Abiotic Production of Methylmercury by Solar Radiation

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Methylmercury [MeHg(I)] in the aerobic surface water of lakes is thought to be rapidly degraded, but contrary to expectations, we show that MeHg(I) concentrations often increase during sunlight hours or remain relatively constant. We hypothesized that there were water column processes that generated MeHg(I) and that these processes were linked to dissolved organic matter (DOM) and solar radiation. A 2-day diurnal pattern of MeHg(I) in surface water with corresponding bottled controls was assessed for two contrasting lakes in Kejimikujik, Nova Scotia, Canada. Following this study, a tangential ultrafiltrator was used to size-fractionate and generate a concentration gradient of DOM from four different lakes located near Lac Berthelot, Quebec, Canada. The watersheds of two of these lakes were not substantially logged whereas the other two had been extensively logged. Different size fractions of DOM as well as different concentrations of DOM were exposed to sunlight for varying periods of time. We observed that, in Kejimikujik, the concentration of MeHg-(I) in surface waters peaked in the early afternoon. Furthermore, this also occurred in bottled water for one of the lakes, Puzzle, eliminating the possibility that in-lake mixing played a role in this pattern. The formation of MeHg-(I) was found to be dependent on the size fraction and amount of DOM present in the water. Specifically, DOM less than 5 kDa or between 30 and 300 kDa generated MeHg-(I) when exposed to sunlight, but larger fractions did not. Furthermore, although data are limited, we found that water from lakes with logged watersheds generated MeHg-(I) when exposed to sunlight, whereas water from lakes with low levels of logging in the undisturbed watersheds did not. Our results demonstrate that MeHg(I) can be formed in freshwaters of certain lakes in response to solar radiation. This photoproduction of MeHg(I) is dependent on DOM concentrations and type, with the importance of water chemistry not yet clear. The significance of this process to freshwater lakes and the mechanism responsible for MeHg(I) photoproduction is still unclear, but a correction in the conventional wisdom that MeHg(I) is rapidly photodegraded is timely.

Introduction

Methylmercury, MeHg(I), is a chemical of concern in many ecosystems because it is a potent neurotoxin that can bioaccumulate in food webs. As a result, there have been numerous investigations into the sources and sinks of MeHg-(I) ([1–6]). Emerging from these investigations is the importance of wetlands and sulfate-reducing bacteria in the production of MeHg(I) ([7–13]). Once formed, MeHg(I) is thought to be transported downstream through adsorption to dissolved organic matter (DOM) ([14, 15]). Once in the lake, the fate of MeHg(I) is less clear. Some studies have found that sediments are a source ([16]) whereas others have found that sediments are a sink of MeHg(I) ([17, 18]). Because source/sink decisions are made relative to water-column concentrations, conflicting reports of a sediment's role as a source or sink maybe due to differences in MeHg(I) formation in the water column itself.

We hypothesized that the water column might be a source of MeHg(I) under certain conditions. Supporting this hypothesis, elevated concentrations of MeHg(I) are photochemically degraded in freshwater lakes with surface degradation rates averaging 18% day^{-1} ([16, 19]). Since MeHg(I) is not thought to be formed in the water column ([20]), concentrations of MeHg(I) at the lake surface in the absence of lake mixing should display a strong diurnal pattern due to the photodegradation of MeHg(I) by sunlight. However, MeHg(I) concentrations peak at noon ([21], this study) and the addition of wide-spectrum microbial inhibitors does not prevent mercury methylation in the Florida periphyton ([22]). Hence, it is possible that abiotic methylation processes are occurring in the water column. Abiotic methylation of mercury can occur under laboratory conditions ([23]) at environmentally relevant concentrations ([24]). If abiotic methylation is occurring, this would explain the results from the Florida Everglades and the conflicting sediment source/sink reports of others.

It is likely that DOM will play a pivotal role in the fate of MeHg(I) in the water column for the following four reasons: (i) Dissolved organic matter is a strong sorbant of MeHg(I) as well as Hg(II) and thereby acts to transport and sequester mercury ([14, 15]); (ii) solar radiation is attenuated by DOM, which thereby reduces the impact of solar radiation at depth in freshwater lakes ([25]); (iii) solar radiation and DOM react to produce oxidants in the water column or DOM itself can be activated to act as an oxidant ([26, 27]); and (iv) DOM contains reactive functional groups and associated counterions that may interact with Hg(II) in various ways including methylation ([28, 29]). Studies investigating DOM–Hg dynamics are all faced with the paradox that investigating the concentration-dependent effect of DOM requires that different lakes with a range of DOM concentrations be sampled. Unfortunately, the DOM structure itself will also vary between lakes. Hence, separating the influence of structure from that of concentration is difficult. Here we make use of the tangential ultrafiltration previously described ([30]) to circumvent this limitation. Tangential ultrafiltration systems have been found to be effective for trace metal investigations ([31]). By using such a system, we can systematically explore the effect of DOM concentration on MeHg(I) dynamics in freshwaters.

