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SPECIATION OF AQUEOUS METHYLMERCURY INFLUENCES UPTAKE BY A FRESHWATER ALGA (*SELENASTRUM CAPRICORNUTUM*)

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Abstract—Uptake of methylmercury (MeHg) by the alga *Selenastrum capricornutum* was measured in freshwater batch culture bioassays. The concentration of MeHg in the alga increased rapidly (within 15 min), reached a maximum by 6 h, and then declined because of growth dilution. The alga's rapid growth rate (doubling time, ~10 h) contributed to the importance of growth dilution. Conditional first-order rate constants were calculated for uptake ($k_1 = 6.95 \times 10^{-9}$ L/cell/h) and growth ($k_G = 0.07$/h). A competitive synthetic ligand, disodium ethylenediaminetetra-acetate, formed strong complexes with MeHg and reduced MeHg uptake, consistent with the biotic ligand model. A conditional equilibrium formation constant (K) for the MeHg–algae complex was estimated to be approximately 10^{16} and was used to model the influence of natural ligands on MeHg bioavailability. Model results suggested MeHg would be most bioavailable at concentrations of dissolved organic matter (DOM) less than 10 mg/L and increasingly unavailable at higher DOM concentrations for the specific humic acid modeled. Similarly, at molar concentrations of sulfide (and, possibly, metal–sulfide clusters) equal to approximately half the MeHg concentration, MeHg was predicted to be unavailable to algae because of the formation of strong 2:1 MeHg–sulfide complexes.

Keywords—Mercury Algae Bioaccumulation Bioavailability Stability constants

INTRODUCTION

Bioaccumulation of methylmercury (MeHg) in aquatic organisms is dependent on two critical steps. First, Hg delivered to watersheds, mainly as inorganic Hg(II), must be converted to MeHg through the activity of microorganisms [1]. Second, for subsequent bioaccumulation to occur, MeHg must be incorporated into aquatic food chains. The uptake of MeHg by algae, as demonstrated by both field and laboratory measurements [2–4], is expected to be important in subsequent transfer to higher trophic levels [5].

The bioavailability of MeHg to algae in aquatic systems may be influenced by the binding of MeHg to various aqueous ligands, colloids, or particles (i.e., the speciation of MeHg). As described in the biotic ligand model (BLM), metal uptake by an aquatic organism involves two steps: Reaction of the metal ion with reactive or sensitive sites on the biological membrane, and transport into the organism, often termed ''internalization'' [6,7]. Binding to the biological membrane is expected to be regulated by competition between aqueous ligands and reactive sites on the biological membrane, the biotic ligand. Subsequent internalization is dependent on the amount of metal bound to biotic ligand sites. The relative strengths and concentrations of the competing ligands should determine the outcome. Exceptions to the BLM include cases in which uptake involves neutral ''lipophilic'' metal species that are transported through the biological membrane [8,9].

The BLM initially was applied mainly to prediction of the bioavailability or toxicity of metals to fish [6,10,11]. In addition, the BLM has been used in modeling the uptake and toxicity of metals to other aquatic organisms, including phytoplankton [7,8,12,13]. Because natural ligands, such as components of dissolved organic carbon (DOC), form strong complexes with MeHg [14–16], it is important to know whether the BLM is applicable to MeHg.

The goal of this investigation was to evaluate the influence of MeHg speciation on the bioavailability of MeHg to algae. Uptake by algae in the laboratory cultures was measured as the amount of MeHg transferred from the aqueous medium to algal cells, and we did not distinguish between binding to the cell surface and internalization. We first investigated the importance of the kinetics of uptake and growth dilution in controlling MeHg concentrations in algae in our experimental system. Then, a conditional equilibrium stability constant for binding of MeHg to algae was estimated using a synthetic ligand, disodium ethylenediaminetetra-acetate (EDTA), as a competitive ligand and as a surrogate for a natural ligand. Finally, the conditional stability constant was used to model the expected binding of MeHg to algae in the presence of natural ligands.

