Methylmercury Photodegradation: Laboratory and Field Studies in the St Louis River

Jeff Jeremiason[1], Joshua Portner[1], Doug Latch[2], and George Aiken[3]

[1]Gustavus Adolphus College, St Peter, MN.

[2]Seattle University, Seattle, WA.

[3]US Geological Survey, Boulder, CO.

I. Abstract

This study examined the potential for photodegradation of methylmercury ($MeHg^+$) in the St Louis River using both field and laboratory studies. $MeHg^+$ is a photoreactive chemical that is principally bound to reduced sulfur groups associated with dissolved organic matter in the environment. The formation of $MeHg^+$ within the watershed and the fate of $MeHg^+$ in the St Louis River, a river system within elevated sulfate levels, is of primary concern. Laboratory results suggest that $MeHg^+$ photodegradation is expected to occur relatively rapidly in surface waters. Field experiments indicate, however, that degradation rates will significantly decrease at depth due to the highly colored St. Louis River water. Experiments with chemically distinct dissolved organic matter (DOM) isolates suggest that the source of the organic matters little in the photochemistry if there is enough DOM present to strongly bind $MeHg^+$ at reduced S sites, DOM concentration does not play a role in photodemethylation (other than to screen light). Experiments with simple thiol ligands yielded much slower degradation rates than found in DOM experiments, indicating that the binding environment or proximity to NOM is important to the $MeHg^+$ demethylation.

II. Introduction

The degradation of methylmercury ($MeHg^+$) can occur through both biological and photochemical pathways (Marvin-DiPasquale et al. 2000). Depending on the conditions (e.g. water clarity and DOC content), photodecomposition is the primary degradation mechanism in lakes that can account for up to 80% of the consumption of methylmercury inputs (Hammerschmidt and Fitzgerald 2006, Hines and Brezonik 2007). Methylmercury is degraded by both photosynthetically active radiation (PAR 400-700 nm) and ultraviolet radiation (UV), but studies have produced wavelength-specific rate constants showing that UVB radiation (280-320 nm) degrades methylmercury more rapidly than UVA radiation (320-400 nm) or PAR (Lehnherr and St. Louis 2009, Fernández-Gómez et al. 2013). Complexation by dissolved organic matter (DOM) and thiol-based ligands also affect methylmercury photodegradation rates (Zhang and Hsu-Kim 2010), but the degree to which the rate changes varies with different studies (Zhang and Hsu-Kim 2010, Tai et al. 2014). Although attempts have been made to gain insight into multiple variables affecting photodegradation, many reaction pathways are possible making the relative importance of various factors dependent on experimental conditions.

Two basic mechanisms have been considered: First, direct absorption of light at the C-Hg bond or by the Hg-ligand bond could occur with subsequent Hg reduction and/or demethylation. Second, photolysis of $MeHg^+$ by radical species or reactive oxygen species (ROS) is possible. ROS species including superoxide (O_2^-), hydrogen peroxide, singlet oxygen ($^1O_2$), and hydroxl radical (•OH) form in sunlit waters. Absorption of light

by dissolved organic matter (DOM) can produces the above ROS. Other important ROS-forming processes in aquatic systems include the Fenton and photo-Fenton reactions and the absorption of UV light by nitrate and nitrite to generate •OH.

In most freshwater systems, $MeHg^+$ binding to reduced sulfur groups associated with DOM predominantly controls $MeHg^+$ speciation (Black et al. 2012). In freshwater and marine systems with very low DOM levels, chloride or other ligands can control $MeHg^+$ speciation (Black et al. 2012). One recent study reported that $^1O_2$ produced by DOM after absorbing light leads to breakage of the methylmercury C-Hg bond that has been weakened due to Hg binding with a reduced S group on the DOM (Fernández-Gómez et al. 2013). Binding to a reduced S group pulls electrons toward S increasing the electronegativity of C, reducing the C-Hg bond enthalpy leading to susceptibility to electrophilic attack by $^1O_2$. While this area of research into the specific mechanism of photodemethylation is in its relative infancy, several studies suggest that the methylmercury reduced S bond is critical to its photoreactivity (Zhang and Hsu-Kim 2010, Black et al. 2012, Fernández-Gómez et al. 2013).

A recent study by Gomez et al. (2013) shows that different natural water samples with a wide range in DOC and iron concentrations, pH, and aromaticities showed that wavelength-specific $MeHg^+$ degradation rates decreased with increasing absorption coefficients of the water samples. Once accounting for light attenuation caused by the absorbing components in the water samples, however, it was revealed that all of the samples converged to a give a common photodemethylation rate constant at a given irradiation wavelength. This finding is somewhat counterintuitive, as it suggests that so long as there exists reduced S sites for binding, the exact nature and chemical characteristics of the DOC is unimportant to the rate of $MeHg^+$ photodemethylation. Furthermore, they conducted experiments spanning a range of $MeHg^+$:DOC ratios, whereby at high ratios $MeHg^+$ is forced to bind to O and N functional groups due to saturation of the more favorable reduced S binding sites. Given that degradation rates decreased as $MeHg^+$:DOC ratios increased (and binding to O and N ligands occurs), their results corroborate the idea that reduced S:$MeHg^+$ binding is key to photodemethylation. A final significant finding arising from their work is that degradation rates sharply decrease as irradiation wavelength increases from UVB to UVA to PAR regions of the spectrum.

