Bull. Environ. Contam. Toxicol. (1991) 47:743–750

Bulletin of Environmental Contamination and Toxicology

Effect of Natural Levels of Dissolved Organic Carbon (DOC) on Methyl Mercury Formation and Sediment–Water Partitioning

B. M. Miskimmin[1,2]

[1]Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, R3T 2N2, Canada and [2]Current address: Department of Zoology, University of Alberta, Edmonton, Alberta, T6G 2E9, Canada

Elevated methyl mercury concentrations in fish tissue (>0.5 ppm) have been measured in remote high-DOC (brown water) drainage lakes (e.g., Mannio et al. 1986; Minnesota Pollution Control Agency 1985) and in certain low-DOC seepage lakes (Grieb et al. 1990). The role of DOC in methyl mercury production, and movement among particulate/aqueous phases and organic tissue in natural ecosystems is poorly understood. Methyl mercury that is bound to terrestrial DOC could be transported into lakes, which may be of particular importance in lakes that receive high volumes of terrestrial runoff (Lee and Hultberg 1990). In contrast, the main source of methyl mercury in seepage lakes is unlikely to be terrestrial, but may be partly explained by elevated methyl mercury production in low-DOC lake water (Miskimmin 1989).

DOC (as humic substances) has been demonstrated to enhance the solubility of inorganic mercury (Miller 1975), but similar experiments with methyl mercury have been lacking despite the fact that essentially all Hg found in freshwater fish tissue is methyl mercury (Grieb et al 1990). Few published studies report the partitioning of methyl mercury between particulate and dissolved phases in aquatic systems (sand, silt and woodchips, Akagi et al. 1979; pH effect, Miller and Akagi 1979). Methyl mercury in the aqueous phase is potentially available for bioaccumulation directly from water; thus, any characteristics that enhance both the production and solubility of methyl mercury should be examined to further understand aquatic mercury problems.

The purpose of this study was to test the hypothesis that DOC enhances the solubility of methyl mercury. I examined the effect of natural levels of DOC on the partitioning (distribution) of methyl mercury between lake sediments and water using both [^{203}Hg] methyl

mercury formed directly in sediments, and [^{14}C] methyl mercury added to sediment-water mixtures in low levels.

MATERIALS AND METHODS

Sediments for methylation and partitioning experiments were sampled with an Ekman dredge from 4-m depth in East Bay of Lake 239 (93°43′W, 49°39′N), an oligotrophic Precambrian Shield lake at the Experimental Lakes Area (ELA), northwestern Ontario, Canada. The surface 2-3 cm of sediments were immediately transferred to glass bottles that were sealed and refrigerated for up to 2 mo until used in all the experiments.

Concentrations of DOC for the experiments were manipulated by diluting a natural-source DOC concentrate with distilled water. DOC was concentrated by rotary-evaporating water (at 60°C) collected from a stream draining a small bog into Lake 239 at ELA, northwestern Ontario. The concentrate was then passed through a cation exchange column to replace with H^+ any cations remaining after the rotary-evaporation process. The range of experimental DOC concentrations was from 550 to 5200 $\mu mol \cdot L^{-1}$.

Dissolved organic carbon concentrations were measured using a high-temperature acid persulfate digestion followed by infrared detection of CO_2 on a Model 700 Carbon Analyzer (OI Corp., Houston, Texas). Sample water was prepared for measurement by filtration through a pre-combusted glass fiber filter (Whatman GF/C, Fisher).

Two experiments were performed to examine the effect of DOC on the partitioning of methyl mercury between lake sediments and water. The procedure that differentiated the two experiments was that #1 measured methyl mercury actually produced in the samples during a 24-hr period, and #2 involved adding a known amount of methyl mercury directly to the samples.