Logging of freshwater watersheds increases DOM concentrations in the associated lake water ([32, 33]) and also

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increases Hg loads in the freshwater biota (*34, 35*). Others have found that forestry practices do not necessarily influence Hg transport (*36*). We hypothesized that watershed impacts may be altering the interconversion processes of the three primary mercury species that occur within the water column. Recently, we found that the reduction of Hg(II) to Hg(0) by DOM was lower in logged compared to unlogged watersheds (*30*). As Hg(0) production is key to the evasion of mercury from a lake, we postulated that the impairment of Hg(0) formation may be one of the reasons for the increase in mercury observed in the biota of logged lakes. Here, we continue this comparison of logged versus unlogged watersheds but extend our focus to include a subcomponent of MeHg(I) dynamics in the water column. Initially, we assessed MeHg(I) diurnal patterns in two different lakes to evaluate if there were water column methylation processes occurring. We then explored the dependence of this process on DOM. Finally, we compared differences in this methylation process between lake water obtained from logged and unlogged watersheds.

Materials and Methods

Lakes Studied. The diurnal cycle of MeHg(I) was assessed at two lakes in southern Nova Scotia, Canada, on the Atlantic coast. Big Dam West is located at (44°46′25″ W, 65°29′50″ N) and Puzzle is located at (44°32′25″ W, 65°23′08″ N), in Kejimukujik National Park (44°26′ W, 65°12′ N) in Nova Scotia, Canada. Big Dam West is an acidic (pH 5.0), low conductance (30 μS cm^{-1}), brown water lake (94 Hazens) with high dissolved organic carbon (10.5 mg L^{-1}). It has a surface area of 105 hectares, a mean depth of 2.5 m and a flushing rate of 13 times yr^{-1}. Puzzle is also an acidic (pH 5.3), low conductance (20.6 μS cm^{-1}) lake but is clear (20 Hazens) and low dissolved organic carbon (3.6 mg L^{-1}). It is smaller with a surface area of 34 hectares, a mean depth of 2.7 m, and a flushing rate of 2 times yr^{-1}.

Four lakes in northern Quebec, Canada, were chosen to represent a range of DOM concentrations within both logged and nonlogged drainage basins. Lakes K2 (48°17′56″ W, 75°10′08″ N) and N70 (48°05′12″ W, 75°29′09″ N) have catchments where very little logging has occurred (0% and 2% of basin, respectively) and have dissolved organic carbon concentrations of 6.7 and 3.2 mg L^{-1}, respectively. Lakes K3 (48° 18′ 26″ W, 75° 16′ 18″ N) and DF9 (48° 42′ 31″ W, 75° 01′ 03″ N) have logged catchments (26% and 67% of basin, respectively) and dissolved organic concentrations of 4.9 and 13.7 mg L^{-1}, respectively. All lakes are relatively small (<150 ha) and are well-saturated with oxygen (>94%). Lake DF9 is an acidic (pH 6.3), brown water lake with a surface area of 0.270 km^2, a maximum depth of 10.5 m, and a flushing rate of 2.7 times yr^{-1} (*30*).

Diurnal Pattern Analysis. We analyzed ambient MeHg concentrations at a 10 cm depth in Big Dam West and Puzzle lakes every 2 h over a 48 h period for each lake using Teflon (FEP) tubing connected to a floating platform that was located approximately 15 m from shore (*30*). The tubing was exposed to only 7.6% of the surface incident radiation (3.8% of total radiation) for a total of 3 min while in transport to the sample collection point. In addition to surface water analysis, we also analyzed the influence of visible light on diurnal MeHg patterns in lake water by placing lake water in precleaned, 1-L HDPE bottles at the beginning of the study period. High-density polyethylene (HDPE) bottles were selected due to their low binding of ambient MeHg in lake water (*37*). These bottles were incubated on the lake surface throughout the study period and were used to correct for any mixing of the upper irradiated layer with underlying water layers. Similar bottles were covered and submerged in lake water to eliminate the influence of solar radiation on MeHg diurnal patterns in freshwater lakes.