In this study, our ultimate objective was to assess $MeHg^+$ photodegradation rates in the St. Louis River. We conducted a field study in 2013 and examined photodegradation of $MeHg^+$ in SLR water in a laboratory study. We also examined photodegradation of $MeHg^+$ in the laboratory using different types of DOM isolated from four water bodies in the SLR watershed and altered these samples by adding thiol-containing ligands. These water bodies included Lake Manganika, St Louis River, Long Lake Creek, and the Swan River.

III. Methods

A. Field Sampling

A field campaign was carried out in the St Louis River from Aug 7-9, 2013 at the Toivola sampling site located near the bridge on St Louis County Road 52. During the field sampling two sets of experiments were conducted on a single water sample. About twenty liters of St Louis River Water was collected and filtered (0.7 μm glass fiber filters) directly into a 20-L polypropylene carboy late in the day on Aug 6. The filters were ashed at 550°C and the teflon filter holders were soaked in 4 M HCl and thoroughly rinsed with Milli-Q water and dried before use. The filtered water was spiked to a concentration of approximately 15 ng/L of $MeHg^+$, although the absolute concentration was unknown due to uncertainty in the volume of water. The water was stored refrigerated and allowed to equilibrate overnight. In the morning, the water was distributed to 125 mL Teflon (FEP), 10 mL quartz bottles, and 500 to 1000 mL dark Teflon bottles. The dark Teflon bottles served as dark controls during the experiments.

In the first set of experiments, the 125 mL bottles were deployed floating on the surface of the SLR beginning at ~10:00. All bottles were deployed in duplicate and bottles were removed from the river, placed in a cooler, acidified with HCl to 0.5 % by volume in a shaded area, and stored in the dark until analysis. Bottles were removed at time = 0, 15, 30, 60, 90, 120 and 240 minutes. A dark bottle was also acidified at t=0 minutes and removed from the river and acidified at the end of the experiment. In the second set of field experiments, quartz bottles were suspended at the surface, 10 cm, 20 cm, and 40 cm depths. Samples were removed at time = 1 hr, 2hrs, 4hrs, 1 day, 2 days, and 3 days. Samples were acidified and stored in the dark until analysis.

B. Laboratory Studies

DOM isolation. Water was collected from four sites in the SLR watershed in June of 2012 by pumping 60 to 150 L of water through 0.2 μm capsule filters (Table 1). The sites included Lake Manganika, Swan River, St Louis River Mile 94 (Toivola), and Long Lake Creek wetland (Site 5). The water was shipped to George Aiken's US Geological Lab in Boulder, CO which conducted the DOM isolations and separations prior to sending aliquots of the isolate to Gustavus and Seattle University. The DOM was separated into 3 fractions which included the hydrophobic organic acid (HPOA), the transphilic acid fraction, and a hydrophilic fraction. All of our experiments on DOM were conducted on the HPOA fractions from the four Minnesota sites or on

Suwannee River fulvic acid (SRFA) or Pony Lake fulvic acid (PLFA) which are commercially available isolates that represent end members organic matter samples derived almost entirely from either terrestrial (SRFA) and microbial sources (PLFA).

Laboratory photolysis experiments. Laboratory photolysis experiments were conducted at Seattle University to complement the field studies. Two types of photochemical apparatuses were used: (*i*) a Suntest XLS+ solar simulator equipped with a broadband Xe lamp and a special UV filter to closely mimic the solar spectrum and (*ii*) a Luzchem photoreactor with interchangeable UVA and UVB lamps to assess the effects of irradiation wavelength on photodemethylation rates. Experimental design was similar for the two light sources. Briefly, pH buffered samples containing different DOC types and concentrations or model thiol ligands were spiked with $MeHg^+$ (final concentration ~10 ng/L) and placed into quartz culture tubes (d = 1 cm, V = 10 mL). The tubes were then irradiated in one of the two photosystems. Samples were removed at time intervals similar to those described above for field studies and treated to 0.5 % HCl for preservation. The samples were then shipped to Gustavus Adolphus College for $MeHg^+$ quantification. In addition to assessing how broadband, UVA, and UVB irradiation impacted $MeHg^+$ photodemethylation, pH (6 – 8), DOM concentration (5 – 30 mg/L), DOM type (terrestrial versus microbial origin), and the effect of thiol ligands on $MeHg^+$ demethylation rates were assessed. Mercaptoacetic acid (MAA) and glutathione (GSH) were used as relatively simple thiol compounds to model potential reduced S binding sites on DOM. Whole water samples from the St. Louis River were also analyzed in the same way after being spiked with $MeHg^+$ (~15 ng/L).

C. Analytical Methods

Aliquots of one mL to two mL were analyzed for methylmercury content. $MeHg^+$ was analyzed by isotope dilution on an Agilent 7700 Inductively Coupled Plasma Mass Spectrometer (ICP-MS) with sample introduction via a MERX-M system (Brooks Rand).