For the first experiment, specific rates of mercury methylation were measured using the radiochemical method of Furutani and Rudd (1980). The term "specific rate" of methylation refers to percent of radio-labelled $HgCl_2$ added that was methylated over the time of the experiment. The $^{203}HgCl_2$ was added to sediment/water samples and any $^{203}Hg^+$ methylated over a 24-hr period was extracted. Methylation measurements were done using Hg^{+2} that was elevated over <u>in situ</u> concentrations because concentrations of Hg that are biologically available are unknowable. Measurements are made comparable by using the same artificial concentrations in all incubations. The amount of

inorganic mercury added is kept constant; thus, the effect of other variables on the specific rates of methylation can be compared.

The first experiment was designed to determine the effect of DOC in sediment pore water and overlying water on inorganic mercury methylation, and on the subsequent partitioning of methylated mercury between sediments and water. The pH of all DOC dilutions was adjusted in bulk to 6.2 ± 0.2 with dilute HCl or NaOH. Specific rates of mercury methylation were determined by adding 1.0 μg of Hg^{+2} [0.037 MBq as $^{203}HgCl_2$, New England Nuclear Corp., Billerica, Maine] to 125-mL glass bottles containing 15 mL of sediment mixed with 70 mL of water at one of three DOC concentrations (550, 2800, 3640 μM). The bottles were tightly capped and vigorously shaken by hand to distribute the Hg^{+2}. Duplicate samples plus one acidified control (using 2 mL 4N HCl to kill methylating bacteria) were incubated for 24 hr at 22°C ± 2°C. After incubation, the samples were shaken for 15 sec to disperse $CH_3{}^{203}Hg^+$ produced in the sediments, then each bottle was centrifuged at 1800 g (3200 rpm) for 15 min. Measured volumes of supernatant were transferred to 125-mL separatory funnels through a 54 μm mesh in a small glass funnel. Methyl mercury could then be extracted from the water and sediments independently. The supernatant in the separatory funnels (aqueous phase) and sediments in the bottles were acidified with 2.0 mL of 4N HCl. Sediments were rewetted with 50 mL distilled water to replace the overlying water and pore water (=supernatant) before any further reagents were introduced.

Methyl mercury produced during the 24-hr incubation period was quantified. The remaining extraction steps are described in Furutani and Rudd (1980). Briefly, the method is a step-wise extraction of the methylated Hg into toluene, then 2.5 mM sodium thiosulfate in 20% ethanol, and finally, 1:1 3M potassium iodide:benzene. An aliquot of the benzene phase is added to scintillation cocktail for scintillation counting.

Results of the assay were reported as the percentage of the isotope added that was methylated and was found in either sediment or overlying water. Significant differences ($P \leq 0.05$) among treatments were evaluated by analysis of variance (ANOVA).

The second experiment was designed to determine the role of DOC in the partitioning (distribution) of methyl mercury between lake sediments and water using a modification of the suspension technique of Nyffeler et al. (1984). A gradient of five DOC concentrations was

obtained by dilution of the DOC concentrate previously described. The pH of each dilution was adjusted to 6.2 (±0.2) before sediment addition. The range of DOC concentrations was 700 to 5200 μM; the lowest DOC concentration was distilled water, enhanced to 700 μM by the addition of sediment. Ten mL subsamples of each dilution in duplicate 125-mL flasks were amended with 0.002 μg methyl mercury (9.2×10^{-6} μmoles Hg; as $^{14}CH_3HgI$, Amersham Laboratories, Buckinghamshire, England). The solution was then equilibrated by rotating at 160 rpm in a mechanical shaker for 1.5 hr. From a suspension of sediment particles, 500 μL of water containing 15 mg of sediments was added to the $^{14}CH_3HgI$-amended water samples. The sediment-water mixture was returned to the shaker that functioned to keep the sediment in suspension.