Big Dam West was sampled between 157.75 and 159.83 GMT, 2001. During this period, air temperature averaged 16.7 °C (range 8.6–23.7 °C), relative humidity averaged 70% (range 29–97%), visible light (400–750 nm) during the day averaged 0.4 kW m^{-2} (range 0.001–1.197 kW m^{-2}), and the wind speed averaged 0.8 m s^{-1} (range 0–8.3 m s^{-1}). Solar radiation was measured with a Li-Cor LI200X silicon pyranometer calibrated against an Eppley precision spectral pyranometer. Puzzle was sampled between 162.92 and 164.75 GMT, 2001. During this period, air temperature averaged 17.4 °C (range 10.4–24.5 °C), relative humidity averaged 81% (range 40–97%), visible light during the day averaged 0.3 kW m^{-2} (range 0.001–1.000 kW m^{-2}), and the wind speed averaged 0.4 m s^{-1} (range 0–5.7 m s^{-1}).

MeHg diurnal data was nonnormally distributed (Anderson-Darling normality test, $P < 0.002$) but log transformation of the data corrected this (Anderson-Darling normality test, $P < 0.510$). Variances between treatments were homogeneous and an initial GLM indicated that there was no difference ($P < 0.97$) between subsequent days for each lake and that there was no interaction between days and lakes ($P < 0.208$). Thus, we considered analysis on subsequent days as replicates for that particular time; for example, samples taken at 08:00 on days 1 and 2 were considered as replicates. Considered in this manner, a general linear model (GLM) testing the effect of treatments (surface water, HDPE + solar radiation, HDPE + dark), time of sampling (2:00 to 24:00), and lakes (Big Dam West, Puzzle) found a nonsignificant interaction between treatments and time ($P < 0.197$) but a significant time interaction ($P < 0.004$).

Ultrafiltration of Lake Water from Quebec Lakes and a Comparison between Logged and Nonlogged Watersheds. To prepare dilutions of lake water, water was fractionated on a Centramate PE lab tangential flow system (Pall Corp., Mississauga, Ontario, Canada) as previously described (*30*). Briefly, lake water was passed through a 0.2 μM Omega poly-(ether sulfone) cassette filter and this sterilized lake water referred to as "whole water". A portion of the whole water for each lake was then filtered further through an Omega poly(ether sulfone) 1 kDa filter ("1 kDa water") to remove most of the DOC while allowing dissolved ions to remain in the filtrate. Dilutions of the whole water from each lake were prepared by use of the 1 kDa filtered water, to produce samples with a range of DOC concentrations while leaving dissolved ions in the water unaltered. Dilutions were prepared in 1 L Teflon bottles at 0%, 10%, 50%, and 100%.

Dilutions of Sterilized Lake Water. As previously described (*30*), dilutions of whole water (0%, 10%, 50%, and 100%) were placed in clear and black 1-L FEP Teflon bottles, which were then partially submerged in lake water by use of a floating platform and exposed to solar radiation for a total of 10.5 h. Solar radiation was measured every 15 min on an Optronics Laboratories 754 spectroradiometer with quartz spectral probe. Scans were taken at 10 nm intervals between 280 and 800 nm and integrated to obtain measurements of cumulative UVB (300–320 nm), UVA (320–400 nm), and photosynthetically active radiation (PAR) (400–700 nm). Water temperature was measured at 3.5-h intervals with a digital thermometer.

Fractionation of Lake DF9 Water. To investigate which fraction of DOM was responsible for photomethylation, a series of 300, 30, and 5 kDa filters were used. For the 300 kDa filter, a T-screen filter made with Pall's Omega membrane [low protein binding, modified poly(ether sulfone)] was used, and for the 30 and 5 kDa filters, suspended screens (catalogue nos. OS030C11 and OS005C11) were used. The 300 and 30 kDa filters were used with an inlet PSI of 8 and no back pressure. To filter lake water with the 5 kDa filter, a back pressure of 8 PSI was used with an inlet PSI of 10. Filtration rates of ca. 200, 254, and 112 mL min^{-1} for the 300, 30, and

5 kDa membranes, respectively, were used. As recommended (*31*, *38*), membranes were preconditioned with lake water for 120 min before use and a concentration factor of 2 was used. Under these conditions, mass balances ($n = 3$) of MeHg for the 300, 30, and 5 kDa filters were 109%, 81%, and 96%, respectively. Filter performance was evaluated before and after filtering lake water and it was found that the filtration rate of MilliQ water for the 300, 30, and 5 kDa filters had decreased by 50%, 17%, and 26%, respectively, after filtration of 200 L of lake water. These incubations were performed once in the morning and once in the afternoon of the same day. Over the course of the incubation there was no change in the DOM or pH of the incubated water (data not shown). Water temperatures for the morning incubation increased from 18 to 21.8 °C and in the afternoon from 21.8 to 23.3 °C (data not shown). The absence of bacteria at the end of the incubation was verified by direct visual counts (*39*).

