at wavelengths less than 400 nm in sunlight, and it is possible that the fluorophores in the Ex/Em maximum at 320/450 nm may be involved in the photoproduction of OH·. The greater intensity of the XAD fluorphores relative to the other isolates at this maximum may in part explain its consistently higher rate of hydroxyl radical production.

Fluorescence ratio analysis showed little difference among the three methods of isolation for Toolik Lake DOM. The fluorescence ratio of the XAD, C-18, and TFUF isolated DOM were 1.18, 1.25, and 1.20, respectively. These values reflect the highly terrestrial nature of the DOM (McKnight et al. 2001). Because there was little variability in the values (average = 1.21 ± 0.04) it appears that this method can not distinguish between fractions of DOM derived from a single source, and is better suited for elucidating the precursors involved in DOM formation where the respective algal and terrestrially derived fluorescence ratios are close to 2 and 1.

## Discussion

Our action spectrum results showed that the maximum OH· production rate occurred at 308 nm, which generally corroborates the wavelength at which the maximum quantum yield occurs for OH· (~310 nm) from irradiated Suwannee River fulvic acid and Delaware surface water DOM (under aerobic conditions) as determined by Vaughan and Blough (1998). Moreover, their quantum yield action spectrum followed the same general trend observed by us. Similarly, Mopper and Zhou (1990) measured photochemical production rates of OH· in irradiated natural samples and found DOM to be the main source of OH· in marine and freshwater systems with OH· production rates ranging from $2.8\times10^{-12}$ M s$^{-1}$ for open-ocean surface water ($>95\%$ from DOM photolysis) to $420\times10^{-12}$ M s$^{-1}$ for a DOM-rich freshwater wetland (Everglades, FL). Our experiments resulted in OH· production rates of $47–178\times10^{-12}$ M s$^{-1}$ at DOC concentrations of about 75 mg/l (equivalent to 1 absorbance unit). Because the actual DOM concentrations in Toolik Lake are significantly lower (range of 7–8 mg/l DOC depending upon the season) the actual production rates are about ten times smaller ($4.7–17.8\times10^{-12}$ M s$^{-1}$). This in-lake estimate of OH· production obviously ignores the presence of other potential non-DOM OH· sources (nitrate and iron complexes) or scavengers (carbonate and bicarbonate) that could be present in an unaltered lake water. Interestingly, our results are comparable to values reported by Mopper and Zhou (1990) for OH· production in deep Gulf Stream water ($14.7\times10^{-12}$ M $s^{-1}$) and deep ocean water ($15.9\times10^{-12}$ M $s^{-1}$).

For further comparison to other environments, the OH· production rate from Suwannee River fulvic acid (measured at 320 nm) by Vaughan and Blough (1998) was approximately $2\times10^{-11}$ M $s^{-1}$ at a light intensity similar to that used for our laser experiments ($1.2\times10^{-3}$ W $cm^{-2}$), at a solution concentration of 10 mg/l (equivalent to approximately 4 mg C/l assuming that 40% of their fulvic acid is carbon). This corresponds to an OH· production rate of $5\times10^{-12}$ M $s^{-1}$ $ppm^{-1}$ C. By interpolating our results to 320 nm, Toolik Lake XAD-8 DOM produced OH· at a rate of approximately $2\times10^{-12}$ M $s^{-1}$ $ppm^{-1}$ C. Despite the obvious geographical differences between these two DOM samples, the major organic matter precursors to both organic matter pools are likely derived from terrestrial higher plant material albeit from vastly different terrestrial ecosystems (Michaelson et al. 1998). Our results indicate that the photochemical behavior (with respect to OH· production) of DOM derived from predominantly allochthonous organic material may be similar irrespective of location. Alternatively, these results could be an artifact of the method used to isolate the DOM from its matrix. Both the Suwannee River and our Toolik Lake sample were isolated by XAD-8 chromatography, and it is conceivable that this method preferentially isolates similar moieties regardless of the nature of the whole water DOM. Because our C-18 DOM isolate resulted in the same hydroxyl radical production rate we believe that it also concentrates structurally similar DOM components. Conversely, the TFUF DOM sample yielded a statistically different OH· production rate at two of the three wavelengths. Thus, measuring OH· production from a wider variety of samples will provide better constraints on how the DOM composition and its method of isolation will influence OH· generation by natural sunlight.