At four time intervals ranging from 2 hr to 7 d, the duplicate flasks of each DOC concentration were removed and the contents filtered through two stacked Nucleopore filters of 0.4 μm pore size and 25 mm diameter. Filters were dissolved in glass scintillation vials with 1.0 mL ethyl acetate and counted on a LKB-Wallac RackBeta scintillation counter after the addition of 14 mL scintillation cocktail (ACS, Amersham). Filtered water was subsampled, added to scintillation cocktail and counted. The first set of samples revealed that activity on the second of the two filters was undetectable, therefore, for subsequent samples only one filter was used for each filtration. The distribution ratio, K_d, was calculated using Equation 1:

$$K_d = C_s / C_w, \qquad (1)$$

where C_s is the concentration of $^{14}CH_3Hg^+$ in sediments (dry weight), and C_w is the concentration of $^{14}CH_3Hg^+$ in water, both in units of $ng \cdot kg^{-1}$.

Results of the partitioning experiment are reported as a linear regression of K_d and DOC concentration, and K_d at each DOC concentration over time. DOC concentrations were measured as previously described, on a set of samples from each experiment that were not amended with a radioisotope, to avoid contamination of equipment. The samples were otherwise identical to the experimental treatments.

RESULTS AND DISCUSSION

For the short time scale of the methylation experiments (24 hr), concentrations of $CH_3{}^{203}Hg^+$ in water overlying sediments increased with increasing DOC concentration (Figure 1). Ten times more methyl mercury was extracted from each of the 15 mL of sediments (0.17% ± 0.02% methylated g^{-1} d.w.$\cdot d^{-1}$) as in the 70 mL of

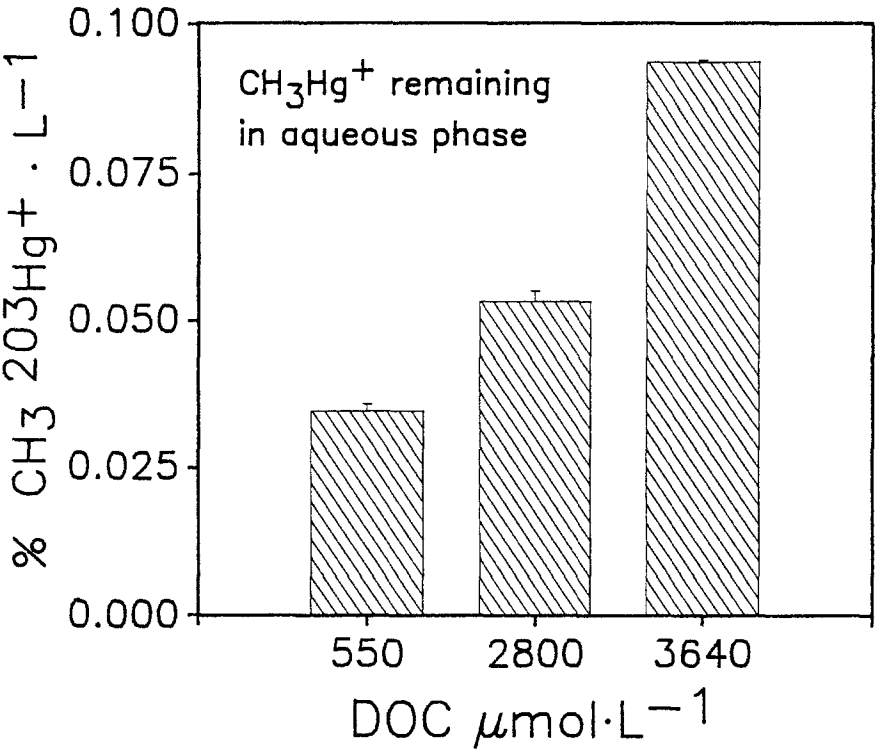

Figure 1. Percent [^{203}Hg] methyl mercury found in water overlying sediments after incubation for 24 hr at 22°C. Values are the percent of $^{203}HgCl_2$ added to sediment-water mixture that was methylated and extracted from the water. Error bars are std. dev.

overlying water. Thus, DOC had no effect on methyl mercury produced in sediments, but clearly affected the apparent water solubility of methyl mercury produced in those disturbed sediments.