Estimation of Photoproduction and Photodegradation Efficiencies. We used a bivariate scattergram, which allowed us to calculate unbiased values for K_1 (slope) and K_2 (intercept) of the principal axis of the relationship between initial MeHg concentrations and the amount of MeHg formed over the time period in question. The slope of the principal axis of the relationship between the observed and predicted values is

$$\text{slope}_{(\text{principal axis})} = \text{covariance}/[\lambda_1 - \text{variance}_{(\text{obsd values})}] \tag{1}$$

where λ_1 is the first eigenvalue of the variance–covariance matrix. We estimated the uncertainty of the slope and intercept from the eigenvalues of the principal and secondary axes (*55*, section 15.7). We evaluated the degree of association between the observed and predicted values using the product–moment correlation coefficient (*r*) and the coefficient of determination (r^2) (*55*, section 15.2). Note that in this case, because there was error associated with both the independent and dependent variables, it was necessary to calculate the coefficient of determination by multiplying the correlation coefficient by the ratio of the variance of the predicted values to the variance of the observed values. Tests of the significance of the differences between the correlation coefficients were preformed by use of the *z* transformation (*55*, section 15.5).

Chemical Analysis of Hg Species. Total Hg in lake water was analyzed by use of $SnCl_2$ reduction in cold vapor-atomic fluorescence spectroscopy as outlined in EPA Method 1631. The method detection limit (3σ of all blanks) for total Hg was 0.31 ng L^{-1} ($n = 8$) and a percent recovery of 97% ± 5% ($n = 8$). MeHg(I) was analyzed by use of a sulfhydryl cotton fiber preconcentration step developed in the mid-1980s (*40*) and subsequently modified (*41*). These modifications used a combination of a H_2SO_4/KBr/$CuSO_4$ elution step, which minimizes artifact formation (*42*), and a single column concentration step, which improves the reproducibility of percent recoveries (*40*). As described previously, we increased the precision of our analysis by performing solid-phase extraction of MeHg(I) within 10 min of sample collection (*43*). Briefly, MeHg(I) was analyzed by immediately acidifying the lake water to pH 3.0 with 20% HCl, buffered with acetic acid buffer, and then concentrated by passing the acidified lake water through sulfhydryl cotton solid-phase extraction via a field-portable multichannel peristaltic pump. The MeHg(I) sorbed on the sulfhydryl cotton within 8 h and was eluted into 7 mL glass vials with acidic KBr/$CuSO_4$. Scintillation vials were stored for less than 14 days at 4 °C before GC/AFS analysis (*41*). Spike recoveries ($n = 5$) of MeHg(I) in acidic KBr/$CuSO_4$ under these conditions averaged 91%. The detection limit for the entire analytical protocol of MeHg(I) was 20 pg L^{-1}, which was calculated as the mean of the blanks ($n = 13$) plus 3 times the standard deviation. Matrix spike
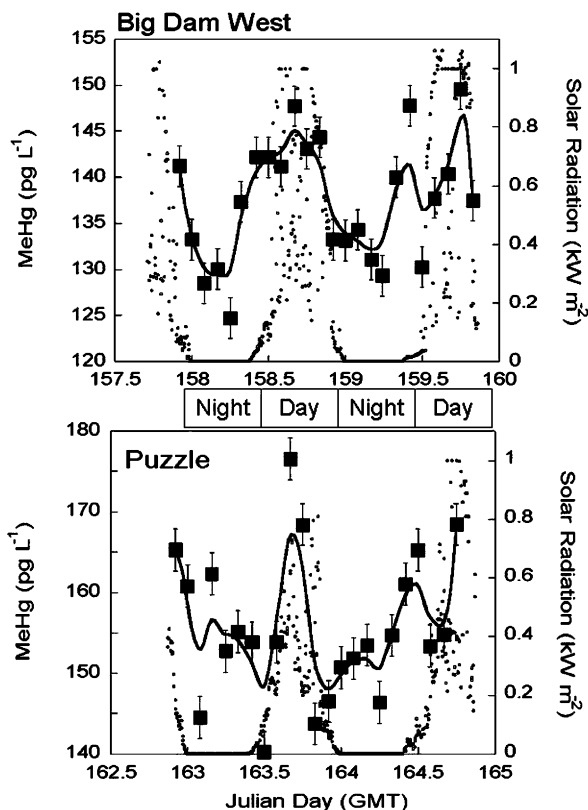

FIGURE 1. Diurnal pattern of ambient MeHg (■) at the surface and solar radiation (●) in two freshwater lakes over a 48 h period. Each data point is the average of duplicate measurements. Error bars are the standard deviation of the estimate of the mean from the general linear model used to analyze the data. The smoothed curve was generated by use of cubic spline interpolation.