Methylmercury concentrations were measured using standard ethylation/isotope dilution techniques. Sample aliquots were spiked with a known amount of isotopically enriched methylmercury ($Me^{201}Hg$) and the pH was adjusted to ~4.8 by neutralizing the hydrochloric acid with potassium hydroxide and the addition of sodium acetate buffer. $MeHg^+$ species were ethylated using sodium tetraethyl borate followed by analysis on an Agilent 7700 ICP-MS (inductively coupled plasma – mass spectrometer) connected to a Brooks Rand MERX system using an isotope dilution method (Hintelmann and Evans 1997).

IV. Results and Discussion

St Louis River Field Study

This initial field study will allow us to develop further, more detailed studies and help to direct laboratory studies.

The results from the two concurrent studies are presented in Figures 1 and 2. Figure 1 details a 4 hour study conducted from 10:00 to 14:00 at the Toivola site on the St Louis River on August 7, 2013. The spiked (~ 15 ng/L of $MeHg^+$) 125-mL Teflon bottles were deployed in duplicate on the surface in about 1 meter of water. August 7 was a partly sunny day with high cumulus clouds covering about 50% of the sky. St. Louis River water at the time was typical of mid-summer with DOC levels of about 35 mg/L. Over the four hour period $MeHg^+$ concentration dropped from about 14.6 ng/L to 13.0 ng/L, a decrease of about 10%. The spiked $MeHg^+$ concentrations are 10-15 times higher than ambient levels at this time of year, but at these levels, all the $MeHg^+$ is predicted to be bound to reduced sulfur sites on DOM. At 35 mg/L of DOC the binding of the spiked $MeHg^+$ would still be controlled by binding with DOM. Assuming S constitutes 0.2% of C in the DOM, the concentration of reduced thiol sites is estimated to be ~ 2 µM compared to the spiked $MeHg^+$ of 75 pM. As Gomez et al. (2013) have shown and others have surmised, $MeHg^+$ photodegradation rates in spike experiments should be equivalent to those at ambient levels so long as the $MeHg^+$ to DOC levels are low enough that binding is dominated by reduced S sites (refs). Our experiments fall well within the strong reduced S binding regime. Dark bottles collected before and after the 4-hr experiment demonstrate that biological degradation was insignificant.

The second set of experiments was conducted using 10 mL quartz tubes spiked with the same water as the Teflon bottles. Quartz tubes have the advantage of not attenuating visible or UV light, but could be hampered by adsorption of $MeHg^+$ to the quartz. The quartz tubes were suspended at depths of 0, 10, 20, and 40 cm. Wire was wrapped around the caps of the tubes and attached to a wooden stake that was driven into the river bed. Deeper tubes had progressively longer wires to prevent shading from above. Sets of samples were removed at 1 hr, 2 hr, 4hr, 1 day, 2 days, and 3 days (Figure 2).

The first depth profile collected at hour one yielded peculiar data, particularly the low value found for the surface sample. Following collection, the samples were immediately placed in a cooler and acid preservative was quickly added while in the shade. The same procedure was followed for every set of samples. Sorption to the glass could be an issue, but it is unclear why this set of samples would be any different than the others. Ignoring the t = 1 hour samples, photodegradation was minimal below the surface as no changes in $MeHg^+$ concentration were observed over 3 days at 10, 20, and 40 cm. At the surface, the concentration decreased to 6.6 ng/L after 3 days (~50 % of the spike concentration). An initial concentration was not measured in the

quartz tubes, but assuming no change in the $MeHg^+$ concentration at 40 cm depth, we estimate the initial concentration to be approximately 13.5 ng/L. After four hours the concentration had decreased to 11.9 ng/L, a decrease of about 12%, similar to the Teflon bottles.

These data show that while photodemethylation is expected to be a significant loss process at the surface of St. Louis River, loss rates drop off precipitously at depth. This is a common photochemical phenomenon due to the significant light attenuation brought about by the highly absorbing St. Louis River water. At 35 mg/L DOC, this so-called inner filter effect is expected to be quite large, and our data from experiments performed at depth reflect this expectation.

The significantly faster photodemethylation in surface experiments performed in quartz tubes relative to those performed in Teflon bottles could be due to the Teflon bottles filtering light important wavelengths for $MeHg^+$ degradation. A second possibility is that the different geometries of the Teflon bottles and the quartz tubes plays a role. The quartz tubes have a small diameter (~1 cm) and thus a short optical path length that limits the inner filter effect (i.e. light attenuation). Inner filter effects may be occurring in the 125 mL Teflon bottles given the high DOC of the St. Louis River water and the significantly longer path lengths of the bottles.

These field experiments indicate that some $MeHg^+$ loss due to photodemethylation is expected to occur in the St. Louis River, but this process is limited to very near surface depths due to significant light attenuation brought about by the high DOC content of the water. It is expected, however, that as the water flows downstream and is diluted (and thus the DOC content and inner filtering decreases) photodemethylation will also become a significant $MeHg^+$ loss process at deeper depths.