During the Arctic summer, significant photobleaching of DOM in surface waters can occur because of continual 24-h solar irradiance (even though the actual photon flux follows a strongly diurnal pattern). Moreover, in the tundra, the lack of shading exacerbates this problem. We observed decreases in both the absorbance of the ultrafiltered DOM sample at 308 nm and changes in hydroxyl radical production as a function of increased irradiation times (Figure 3). It is conceivable that over time the destruction of chromophores may render the DOM phase less effective at the production of reactive transients that can participate in the biogeochemical cycling of carbon in these systems. Further studies will need to include controlled photobleaching studies to determine the extent to which irradiated DOM will produce OH· before the active chromophores are exhausted. Finally, the degree to which this occurs is dependent upon how quickly the surface water body receives new DOM. Based upon our own observation of lakes in the vicinity of Toolik Lake, we have sampled waters that may have experienced severe photobleaching (indicated by their extremely low DOM levels and light absorbance).

Our study we believe is the first to measure the production of hydroxyl radicals from photo-irradiated Arctic DOM. Our findings corroborate the results of other researchers studying hydroxyl radical production by DOM collected from lower latitude surface waters (Vaughan and Blough 1998; Guo and Zepp 1998; Southworth and Voelker 2003; White et al. 2003). While we have not identified the exact pathway(s) for photoproduction of $OH\cdot$ from our irradiated samples e.g., excited state quinones, photo-Fenton pathways, etc., we have shown that the production rates are similar to values reported worldwide in both freshwater and marine environments. Because the oxidation of DOM by photo-generated $OH\cdot$ is potentially an important abiotic mineralization mechanism (Guo and Zepp 1998; White et al. 2003) we believe that continued release of organic matter originally sequestered in permafrost to Arctic surface waters (due to changes in climate) may have a profound impact on the cycling of carbon.

Finally, many researchers rely on DOM isolated from environmental systems to conduct reproducible, controlled experiments in the laboratory, however, extrapolation of photoreactivity based upon these reconstituted isolates to the entire DOM pool in a surface water could be misleading. This is especially true for DOM isolated from remote regions of the world e.g., the Arctic, as the transport of large volumes of water would be prohibitively expensive and may alter the composition of the DOM over time if stored improperly. As we have demonstrated in this study, different fractions of the DOM pool may have unique photoreactive behavior. Thus, results from such studies may be unique to that fraction of DOM isolated from the surface water, and may not reflect processes that actually occur in the environment.

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## References

Alberts J.J. and Takacs M. 2004. Total luminescence spectra of IHSS standard and reference fulvic acids, humic acids and natural organic matter: comparison of aquatic and terrestrial source terms. Org. Geochem. 35: 243–256.

Aiken G.R., McKnight D.M., Thorn K. and Thurman E.M. 1992. Isolation of hydrophilic organic acids from water using nonionic macroporous resins. Org. Geochem. 18: 567–573.

Aluwihare L.I. and Repeta D.J. 1999. A comparison of the chemical characteristics of oceanic DOM and extracellular DOM produced by marine algae. Mar. Ecol. Prog. Ser. 186: 105–117.

Amon R.M.W. and Benner R. 1994. Rapid cycling of high-molecular-weight dissolved organic matter in the ocean. Nature 369: 549–52.

Bertilsson S. and Tranvik L.J. 2000. Photochemical transformation of organic matter in lakes. Limnol. Ocean. 45: 753–762.

Dittmar R. and Kattner G. 2003. Recalcitrant dissolved organic matter in the ocean: major contribution of small amphiphilics. Mar. Chem. 82: 115–123.

Dixon R.K., Brown S., Houghton R.A., Solomon A.M., Trexler M.C. and Wisniewski J. 1994. Carbon pools and flux of global forest ecosystems. Science 263: 185–190.

Dria K.J., Sachleben J.R. and Hatcher P.G. 2002. Solid-state carbon-13 nuclear magnetic resonance of humic acids at high magnetic field strengths. J. Environ. Qual. 31: 393–401.

Everett C., Chin Y. and Aiken G.R. 1999. High pressure size exclusion chromatography analysis of dissolved organic matter isolated by tangential-flow ultrafiltration. Limnol. Oceanogr. 44: 1316–1322.

Foreman C.M. and Covert J.S. 2003. Linkages between dissolved organic matter consumption and bacterial community structure. In: Findlay S. and Sinsabaugh R.L. (eds), Aquatic Ecosystems: Interactivity of Dissolved Organic Matter. Academic Press, pp. 343–362.

Gao H. and Zepp R.G. 1998. Factors influencing photoreactions of dissolved organic matter in a coastal river of the southeastern United States. Environ. Sci. Technol. 32: 2940–2946.