recoveries averaged 93% ($\sigma = 4.2$, $n = 10$). Percent deviation of triplicate water samples sampled at the same time ($n = 6$) ranged from 4% to 13% and averaged 6.8% for an analytical variability of 2.8%. Percent deviation of replicate samples for Puzzle and Big Dam West averaged 4.3% ($n = 72$). Recent work (*44*) has found that the MeHg extraction method used here may result in an artificial formation of MeHg from Hg(II) spiked into solution. This artifact formation in natural waters is a function of reaction volume and decreased from 0.032% when 10 mL was analyzed to 0.0023% when 100 mL was analyzed. In this study, we analyzed 500–1000 mL. Spiking of 100 ng L^{-1} into 1 L of natural waters did not result in MeHg(I) artifact formation (data not shown). However, if we assume a maximum artifact formation of 0.0023%, this represents a MeHg(I) artifact formation of only 0.092 pg of MeHg/L.

Results and Discussion

In Puzzle and Big Dam West lakes we observed that MeHg(I) concentrations in surface water as well as water from Puzzle in HDPE bottles exposed to solar radiation displayed a diurnal pattern (GLM, testing time effects; $P < 0.004$) with the highest MeHg(I) concentrations observed at noon for both lakes (Figures 1 and 2). In contrast, dark bottles did not display any diel pattern. During the sampling period, total Hg for Big Dam West was 4.7 ng L^{-1} (σ of 0.89 ng L^{-1}, $n = 47$) and for Puzzle, 2.7 ng L^{-1} (σ of 0.81 ng L^{-1}, $n = 47$) with no diurnal pattern observed. MeHg(I) concentrations over a 2-day period at each lake were positively correlated (Big Dam West, $r = 0.531$, $n = 48$, $p < 0.096$; Puzzle, $r = 0.395$, $n = 48$, $p < 0.239$) with visible solar radiation. The diurnal pattern observed in HDPE bottled and surface water did not differ ($P < 0.197$) (Figure 2), suggesting that diurnal fluctuations of MeHg(I) in
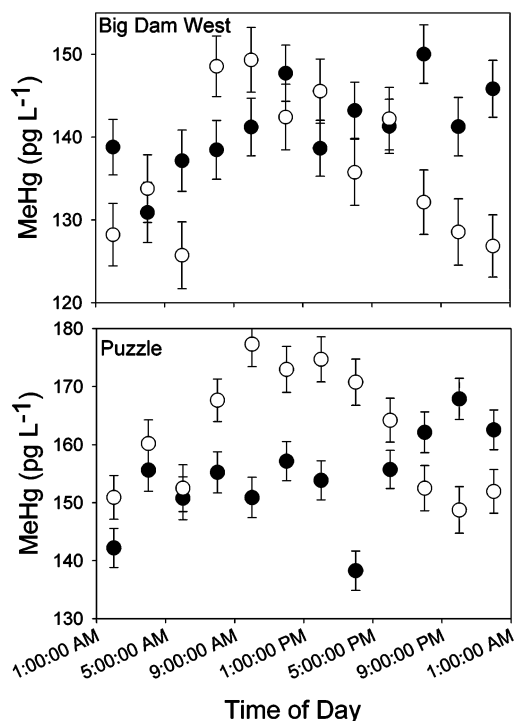
FIGURE 2. Diel pattern of MeHg(I) in water bottled in dark containers (●) compared to water bottled in light containers (○). Each data point is the average of duplicate measurements. Error bars are the standard deviation of the estimate of the mean from the general linear model used to analyze the data.
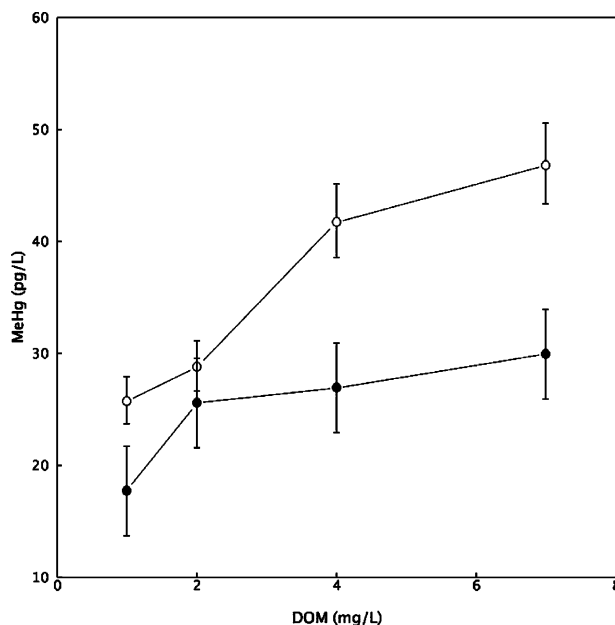