St Louis River Lab Studies

In addition to the field studies described above, $MeHg^+$ degradation in St. Louis River water was also examined in the laboratory. Results of these laboratory experiments are summarized in Figure 3. As expected based on our field studies, these data show that $MeHg^+$ is susceptible to photodegradation in St. Louis River water. In addition to conducting experiments with simulated solar light, $MeHg^+$ degradation in St. Louis River water was also tracked upon exposure to narrow bands of UVA and UVB light. Figure 3 shows that lower wavelength UVB light is much more important to demethylation than UVA light. This finding is consistent with the findings of Gomez et al. (2013). This result could be due to better overlap with the absorption spectrum of the $MeHg^+$:DOM complex (i.e. direct photolysis) or to the DOM-initiated generation of more reactive species capable of degrading $MeHg^+$ (i.e. indirect photolysis). More experiments are needed to elucidate which process is occurring (or to determine if both processes may be operative to some degree).

Experiments with DOM isolates

To gain a firm understanding of how general environmental photochemical results are and to better predict how these results may change due to season and location, experiments are often performed using relatively well characterized DOM isolates originating from a range of natural waters. To that end, we performed experiments using a DOM isolate of terrestrial origin (SRFA) and one derived from microbial sources (PLFA). These isolates have distinct structural and functional characteristics, leading to differences in ability to generate photochemically produced reactive intermediates (Grandbois et al. 2008) (Guerard et al. 2009). Performing experiments with these isolates will allow for insights into photochemical loss processes and may be instructive in assessing the extent to which indirect photochemistry occurs.

Figure 4 is illustrative of the results obtained in our experiments with DOM isolates. Photodegradation of $MeHg^+$ readily occurs in the presence of PLFA (and is faster than in the absence of PLFA; data not shown), though rates do not scale with PLFA concentration. This is consistent with direct photolysis or intra-DOM indirect photoreactions under conditions where all of the $MeHg^+$ is bound to reduced S sites even at the lowest DOM concentration tested. Given the $MeHg^+$ and DOM concentrations used in all of our experiments, we expect this to be the case. Results with SRFA were very similar to those of PLFA, suggesting that the source of DOM does not matter as long as enough is present to bind $MeHg^+$ to thiol sites. This result is consistent with that reported by Gomez et al. Figure 5 shows that pH does not impact degradation rates (at least over the range from 6 to 8). If direct photolysis of the $MeHg^+$:DOM complex is operative, pH certainly would not be expected to make a difference in this process. Only slight changes in reactive intermediate production would be expected to occur over this pH range, meaning that these pH data do not provide much insight into the $MeHg^+$ degradation mechanism. Nevertheless, this finding is useful in that it shows that pH is not a variable that needs to be considered in predicting $MeHg^+$ photodegradation rates in the field.

Effect of added thiol ligands

Because binding of $MeHg^+$ by organic matter is dominated by reduced S sites, we performed experiments with simple thiol ligands in an effort to characterize the photochemical behavior of these complexes. Figure 6 shows a comparison of $MeHg^+$ loss in NOM solutions relative to that observed using the simple thiol ligands MAA and GSH. It is readily apparent from this plot that $MeHg^+$ loss is much slower with the simple thiol ligands than in NOM solutions. When NOM solutions are mixed with these thiol ligands in the same solution, intermediate rates are observed. Figure 7 shows this effect with SRFA. That the model thiol ligands gave such different degradation rates than NOM was not expected. While we cannot offer a detailed explanation

of this result, it likely speaks to a much different binding environment within the NOM (e.g. the electron withdrawing or donating characteristics of the reduced S sites may differ from MAA and GSH) or that an intra-NOM indirect photolysis mechanism is occurring. In the latter case, binding to MAA and GSH would pull $MeHg^+$ away from the relatively high reactive intermediate concentration within the NOM macromolecules and into the bulk water regime where indirect photoreactions occur more slowly.

V. Conclusions

Our results suggest that $MeHg^+$ photodegradation is expected to occur relatively rapidly in surface waters. Field experiments indicate, however, that degradation rates will significantly decrease at depth owing to the highly colored St. Louis River water. It is expected that more $MeHg^+$ loss will occur in dilute, less colored waters downstream, as the photic zone increases. Results from laboratory studies show that UVB drives the $MeHg^+$ photodegradation. Experiments with the NOM isolates SRFA and PLFA indicate that the source of the organic matters little in the photochemistry and that so long as there is enough NOM present to strongly bind MeHg+ at reduced S sites, NOM concentration does not play a role in photodemethylation (other than to screen light as [NOM] or depth increases). Experiments with simple thiol ligands yielded much slower degradation rates than found in NOM experiments, indicating that the binding environment or proximity to NOM is important to the $MeHg^+$ demethylation.