MATERIALS AND METHODS

Batch algal cultures of *Selenastrum capricornutum* (UTEX 1648; University of Texas at Austin Culture Collection, Austin, TX, USA) were used to assess MeHg bioavailability to freshwater algae. *Selenastrum capricornutum* is a relatively small (length, ~10 μm), crescent-shaped, unicellular green alga. After attempting experiments with various algae (other greens, cryptophytes, and diatoms), *S. capricornutum* was chosen because of its ease of growth in the laboratory, previous use in other studies [2,3,8,17], presence in natural samples, edibility by zooplankton, and use as a U.S. Environmental Protection Agency test organism [18]. Algal cultures were grown axe-

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nically in 500-ml, acid-leached, polycarbonate Erlenmeyer flasks (Corning, Acton, MA, USA), which were covered with two-position screw caps. During culturing, screw caps were kept on flasks in the loose position to provide air exchange for growth. The flasks were placed within specially fabricated, all-acrylic, clear growth chambers, which were illuminated by continuous cool white fluorescent lighting (155 $\mu E/m^2/s$), kept under constant high-efficiency particulate air filtration and within an environmental chamber held at 20 ± 1°C (temperature in flasks, 21 ± 1°C). Cultures were periodically swirled by hand at least twice a day.

Both container type (polycarbonate flasks or Teflon® bottles) and preconditioning methodologies were tested regarding their suitability for bioassay experiments to ensure that all added MeHg could be recovered at the end of the bioassay [19,20]. Flasks or containers typically are preconditioned at experimental metal concentrations before the experiment. We found both preconditioning polycarbonate flasks and using Teflon containers were unnecessary, but proper polycarbonate flask cleaning was important: Immersion for 2 to 3 d in 20% reagent-grade HNO_3 at room temperature, filling with 10% trace metal–grade (TMG) HCl, and holding in a 40°C oven overnight (clean flasks stored completely filled with 1% TMG HCl). Although our bioassay was run under continuous illumination, no significant photodegradation or loss of MeHg occurred [19,20]. Evasion losses of Hg also were checked in a control experiment, in which the headspace of Hg-spiked flasks (both with and without algae) were purged continuously for 24 h with Hg–clean air and passed through gold traps. The gold traps were then analyzed for Hg and were less than the limit of detection.

The alga was maintained in Fraquil medium [21] with dissolved salts prepared according to the method described by Morel et al. [21] and nutrients according to the method described by Price et al. [22] and then pumped separately (~7 ml/min) through Teflon columns, each containing 3.0 g of clean Chelex® 100 resin (200–400 mesh; Bio-Rad Laboratories, Richmond, CA, USA) to remove any trace-metal contaminants.

Chelex resin was cleaned in several steps, analogous to the method described by Price et al. [22], to remove any strong ligands attached to the Chelex. First, Chelex was placed in a clean Teflon bottle with 2.5 M TMG HNO_3 and placed on a shaker for 30 min. Acid was filtered off using a 1.0-μm Teflon filter apparatus (diameter, 47 mm) held in a Teflon tower. While still in the tower, Chelex was rinsed twice with 100 ml of Milli-Q® deionized water (MQ; Millipore, Billerica, MA, USA). Rinsing was repeated using 1.0 M TMG HNO_3 followed by 2.0 M TMG NH_4OH. Chelex was then soaked for at least 1 h in a pH 6.5 buffer (4 g or 4 ml of TMG acetic acid, 5.3 g or 6 ml of TMG NH_4OH, and 90 ml of MQ). Chelex was then separated from the buffer by filtration, rinsed once with 50 ml of MQ, filtered dry, and stored in a clean Teflon jar.

After all, Fraquil components were combined, and the medium was sterilized by filtration through an acid-leached, 0.2-μm polypropylene capsule filter. Medium was assayed routinely for sterility using Luria-Bertani broth (Becton Dickinson, Sparks, MD, USA). The background Hg concentrations in the medium were less than the limit of detection for MeHg (0.02 ng/L) but were detectable for total Hg (~1.0 ng/L). Replicate control flasks without Hg additions (two medium-only flasks and two medium-with-algae flasks) were assayed with each experiment.