FIGURE 3. MeHg photoaccumulation in freshwater with increasing concentrations of dissolved organic matter (DOM) exposed to 7.5 h of solar radiation (○) and dark controls (●). Each point is the estimate of 32 replicates (○) or eight replicates (●). Error bars are the standard deviation of the estimate.

the surface water were not due to the mixing of deep water with surface waters. We are not certain why MeHg(I) only decreased at night in bottled controls exposed to sunlight as opposed to the dark controls. As these bottles were not sterilized, it is possible that biological reactions occurred at night that consumed MeHg(I). In addition, the exposure to light on a daily basis would have certainly resulted in different microbial communities in the light compared to the dark bottles and this may be the reason these treatments differed. For these reasons, it is not surprising that MeHg(I) levels in bottled controls, 148 pg L^{-1}, differed ($P < 0.05$) from the average levels observed in the lake water, 142 pg L^{-1}.

As can be seen in Figures 1 and 2, Puzzle and Big Dam West displayed different sensitivity to photoproduction. For Puzzle, MeHg(I) concentrations were higher ($P < 0.05$) in HDPE bottles exposed to solar radiation (163 pg L^{-1}, $\sigma = 2.1$, $n = 24$) compared to dark controls (155 pg L^{-1}, $\sigma = 2.0$, $n = 24$), whereas in Big Dam West, HDPE bottles exposed to solar radiation contained similar levels (137 pg L^{-1}, $\sigma = 1.8$, $n = 24$) compared to dark controls (140 pg L^{-1}, $\sigma = 1.8$, $n = 24$). This suggests that lake chemistry plays an important role in this unexpected diurnal MeHg(I) cycle, and as we demonstrate below, one critical factor appears to be DOM. Since the lake water and the bottled controls were not sterilized, the observed diel increases in MeHg(I) may have been due to biological activity.

We do not consider the results presented in Figure 1 as conclusive. The variation between night- and daytime MeHg-(I) concentrations is close to the limits of sensitivity for the current generation of analytical equipment. However, the results do bring into the question the common idea that MeHg(I) is rapidly degraded in aerobic freshwaters. In our opinion, Figure 1 shows either that photodegradation of MeHg(I) does not occur or that there are mixing-independent, water column processes that regenerate MeHg(I) lost due to photodegradation. We tested for biological mercury methy-lation in the water column during the diurnal cycle by

assaying for mercury methyltransferase activity (*45*) in samples from Big Dam West and Puzzle but found no detectable activity ($n = 132$). It is possible that biological mercury methylation occurred in the water column that did not use methyltetrahydrofolate as a methyl donor, but this is the only reported biological methyl donor for mercury methylation (*46*, *47*). This evidence suggested that there was an extracellular abiotic process occurring in the water column that generates MeHg. However, the diurnal cycle data were not conclusive so further, more controlled experiments were performed in which we filter-sterilized water.

DOM is known to be a key participant in many methylation reactions of metals (*48*, *49*). We investigated the abiotic role of DOM by using the filtrate of a 1 kDa tangential ultrafiltrator to dilute lake water passed through a 0.22 μM filter and thereby created a concentration gradient of DOM. Various DOM concentrations were placed in 1-L Teflon bottles and these bottles were incubated on the lake surface for 7.5 h, with controls placed in black Teflon bottles. In response to solar radiation, levels of MeHg(I) increased ($P < 0.01$) with increasing DOM concentrations. MeHg(I) concentrations were higher ($P < 0.05$) in bottles containing 4.4 or 7.1 mg L^{-1} DOM exposed to solar radiation compared to dark controls (Figure 3). Treatments containing below 1.8 mg L^{-1} were not elevated compared to controls, and there was no DOM dependence in MeHg(I) concentrations of the dark controls ($P < 0.35$). We have observed that approximately 33% of the DOM present in water will pass a 1 kDa filter under these conditions (J. Hill, personal communication). Thus, the dilutions presented here are in fact dilutions of a high DOM sample with a sample containing substantially less DOM that will undoubtedly be of a slightly different structure. Hence, we hypothesized that during the ultrafiltration process a chemical species was generated, perhaps related to the small DOM size that catalyzed mercury methylation. However, use of elevated MeHg(I) concentrations similar to that used by others to investigate photodegradation (*16*, *19*) resulted in photodegradation rates similar to that previously reported (*43* and data not shown). Thus, the observed production of MeHg(I) was related to the trace levels investigated and not the ultrafiltration technique.