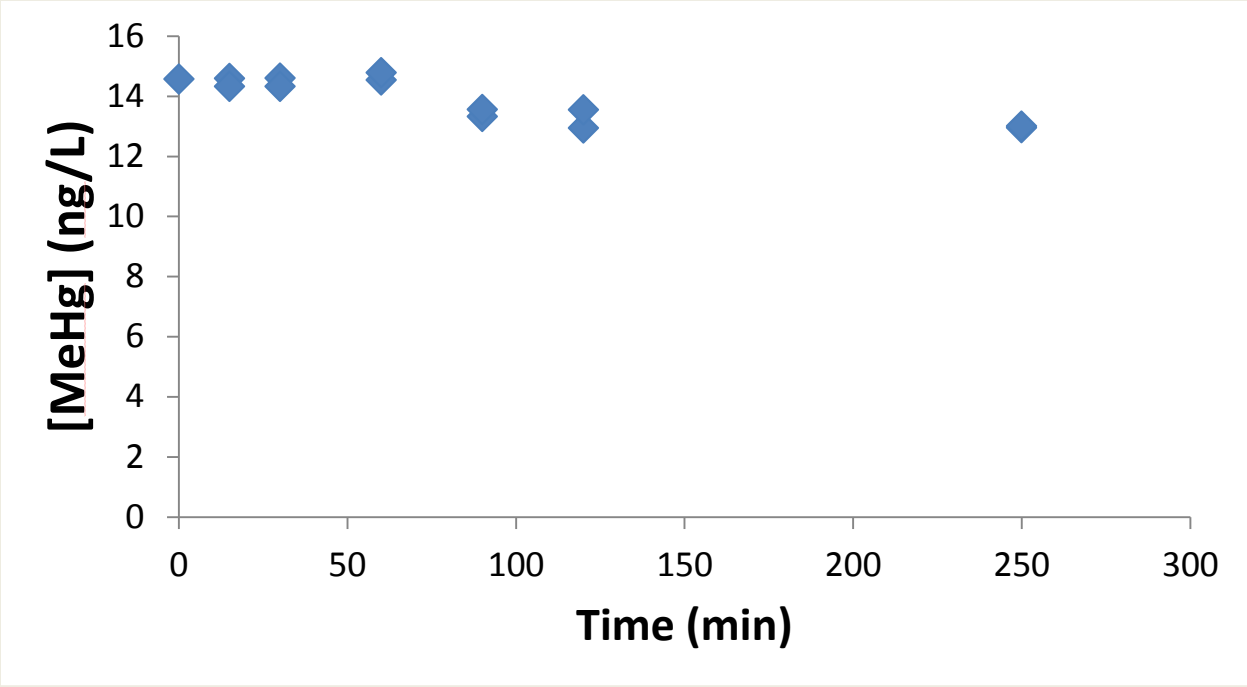

Figure 1. Photodemethylation of $MeHg^{+}$ in St. Louis River water. Samples were irradiated in the field in 125 mL Teflon bottles.

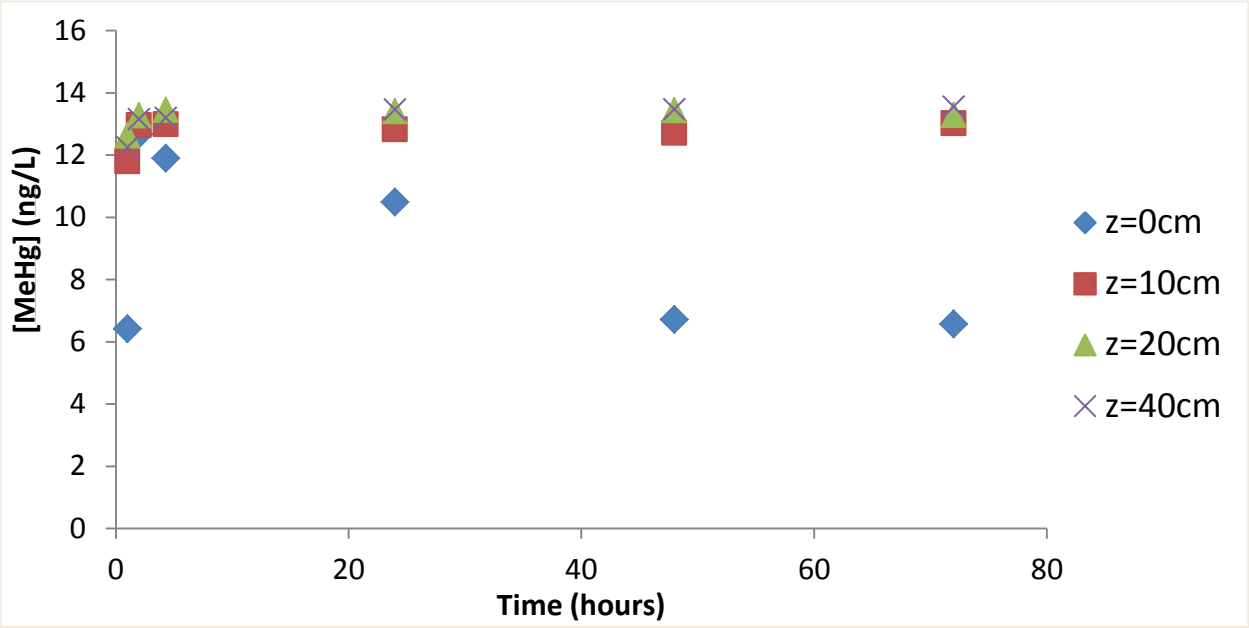

Figure 2. Photodemethylation of $MeHg^+$ in St. Louis River water at various depths. Samples were irradiated in the field in sealed 10 mL quartz tubes.