Before a MeHg uptake experiment was performed, *S. capricornutum* was grown for 7 d in Fraquil medium in which disodium EDTA was 1,000-fold lower (5 nM) than that used by Price et al. [22]. Algae were transferred (~5 ml) to Fraquil medium without EDTA, Cu, and Zn (deficient Fraquil) and grown for five more days to purge (i.e., growth dilute) any trace contaminants in the alga. Algae were then inoculated (4,000 cells/ml) into flasks containing deficient Fraquil for experiments. Deficient medium was used for experiments to avoid competition between MeHg, Cu, and Zn for binding sites on the algae and to avoid binding of MeHg to EDTA rather than algae. *Selenastrum capricornutum* growth achieved exponential growth in both Fraquil with a full complement of trace nutrients and in Fraquil without Cu, Zn, and EDTA for up to 5 d. No signs of toxic effects (i.e., reduced growth) were observed for up to 24 h between a control (no MeHg) and a MeHg-spiked (2 ng/L) treatment [19,20]. Test endpoints included the concentration of MeHg in the medium (ng/L) and algal cells (ag/cell; 1 ag = 10^{-18} g) over a set amount of time. At each time point, independent duplicate (for the MeHg-uptake experiment) or triplicate (for the EDTA experiment), flasks were completely filtered for each measurement. Not counting controls, 14 flasks were in the uptake experiment and 15 in the EDTA experiment.

Processing and analysis of samples

Flow cytometry. Algal densities (cells/ml) were counted using flow cytometry (EPICS XL; Beckman Coulter, Fullerton, CA, USA). Sample aliquots (15 ml) were removed from each flask and centrifuged for 15 min at 2,000 rpm. Ten milliliters of the supernatant were removed, and the remaining 5 ml were resuspended. A 250-μl aliquot of this resuspension was mixed with 50 μl of standardized flow cytometry beads (Bangs Laboratory, Fishers, IN, USA) and counted using the flow cytometer. Each experimental flask was counted in triplicate (three replicates from each centrifuged sample); triplicate count standard deviation was 10% or less.

Dissolved organic carbon. Samples were filtered through a filter tower (diameter, 25 mm) containing an ashed, glass-fiber filter (nominal pore size, ~0.7 μm; Whatman, Maidstone, UK) into ashed, 20-ml glass vials. Two samples were taken from each flask. Dissolved organic carbon was analyzed on a model TOC-V CSH total organic carbon analyzer (Shimadzu, Kyoto, Japan) with a AS1-V autosampler (Shimadzu). Four repeated measures were run on each sample, and the relative standard deviation was less than 2%.

Mercury concentration measurements. A weighed aliquot from each flask was poured through a preashed, quartz-fiber filter (diameter, 47 mm; nominal pore size, ~2.2 μm; Whatman, Maidstone, UK) held in a Teflon filter tower. The quartz-fiber filter contained the algal fraction, and the filtrate was the aqueous fraction of MeHg. We did not distinguish between intra- and extracellular MeHg. Although several rinsing techniques using EDTA were explored for recovery of MeHg bound to the algal cell surface, none was successful (high variability or less than the limit of detection). Filtrate was collected in an acid-cleaned Teflon bottle, acidified to 1% with TMG HCl, and refrigerated. The quartz-fiber filters were stored frozen in acid-cleaned Petri dishes.