TABLE 1. Efficiency of Photomethylation and Photodegradation of MeHg and Inorganic Hg in Lake DF9 by Solar Radiation

fraction of water	correlation coeff[a] (r)	photodegradation (K_1) $(kW\ m^2)^{-1}$	photomethylation (K_2) [pg L^{-1} $(kW\ m^2)^{-1}$]
<5 kDa	−0.81 ($P < 0.02$)	−0.090 (−0.152 to −0.029)[b]	4.55 (3.31−5.79)
<300 kDa	−0.80 ($P < 0.02$)	−0.382 (−0.110 to −0.703)	20.1 (14.4−25.7)

[a] Correlation coefficient of the bivariate scattergram and the significance of the derived principal component relationship. [b] 95% confidence interval of the estimate.

At this point, we investigated the nature of the photomethylation processes with the aim of determining why photomethylation is observed only at ambient MeHg(I) concentrations. We fractionated water from lake DF9 into three fractions, <5 kDa, 5−30 kDa, and 30−300 kDa, and incubated in solar radiation for 180 min. Under these conditions, MeHg(I) concentrations displayed a biphasic pattern with an initial decrease in MeHg(I) concentrations in the first 90 min followed by an increase over the subsequent 90 min (43). This increase was especially strong for the fraction of water containing DOM less than 300 kDa, in which MeHg-(I) was initially degraded to approximately 40 pg L^{-1} followed by an increase ($P < 0.03$) at a rate of 2.5 pg L^{-1} $(kW\ m^{-2})^{-1}$ to 72 pg L^{-1}(43). We postulate that fluctuations in MeHg(I) concentrations result from the net difference of photodegradation and photomethylation. Previous data (16, 19, 43) suggests that photodegradation is a first-order reaction and hence

$$\text{photodegradation } (kW\ m^{-2})^{-1} = [MeHg]K_1 \quad (2)$$

In contrast, photomethylation involves the reaction of Hg(II), DOM, and solar radiation. However, fluctuations at ambient MeHg(I) concentrations are 30−40 pg L^{-1} compared to Hg(II) concentrations of 700−1000 ng L^{-1} and DOM concentrations of milligrams per liter. Thus, the photoproduction of MeHg(I) can be represented as a substrate independent reaction governed by a constant termed K_2. Hence, net changes in MeHg(I) can be expressed as

$$(MeHg_{initial} - MeHg_{final})\ (kW\ m^{-2})^{-1} = MeHg_{initial}\ K_1 + K_2 \quad (3)$$

where $MeHg_{initial}$ is the MeHg concentration at sampling time n and $MeHg_{final}$ is the MeHg concentration at sampling time $n + 1$. K_1 is the degradation efficiency of MeHg in an irradiation-dependent manner, and K_2 is the creation of MeHg in an irradiation-dependent manner. Plotting $(MeHg_{initial} - MeHg_{final})\ (kW\ m^{-2})^{-1}$ versus $MeHg_{initial}$ results in abiotic photodegradation (K_1) and photomethylation (K_2) production efficiency values (Table 1). These values were obtained by use of a bivariate scattergram because the uncertainties in the X values ($MeHg_{initial}$) were also reflected in uncertainties in the Y values ($MeHg_{initial} - MeHg_{final}$) (Figure 4). The fraction of water between 5 and 30 kDa did not have a significant correlation between changes in MeHg(I) and initial MeHg(I) ($r = -0.52$, $t = -0.93$, $n = 14$), so it was not analyzed further. The fraction of DF9 lake water between 30 and 300 kDa was primarily responsible for photomethylation with production efficiencies significantly ($p < 0.05$) greater than that observed for the fraction of water <5 kDa. In contrast, photodegradation efficiencies did not display a difference between water fractions. These results suggest that the type, that is, fulvic versus humic, of DOM may be important in photomethylation reactions.
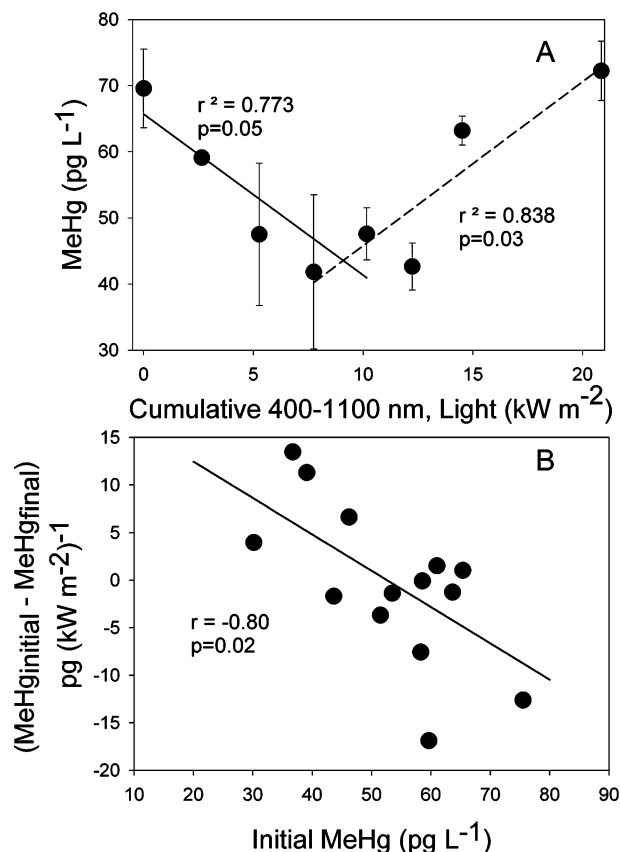