Similarly, the partitioning of $^{14}CH_3Hg^+$ added to sediment-water mixtures showed a decrease in K_d (increase in solubility) with increasing DOC concentration at days 4 and 7 (Figure 2). If this effect occurs <u>in situ</u>, DOC-solubilization may occur in situations both where methyl Hg production is important and where methyl Hg is introduced directly to lake water and sediments from outside sources.

Approximately 80% of the [^{14}C]methyl mercury added in the partitioning experiment was lost either due to demethylation or volatilization. This occurred more slowly with increasing DOC concentration (data not shown). These losses occurred within the first 24 hr and were likely due to the sample pH (which increased

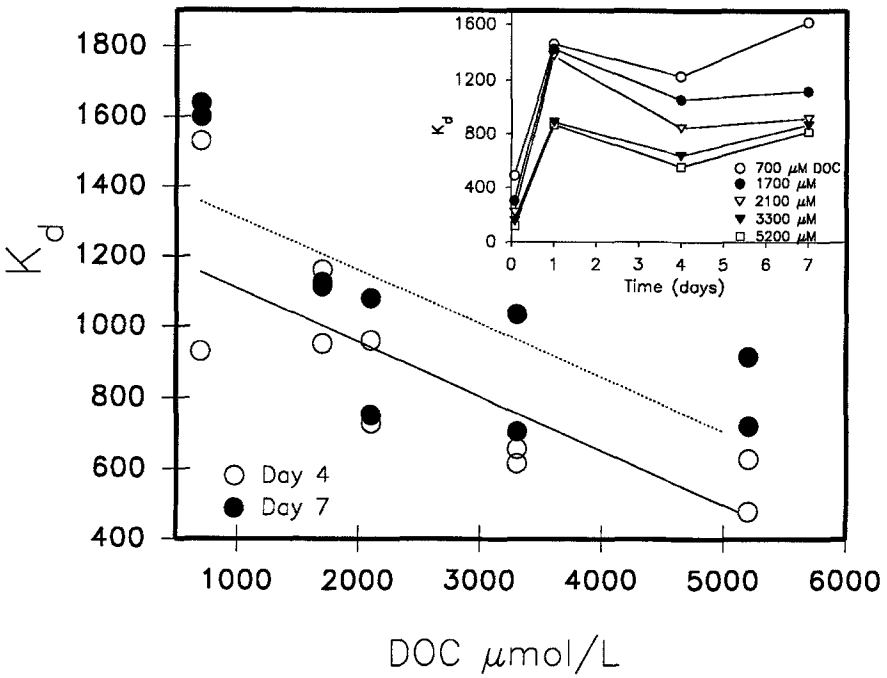

Figure 2. Partition coefficients (K_d) for $^{14}CH_3Hg^+$ at days 4 and 7 in sediment-water solutions with increasing DOC concentration. The lines are a linear regression of days 4 (r=0.80 P<0.01, solid line) and 7 (r=0.74 P<0.05, dotted line). Inset depicts K_d over 7 days at all five [DOC].

from 6.2 to 6.5 ± 0.2), small sample volumes and exposure to air caused by the mechanical rotation.

K_d values for $^{14}CH_3Hg^+$ were low (Figure 2) in comparison to other organic contaminants (DDT, PCB, lindane; Chiou et al. 1986) and metals (^{59}Fe, ^{65}Zn, ^{60}Co, ^{75}Se; Hesslein 1987). While it is acknowledged that the organic composition and other characteristics of the sediments used in partitioning experiments can make large differences in K_d values, these values are within the range found for methyl mercury by Akagi et al. (1979) using a variety of substrates (K_d of 170 for sand, 760 for silt/woodchips and 4200 for woodchips).

While these experiments clearly demonstrate that methyl mercury may be bound by DOC, the ultimate fate of this ycontaminant cannot be inferred. Whether the DOC-bound methyl mercury is available for bioaccumulation by aquatic organisms, or whether uptake from water is reduced in the presence of DOC, is not known. A

negative correlation between methyl mercury in fish and DOC concentrations in seepage lakes in northern Michigan (Grieb et al. 1990) may suggest that methylation rates were lower when DOC concentrations were high (Miskimmin 1989), and/or that DOC-bound methyl mercury was less available for bioaccumulation at high DOC concentrations.