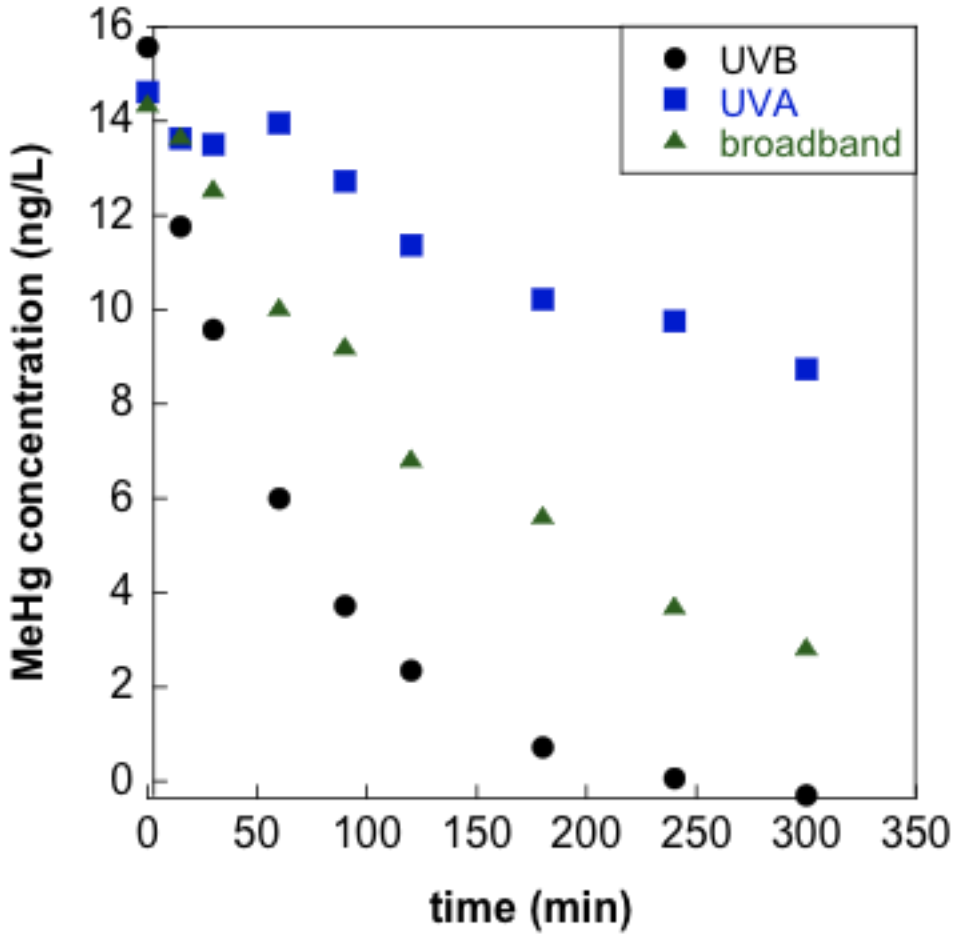

Figure 3. Laboratory studies of the photodemethylation of $MeHg^+$ in St. Louis River water. Samples were irradiated in sealed 10 mL quartz tubes in a solar simulator (green diamonds, “broadband” light mimicking the solar spectrum) and in a photoreactor equipped with either UVA (blue squares) or UVB (black circles) lamps.