FIGURE 4. Calculations of photodegradation and photomethylation rates. (A) Biphasic MeHg dynamics in 300 kDa fraction of freshwater. Error bars indicate the variability between duplications. (B) Data transformation to the form $MeHg_{initial} - MeHg_{final} = MeHg_{initial}K_1 + K_2$. The indicated line is estimated by principal axis analysis used to derive the nature of the relationship between the two variables.
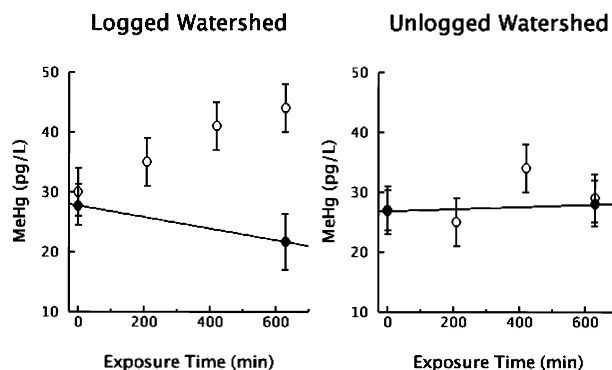

FIGURE 5. MeHg dynamics in sterilized water from logged ($n = 2$) or unlogged ($n = 2$) watersheds exposed to solar radiation (○) or dark controls (●). Each symbol is the estimate of 12 replicates, 6 replicates/lake, and error bars represent the standard deviation of the estimate.

We evaluated the effect of water chemistry and DOM type on photoproduction of MeHg(I) using water obtained from lakes with either logged (lakes DF9 and K3) or unlogged (lakes K2 and N70) watersheds. There were significant ($P < 0.03$) differences in MeHg(I) photoaccumulation between bottles containing water from the different watersheds. Bottles containing water from logged watersheds accumulated MeHg(I) with exposure to solar radiation, in sharp contrast to bottles containing water from unlogged watersheds (Figure 5). The significance of this photomethylation process to the enhanced mercury bioaccumulation in logged watersheds requires further investigation before we can safely reach the

conclusion that photomethylation processes are, at least partially, responsible for the increase in MeHg observed in logged watersheds.

Our results provide an explanation for the results obtained in the Florida Everglades where MeHg(I) peaked at noon and microbial inhibitors do not prevent MeHg(I) formation (*21, 22*). It appears that there is a solar radiation-dependent formation of MeHg(I) in the water column of certain lakes. This process does not appear to be directly mediated by microorganisms. However, it is possible that an extracellular substance secreted by microorganisms may play an important role in the methylation of mercury in the water column (*28*). In addition, our results also provide an explanation for the differential role of sediments observed in some studies. We speculate that the overlying water column was either producing MeHg(I), in cases where sediments were assumed to act as a source, or was not, in cases where the sediments acted as a sink. For example, two lakes investigated in this study did not display substantial MeHg(I) photoproduction, lakes K2 and N70, whereas two other lakes did, lakes K3 and DF9. The reason for this differential behavior of MeHg(I) is not yet known.

Here we have presented evidence that DOM is an important modulator of MeHg(I) formation in the water column. However, this does not exclude the role of other water-borne substances. The method used to fractionate the water excluded all particles larger than 0.22 μM but many other compounds such as algae secretions, chloride ions, and electron mediators such as benzoquinones may also be important in the process. These compounds have been found to be important in the solar-mediated formation of Hg(0) in waters (*50*). Hence, it is possible that many of these compounds may also play a role in MeHg(I) formation.

Our results demonstrate that MeHg(I) is formed in the water column of certain lakes in an abiotic fashion. The exact mechanism of this reaction is not yet clear but DOM appears to be an important player in this reaction. A comparison of waters obtained from logged and unlogged watersheds suggest that photoproduction of MeHg(I) may be tied to some specific water chemistry, and thus, photoproduction of MeHg(I) is not necessarily important in all lakes. The overall importance of this to MeHg(I) balances in freshwater lakes is not yet known, but clearly, conventional wisdom regarding the photodynamics of MeHg(I) in freshwaters need to be reevaluated.

Acknowledgments

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