Approximately 80 ml of acidified filtrate sample, or 80 ml of MQ with quartz-fiber filters, were distilled for MeHg determination using standard reagents and techniques that included blanks, duplicates, and spiked samples [23,24]. Spike recoveries were between 75 and 125%. Analysis was com-

Table 1. The acid–base and complexation reactions used to describe the speciation of methylmercury (MeHg) and disodium ethylenediaminetetra-acetate (Y)

Species formation reactions	Log *K*	Equation	Reference
$Y^{4-} + H^+ \rightarrow HY^{3-}$	10.27	1	[26]
$Y^{4-} + H^+ \rightarrow H_2Y^{2-}$	16.64	2	[26]
$Y^{4-} + MeHg^+ \rightarrow MeHgY^{3-}$	8.21	3	[26]
$Y^{4-} + 2MeHg^+ \rightarrow (MeHg)_2Y^{2-}$	11.56	4	[26]
$HY^{3-} + MeHg^+ \rightarrow MeHgHY^{2-}$	6.80	5	[36]
$Y^{4-} + H^+ + MeHg^+ \rightarrow MeHgHY^{2-}$	17.07	6 = 1 + 5	

pleted using established protocols for gas chromatography and cold-vapor atomic fluorescence spectroscopy detection [25] after ethylation and carbotrap collection. Detection limits were determined from method blanks, which were included in each distillation batch, and averaged 0.03 ng/L of MeHg.

Calculations and modeling

Complexation of MeHg by EDTA. Methylmercury species formed by complexation with EDTA and their stability constants were evaluated from the literature (Table 1). According to Hojo et al. [26], the binding of MeHg to EDTA occurs at the two amine (N) groups. The four carboxyl groups are not sites of complex formation with MeHg but, rather, are ionized at pH greater than 4. We used Y^{4-} to represent EDTA with no N groups protonated and HY^{3-} and H_2Y^{2-} as the two protonated species. Our modeling indicated the species $MeHgHY^{2-}$, $MeHgY^{3-}$, and $(MeHg)_2Y^{2-}$ to be important at our experimental pH (7.3), consistent with the report by Alderighi et al. [27] that the three corresponding complexes are formed between MeHg and 1,2-diaminoethane.

Equilibrium speciation of MeHg and EDTA in Fraquil was modeled using MINEQL+ (Ver 4.5; Environmental Research Software, Hallowell, ME, USA) and component concentrations according to the methods described by Morel et al. [21] and Price et al. [22]. For most runs, the modeled system did not contain Cu and Zn, which is consistent with our experimental conditions. Methylmercury species were added to the thermodynamic database using published stability constants (SETAC Supplemental Data Archive, Item ETC-25-02-02; http://etc.allenpress.com), whereas EDTA speciation was modeled as above. Relevant water-chemistry parameters measured during the experiment were set within MINEQL+ (pH 7.34; 20°C; log partial pressure CO_2, −3.5 atm).

Binding of MeHg to algae. A competitive ligand approach was used to estimate a conditional stability constant for MeHg binding to algae, similar to the methods described by Janes and Playle [11] and Playle et al. [28], with EDTA as the competitive ligand. The MeHg–algae complex was defined in MINEQL+ as a 1:1 complex of MeHg to each algal binding site, with concentrations expressed as mol/L.

RESULTS AND DISCUSSION

MeHg uptake kinetics

Exposure of *Selenastrum* cells to MeHg in the Fraquil medium caused the filterable MeHg concentration in the medium to gradually decrease from an initial concentration of 2 to 0.14 ng/L after 48 h, whereas the concentration of MeHg per cell increased rapidly during the first 6 h and then gradually decreased with time (Fig. 1A). The total MeHg concentration (sum of MeHg in algae plus MeHg in medium) in the flasks remained near 2.0 ng/L throughout the experiment, indicating that losses of MeHg from the experimental system (adsorption, volatilization, and degradation) were small. The overall concentration of MeHg in the cells continued to decrease over 48 h as cell growth continued, whereas MeHg concentration in solution decreased because of adsorption/uptake by the algal cells.