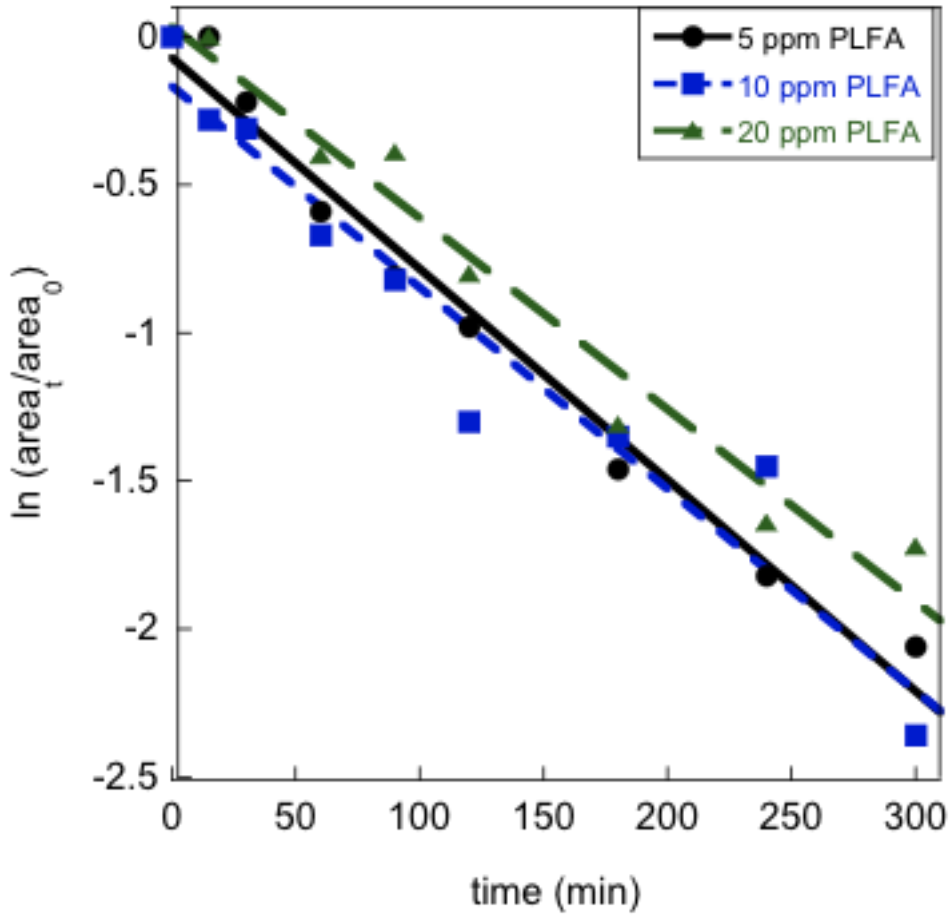

Figure 4. Laboratory studies of the photodemethylation of $MeHg^+$ in solutions with varying amounts of added organic matter isolate (PLFA). Samples were irradiated in 10 mL quartz tubes in a solar simulator.

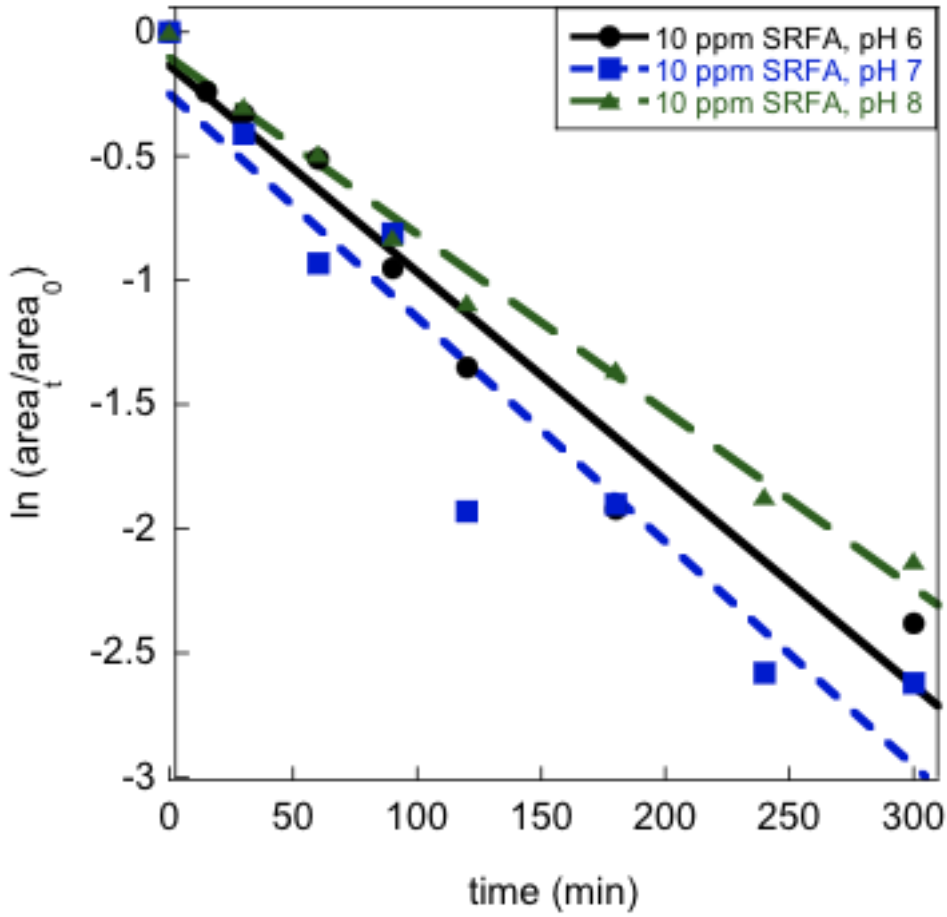

Figure 5. Laboratory studies of the photodemethylation of $MeHg^+$ in solutions with 10 mg/L of added organic matter isolate (SRFA) buffered to different pH values. Samples were irradiated in 10 mL quartz tubes in a solar simulator.