Goldstone J.V., Pullin M.J., Bertilsson S. and Voelker B.M. 2002. Reactions of hydroxyl radical with humic substances: bleaching, mineralization, and production of bioavailable carbon substrates. Environ. Sci. and Technol. 36: 364–372.

Grannas A.M., Miller P.L., Cory R.M., Chin Y.P. and McKnight D.M. Phototransformation of persistent organic pollutants in Arctic surface waters. Environ. Sci. and Technol. in review.

Hatcher P.G. and Wilson M.A. 1991. The effect of sample hydration on $^{13}$C CPMAS NMR spectra of fulvic acids. Org. Geochem. 17: 293–299.

Hedges J.I., Keil R.G. and Benner R. 1997. What happens to terrestrial organic matter in the ocean? Org. Geochem. 27: 195–212.

Kaiser E. 2003. Sources, transformations, and fates of riverine organic matter. Eidgenoessische Technische Hochschule Zuerich, 181 pp. Dissertation written in English.

Kaiser E. and Sulzberger B. 2004. Phototransformation of riverine dissolved organic matter (DOM) in the presence of abundant iron: effect on DOM bioavailability. Limnol. Oceanog. 49: 540–554.

Kaiser E., Simpson A.J., Dria K.J., Sulzburger B. and Hatcher P.G. 2003. Solid-state and multidimensional solution-state NMR of solid phase extracted and ultrafiltered riverine dissolved organic matter. Environ. Sci. Technol. 37: 2929–2935.

Knicker H., Almendros G., Gonzalez-Vila F.J., Ludemann H.D. and Martin F. 1996. $^{13}$C and $^{15}$N NMR analysis of some fungal melanins in comparison with soil organic matter. Org. Geochem. 23: 1023–1028.

Kohler H., Meon B., Gordeev V.V., Spitzy A. and Amon R.M.W. 2003. Dissolved organic matter (DOM) in the estuaries of the Ob and Yenisei and the adjacent Kara-Sea, Russia. In: Stein R., Fahl K., Futterer D.K., Galimov E.M. and Stepanets O.V. (eds), Siberian River Run-off in the Kara Sea. Proc. Mar. Sci. 6: pp. 281–308.

Kowalczuk P., Cooper W.J., Whitehead R.F., Durako M.J. and Sheldon W. 2003. Characterizing CDOM in an organic rich river and surrounding coastal ocean in the S. Atlantic Bight. Aquatic Sci. 65: 384–401.

Lara R.J., Rachold V., Kattner G., Hubberten H.W., Guggenberger G., Skoog A. and Thomas D. 1998. Dissolved organic matter and nutrients in the Lena River, Siberian Arctic: characteristics and distribution. Mar. Chem. 59: 301–309.

Lobbes J., Fitznar H.P. and Kattner G. 2000. Biogeochemical characteristics of dissolved and particulate organic matter in Russian rivers entering the Arctic Ocean. Geochim. Cosmochim. Acta 64: 2973–2983.

McKnight D.M., Boyer E.W., Westerhoff P.K., Doran P.T., Kulbe T. and Andersen D. 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. Limnol. Oceanog. 46: 38–48.

Michaelson G.J., Ping C.L., Kling G.W. and Hobbie J.E. 1998. The character and bioactivity of dissolved organic matter at thaw and in the spring runoff waters of the arctic tundra north slope, Alaska. J. Geophys. Res. 103: 28939–28946.

Miller P.L. and Chin Y.P. 2002. Photoinduced degradation of carbaryl in wetland surface water. J. Agric. Food Chem. 50: 6758–6765.

Miller W.L., Moran M.A., Sheldon W.M., Zepp R.G. and Opsahl S. 2002. Determination of apparent quantum yield spectra for the formation of biologically labile photoproducts. Limnol. Oceanog. 47: 343–352.

Mopper K. and Zhou X. 1990. Hydroxyl radical photoproduction in the sea and its potential impact on marine processes. Science 250: 661–664.

Mopper K. and Kieber D.J. 2000. Marine photochemistry and its impact on carbon cycling. In: deMora S., Serge D. and Vernet M. (eds), The Effects of UV Radiation in the Marine Environment. University Press, Cambridge, pp. 101–129.

Moran M.A. and Covert J.S. 2003. Photochemically mediated linkages between dissolved organic matter and bacterioplankton. In: Findlay S., Sinsabaugh R.L. (eds), Aquatic Ecosystems: Interactivity of Dissolved Organic Matter. Academic Press, pp. 243–263.

Moran M.A. and Zepp R.G. 1997. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. Limnol. Oceanogr. 42: 1307–1316.