The change in MeHg concentration in algal cells over time reflects the difference between rates of uptake and elimination

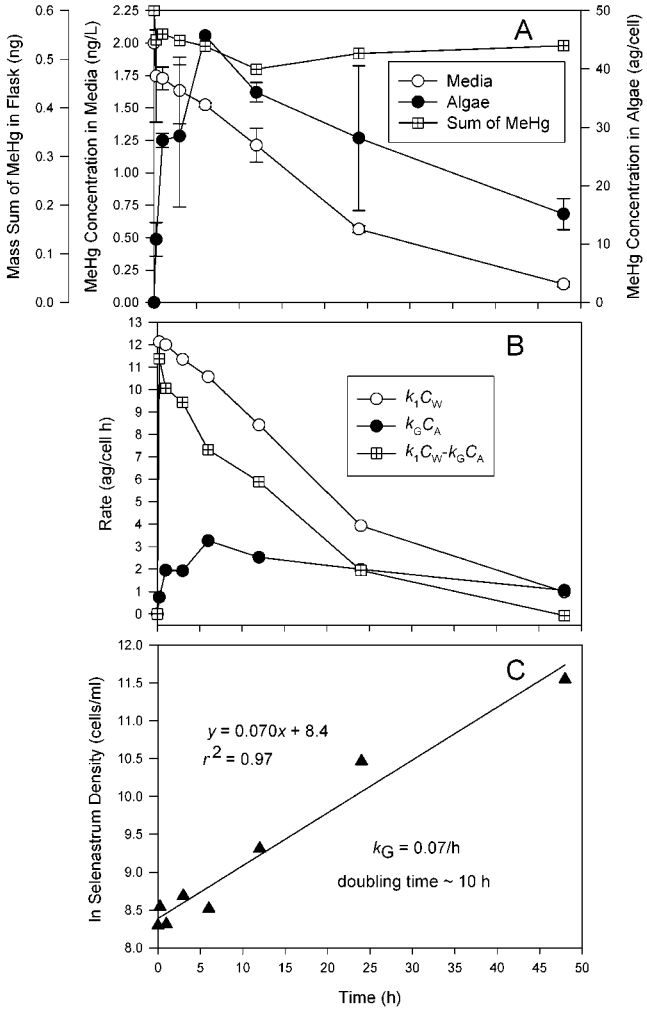

Fig. 1. (**A**) Uptake of methylmercury (MeHg; 2.0 ng/L) by *Selenastrum capricornutum* over 48 h (1 ag = 10^{-18} g). Each point represents the average concentration in two flasks sacrificed at the given time. Error bars represent one standard deviation. (**B**) Influences of gross uptake rate (k_1C_W) and growth dilution rate (k_GC_A) on the net uptake rate of MeHg by *S. capricornutum*. The net uptake rate (difference) assumes that the elimination rate (k_2C_A) is negligible. (**C**) Changes in algal density over 48 h and fit of the experimental data (triangles) to first-order growth model (line).

of MeHg and dilution by growth [29]. For algae, growth mainly involves an increase in cell abundance with time. A basic first-order model for net uptake is

$$\frac{dC_A}{dt} = k_1 C_W - k_2 C_A - k_G C_A \quad (1)$$

where C_W and C_A are the concentrations of MeHg in water and algal cells, respectively, and k_1, k_2, and k_G are first-order rate constants for uptake, elimination, and growth, respectively. Here, all filterable MeHg in the medium is assumed to be available for uptake. In our system, both C_W and the abundance of algal cells (A) change with time, so the accumulation of MeHg in algal cells actually depends on both C_W and A. Therefore, when loss terms (elimination and growth) are very small, the uptake rate can be expressed as

$$\frac{dC'_A\ (\text{ng L}^{-1})}{dt\ (\text{h})} = k_1\ (\text{L cell}^{-1}\cdot\text{h})\cdot C_W\ (\text{ng L}^{-1})\cdot A\ (\text{cell L}^{-1}) \quad (2)$$

illustrating that the uptake of MeHg into algal cells on a per volume of medium basis (C'_A) is dependent on both C_W and A. However, on a per cell basis (C_A), the uptake rate depends only on C_W. A pseudo–first order rate constant (k'_1) for the uptake of MeHg was calculated from experimental data using Equation 3. This rate constant varies with algal cell abundance (A), and Equation 4 can be used to obtain the corresponding first-order rate constant, k_1:

$$\frac{dC'_A\ (\text{ng L}^{-1})}{dt\ (\text{h})} = k'_1\ (\text{h}^{-1})\cdot C_W\ (\text{ng L}^{-1}) \quad (3)$$

$$k_{1,t} = \frac{k'_{1,t}}{A_t} \quad (4)$$

We estimated k'_1 by applying Equation 3 to data at the early stages of uptake (i.e., 0–6 h), where C_A is small. Because $dC'_A \cong -dC_W$, we regressed ln $C_{W,t}$ versus time:

$$\ln(C_W) = -k'_1 t + \ln C_{W,0} \quad (5)$$

for the 0- to 6-h time period ($\ln C_W = -0.035t + 0.61$, $r^2 = 0.75$), giving $k'_1 = 0.035$/h. At 1 h, the cell abundance (A_t) was 5×10^6 cells/L. Solving Equation 4, we obtain $k_1 = 6.95 \times 10^{-9}$ L/cell/h for the uptake rate constant in our system.

The importance of growth dilution on the net accumulation rate was evaluated from the data obtained on cell growth over 48 h (Fig. 1C) [20]. First, applying a first-order growth model ($\ln A = \ln A_0 + k_G t$, where A is cell abundance) to our algal abundance versus time data, we obtained the relation ln cell density (cells/ml) = $0.07t + 8.4$ ($r^2 = 0.97$). Thus, $k_G = 0.07$/h and $t_{double} \sim 10$ h, where k_G is the pseudo–first order growth rate constant and t_{double} is the doubling time. The importance of growth dilution increases with time, as cell abundance increases and the uptake rate ($k_1 C_W$) decreases. At 48 h, the calculated uptake rate ($k_1 C_W$) is 0.98×10^{-9} ng/cell/h and the growth dilution rate ($k_G C_A$) is 1.05×10^{-9} ng/cell/h. Thus, at 48 h, uptake has removed much of the MeHg from solution, and the rate of dilution by growth is relatively large, corresponding to 108% of the uptake rate. For comparison, growth dilution was only 6% of uptake at 15 min. Our data did not allow evaluation of k_2 and elimination rate, because the MeHg concentration was not at steady state where $dC_A/dt = 0$.

Our calculated uptake rate ($k_1 C_W$) of 12 ag/cell/h at 15 min is similar to the rate reported by Moye et al. [3] of 11.4 ag/cell/h (5.28 amol/cell/h) for *S. capricornutum* over a 5-min exposure, although we would expect our lower concentration (0.01 vs 0.19 nmol/L) to result in a lower uptake rate. However, Moye et al. [3] used a different medium (Allen's) as well as a higher algal density (5×10^5 cells/ml) and continuous stirring, so the results may not be directly comparable.

Competitive ligand approach using EDTA

In the absence of EDTA, modeling indicated that the dominant species of MeHg at the experimental pH (7.3) are $MeHgOH^0 > MeHgCl^0$. Thus, uptake could involve transport of a neutral species through the cell membrane or binding at the surface with displacement of these relatively weak ligands. As the EDTA concentration was increased from 10 to 10,000 μM, MeHg complexes with EDTA became more abundant [20]. The main complex of MeHg with EDTA in Fraquil was $MeHgHY^{2-}$.

A conditional stability constant for the binding of MeHg to algal cells was estimated from measurements of MeHg uptake by algal cells in the presence of varying levels of EDTA using 24-h exposures (Fig. 2A). To model the competition for MeHg between algal cells and EDTA, we needed a measure of total binding sites per algal cell. The density of algal binding sites was estimated to be 4.45×10^{-20} mol/cell based on the amount of MeHg associated with the cells after exposure to the initial concentration of MeHg (9.28 PM) in the absence of EDTA (i.e., no EDTA control). We then multiplied the binding site density by the abundance of algal cells (~15,000 cells/ml) in the no-EDTA control to obtain the total binding site concentration (6.67×10^{-13} mol/L). For subsequent modeling scenarios in the present study, we kept this concentration constant.