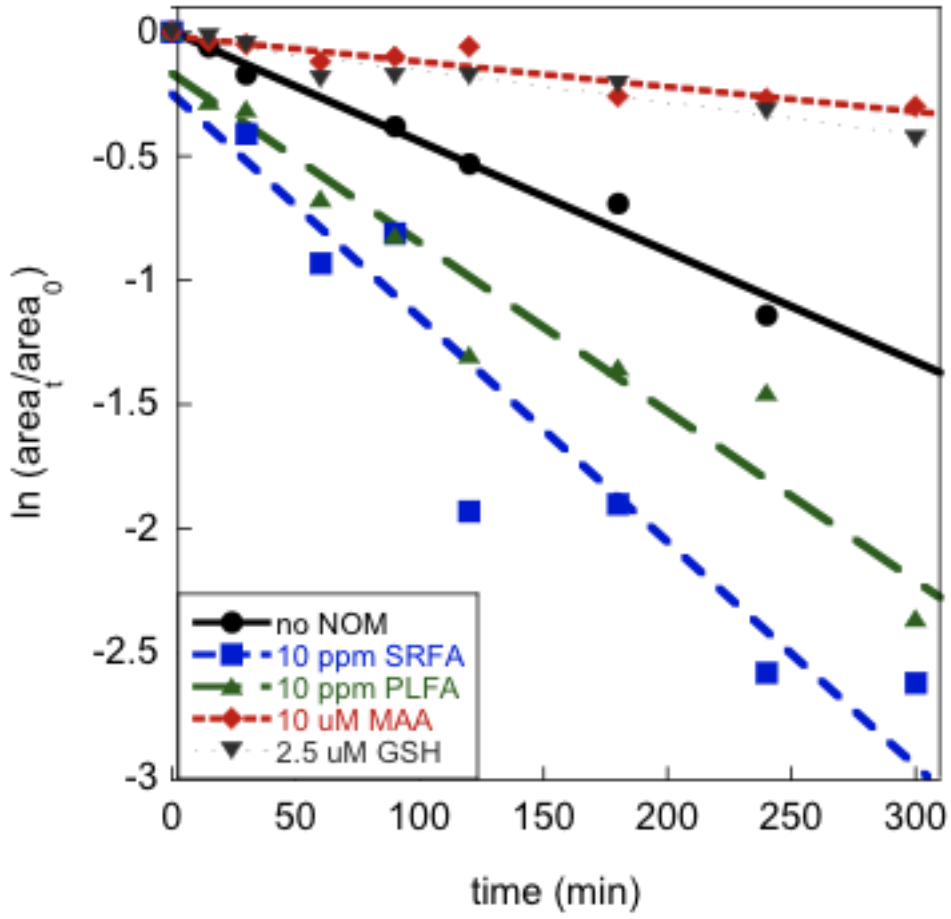

Figure 6. Laboratory studies of the photodemethylation of $MeHg^{+}$ in solutions with different reduced S substrates (including DOM isolates SRFA and PLFA). Samples were irradiated in 10 mL quartz tubes in a solar simulator.

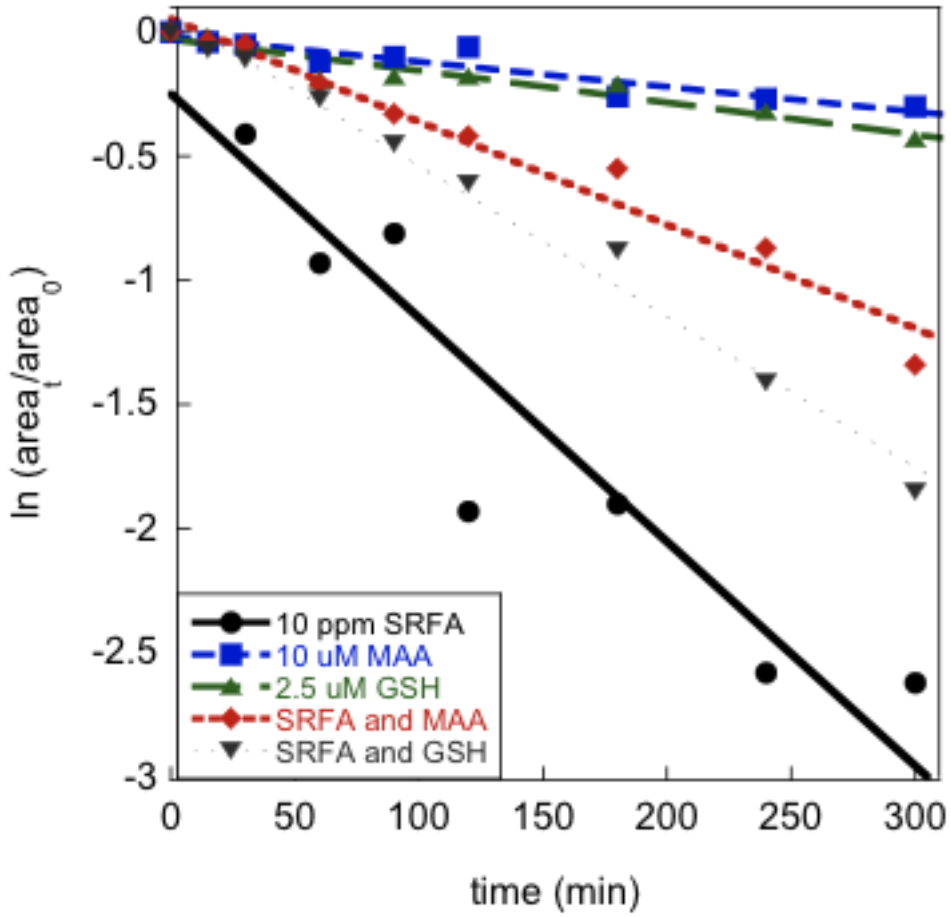

Figure 7. Laboratory studies of the photodemethylation of $MeHg^{+}$ in solutions with mixed solutions of DOM and simple thiol ligands. Samples were irradiated in 10 mL quartz tubes in a solar simulator.

VI. References

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