Moran M.A. and Zepp R.G. 2000. UV radiation effects on microbes and microbial processes. In: Kirchman D.L. and Mitchell R. (eds), Microbila Ecology of the Oceans. J. Wiley and Sons, pp. 201–228.

Moran M.A., Sheldon W.M. and Sheldon J.E. 1999. Biodegradation of riverine dissolved organic matter in five estuaries of the southeastern US. Estuaries 22: 55–64.

Nelson P.N., Baldock J.A. and Oades J.M. 1993. Concentration and composition of dissolved organic carbon in streams in relation to catchment soil properties. Biogeochem. 19: 27–50.

Obernosterer I. and Benner R. 2004. Competition between biological and photochemical processes in the mineralization of dissolved organic carbon. Limnol. Oceanog. 49: 117–124.

Qian J., Mopper K. and Kieber D. 2001. Photochemical production of the hydroxyl radical in Antarctic waters. Deep Sea Res. Part 1 48: 741–759.

Opsahl S. and Benner F. 1998. Photochemical reactivity of dissolved lignin in river and ocean waters. Limnol. Oceanogr. 43: 1297–1304.

Opsahl S., Benner R. and Amon R.M.W. 1999. Major flux of terrigenous organic matter through the Arctic Ocean. Limnol. Oceanogr. 44: 2017–2023.

Randall C.E., Harvey V.L., Manney G.L., Orsolini Y., Codrescu M., Sioris C., Brohede S., Haley C.S., Gordley L.L., Zawdony J.M. and Russell J.M. 2005. Stratospheric effects of energetic particle precipitation in 2003–2004. Geophys. Res. Lett. 32, LO5082, doi:10.1029/2004GL022003.

Repeta D.J., Quan T.M., Aluwihare L.I. and Accardi A.M. 2002. Chemical characterization of high molecular weight dissolved organic matter in fresh and marine waters. Geochim. Cosmochim. Acta 66: 955–962.

Rex M., Harris N.R.P., der Gathen P., Lehman R., Braathen G.O., Reimer E., Beck A., Chipperfield M.P., alfier R., Allaart M., O'Conner F., Dier H., Dorokhov V., Fast H., Gil M., Kyro E., Litynska Z., Mikkelsen I.B., Molyneux M.G., Nakane H., Notholt J., Rummukainen M., Viatte P. and Wenger J. 1997. Prolonged stratospheric ozone loss in the 1995–96 Arctic winter. Nature 389: 835–838.

Schwede-Thomas S.B., Chin Y.P., Dria K.J., Hatcher P.G., Kaiser E. and Sulzberger B. 2005. Characterizing the properties of dissolved organic matter isolated by XAD and C-18 solid phase extraction and ultra filtration. Aquatic Sci. 67: 61–71.

Shriner R.L., Fuson R.C., Curtin D.Y. and Morrill T.C. 1980. Systematic Identification of Organic Compounds. John Wiley and Sons, New York.

Sirju A.P. and Shepson P.B. 1995. Laboratory and field investigation of the DNPH cartridge technique for the measurement of atmospheric carbonyl compounds. Environ. Sci. Technol. 29: 384–392.

Southworth B.A. and Voelker B.M. 2003. Hydroxyl radical production via the photo-Fenton reaction in the presence of fulvic acid. Environ. Sci. Technol. 37: 1130–1136.

Tranvik L.J. and Kokalj S. 1998. Decreased biodegradability of algal DOC due to interactive effects of UV radiation and humic matter. Aquat. Microb. Ecol. 14: 301–307.

Vaughan P. and Blough N.V. 1998. Photochemical formation of hydroxyl radical by constituents of natural waters. Environ. Sci. Technol. 32: 2947–2953.

Voelker B.M. and Sulzberger B. 1996. Effects of fulvic acid on Fe(II) oxidation by hydrogen peroxide. Environ. Sci. Technol. 30: 1106–1114.

Voelker B.M., Morel F.M.M. and Sulzberger B. 1997. Iron redox cycling in surface waters: effects of humic substances and light. Environ. Sci. Technol. 31: 1004–1011.

White E.M., Vaughan P.P. and Zepp R.G. 2003. Role of the photo-Fenton reaction in the production of hydroxyl radicals and photobleaching of colored dissolved organic matter in a coastal river of the southeastern United States. Aquatic Sci. 65: 402–414.

Zepp R.G., Faust B.C. and Hoigne J. 1992. Hydroxyl radical formation in aqueous reactions (pH 3–8) of iron(II) with hydrogen peroxide: the photo-Fenton reaction. Environ. Sci. Technol. 26: 313–319.