Our approach assumed that the maximum number of binding sites per cell occupied by MeHg in any EDTA treatment would be the number of sites occupied in the corresponding no-EDTA control. We also assumed that all sites would be occupied in the no-EDTA control after 24 h, based on data from our uptake versus time experiment (Fig. 1A), and that binding to surface sites was large compared to internalization. Thus, our calculated value of binding site abundance is operational and might change if conditions were changed, including selection of a different (higher) initial concentration of MeHg. In fact, binding sites per cell measured in this way differed between our uptake-versus-time (Fig. 1A) and EDTA-competition (Fig. 2A) experiments (4.45 vs 13×10^{-20} mol/cell for 24-h exposures). The estimation of binding site abundance is one of the difficulties encountered in developing parameters for a BLM [7]. Consequently, the log K for binding of MeHg to algae obtained using this site density should be regarded as strictly operational (i.e., applicable to our specific experimental conditions).

Measurement of MeHg uptake by algae at varying EDTA concentration showed that low EDTA concentrations (≤10 μM) had little influence on MeHg concentration in algae (Fig. 2A). However, as the EDTA concentrations increased, the MeHg–algae concentration decreased, indicating that complexation of MeHg by EDTA was limiting the bioavailability of MeHg to algae. The concentration of MeHg in the algae was very low, near the detection limit, at EDTA concentrations of 100 μM and greater (Fig. 2A).

We estimated the stability constant for binding of MeHg to algae ($K_{MeHgAlgae}$) by competitive ligand modeling. With the total MeHg concentration, stability constants for MeHg–EDTA complexes, and concentration of algal binding sites held constant as knowns, we modeled the concentration of algal-as-