The main source of methyl mercury in drainage lakes may be quite different from that of seepage lakes, even though the in-lake DOC influences may be similar. Low-DOC lakes usually have long water residence times whereas high-DOC lakes are relatively fast-flushing (Schindler 1971). These characteristics are important because it has been demonstrated that terrestrial inputs of mercury may be most important in drainage lakes with short water residence times (Lee and Hultberg 1990), but in slower flushing lakes, in-lake methyl mercury production likely predominates (Winfrey and Rudd 1990). The present study disallows drawing conclusions about terrestrial systems, but does indicate the potential for DOC-solubilization of methyl mercury and is consistent with Lee and Hultberg's (1990) proposition that fast-flushing (high DOC) lakes may have mercury problems because of a terrestrial source. The watershed may be very important in introducing methyl mercury to fast-flushing, high-DOC lakes, and once in the water column, DOC-bound methyl mercury may be resistant to entering lake sediments.

Acknowledgments. I thank P.J. Curtis and J.W.M. Rudd for reviewing the manuscript, J.W.M. Rudd, C.A. Kelly and R. Hesslein for advice on the project, and S.G. Lawrence and M. Holoka for providing the DOC concentrate. This research was supported by by the Department of Fisheries and Oceans, Canada.

REFERENCES

Akagi H, Mortimer DC, Miller DR (1979) Mercury methylation and partition in aquatic systems. Bull Environ Contam Toxicol 23:372-376

Chiou CT, Malcolm RL, Briton TI, Kile DE (1986) Water solubility enhancement of some organic pollutants and pesticides by dissolved humic and fulvic acids. Environ Sci Technol 20:502-508

Furutani A, Rudd JWM (1980) Measurement of mercury methylation in lake water and sediment samples. Appl Environ Microbiol 40(4):770-776

Grieb TM, Driscoll CT, Gloss SP, Schofield CL, Bowie GL, Porcella DB (1990) Factors affecting mercury accumulation in fish in the upper Michigan peninsula. Environ Toxicol Chem 9:919-930

Hesslein RH (1987) Whole-lake metal radiotracer movement

in fertilized lake basins. Can J Fish Aquat Sci 44(Supp 1):74-82.
Lee Y-H, Hultberg H (1990) Methylmercury in some Swedish surface waters. Environ Toxicol Chem 9:833-841
Mannio J, Verta M, Kortelainen P, Rekolainen S (1986) The effect of water quality on the mercury concentration of northern pike (Esox lucius) in Finnish forest lakes and reservoirs. Publications of the Water Research Institute, National Board of Waters, Finland, No 65: 32-43
Miller DR, Akagi H (1979) pH affects mercury distribution, not methylation. Ecotox Environ Safety 3:36-38
Miller DR (1975) The role of humic acids in the uptake and release of mercury by freshwater sediments. Verh Internat Verein Limnol 19:2082-2086
Minnesota Pollution Control Agency (1985) Mercury (Hg) in northeastern Minnesota fish. Division of Water Quality, Monitoring and Analysis Section, p 81
Miskimmin BM (1989) The influence of dissolved organic carbon on methyl mercury production and sediment-water partitioning in Precambrian Shield lakes. MSc Thesis University of Manitoba, Winnipeg, p 104
Nyffeler UP, Li Y-H, Santschi PH (1984) A kinetic approach to describe trace-element distribution between particles and solution in natural aquatic systems. Geochim Cosmochim Acta 48:1513-1522
Schindler DW (1971) A hypothesis to explain differences and similarities among lakes in the Experimental Lakes Area, northwestern Ontario. J Fish Res Board Can 28:295-301
Winfrey MR, Rudd JWM (1990) Environmental factors affecting the formation of methylmercury in low pH lakes: a review. Environ Toxicol Chem 9:853-869

Received February 18, 1991; accepted June 24, 1991.