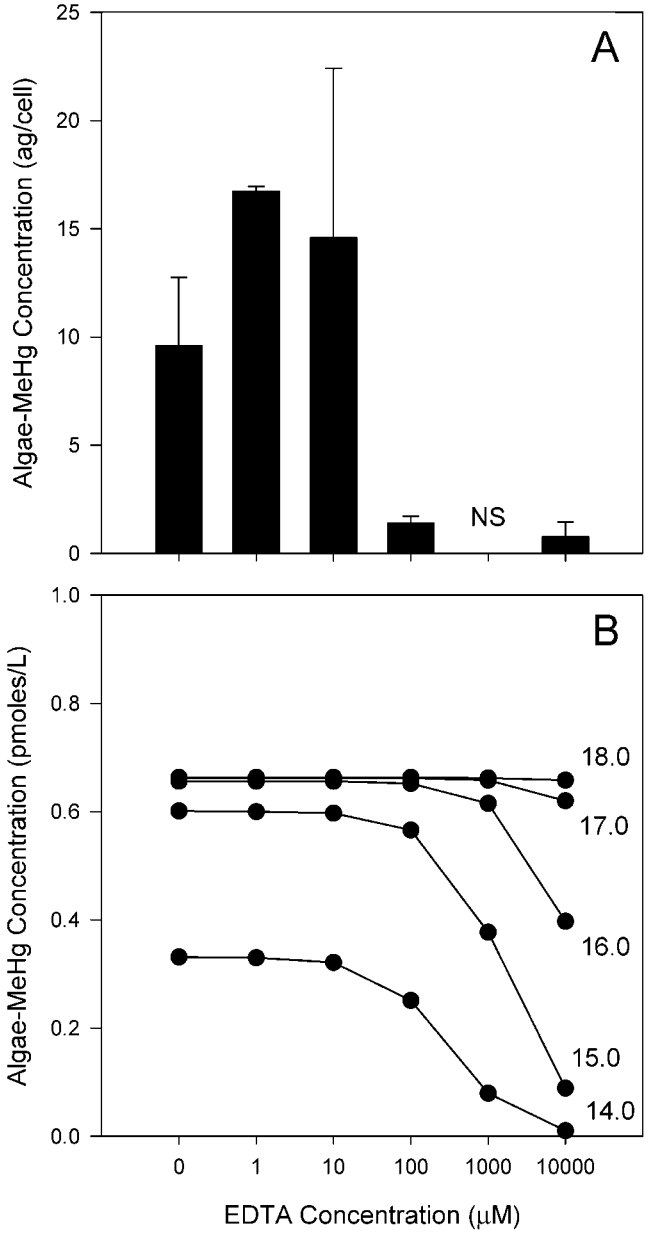

Fig. 2. Influence of a competitive ligand (disodium ethylenediaminetetra-acetate [EDTA]) on uptake of methylmercury (MeHg) by *Selenastrum capricornutum* during a 24-h incubation period. (**A**) Experimental results with MeHg–algae concentration (MeHg bound to algae) expressed as ag/cell. NS = no sample. (**B**) Modeled association of MeHg with algal cells, where algal abundance and associated binding site density was kept constant (0.667 pmol/L), and the MeHg–algae equilibrium binding constant was decreased stepwise from 10^{18} to 10^{14}.

sociated MeHg for various values of a stability constant for binding of MeHg to algae (log $K_{\text{MeHgAlgae}}$) and EDTA concentration (Fig. 2B). The results indicate log $K < 15$ underestimates the binding of MeHg in the absence of EDTA and log $K > 17$ predicts little competition by EDTA, even at the highest EDTA concentrations. The best fit was achieved with log $K_{\text{MeHgAlgae}} \cong 16$ (Fig. 2B), because competition by EDTA is predicted at concentrations similar to those indicating competition in our data (Fig. 2A).

Several factors may influence the accuracy of our estimated log $K_{\text{MeHgAlgae}}$. First, the estimate of total binding site concentration per cell is an approximation (see above). Second, the distribution of MeHg between algae and the aqueous medium may not be at equilibrium, because concentrations change with time as a result of algal growth, an inherent characteristic of batch systems. This could cause underestimation of log *K*. Third, uptake may include both binding to algal surface sites and internalization, possibly causing an overestimation of log *K*. These problems are common to most applications of the BLM [7]. Fourth, we assumed that MeHg uptake was influenced by EDTA only through complexation of MeHg by EDTA. However, we observed an effect of EDTA on algal growth at high EDTA concentrations, indicating that EDTA may have had direct effects on algal cells (see below). In addition, because the fraction of MeHg bound by algae was small (<5%) in our EDTA-competition experiments (Fig. 2A) as a result of low algal abundance, we modeled changes in a small fraction of the overall MeHg. Furthermore, our results are based on *S. capricornutum* and might differ for other algal species. Binding sites are related to properties of the cell wall, which is composed of cellulose or other polymers for *S. capricornutum*, a Chlorophyte [30], but contains different constituents in diatoms and cyanobacteria (silica or peptidoglycan, respectively). For example, Miles et al. [2] found that uptake rates were higher for two green algae (*S. capricornutum* and *Cosmarium botrytis*) than for a diatom or cyanobacterium. Finally, we have not included the potential for competition by other metals for algal binding sites to decrease the association of MeHg with the algal surface [13].

Generally, EDTA is used in algal media to keep metal ions in solution or to control metal speciation. To achieve competition, concentrations of EDTA generally were much higher than those used for maintenance cultures (5 nM). Algal abundance at 24 h decreased with increasing EDTA concentrations, indicating that high EDTA concentrations slowed algal growth. However, algal cells remained viable, because abundance at least doubled from the inoculation densities in all treatments. Growth may have been limited by the low bioavailability of essential metals, particularly Zn, at high EDTA concentrations [31]. However, EDTA also may have altered the MeHg binding or uptake properties of the cells, a potential problem when algal cells are exposed to competing ligands [13].

Because log $K_{\text{MeHgAlgae}} = 16$ is a large value in comparison to the stability constants of other ligands in our model, the modeled algal binding sites were occupied completely by MeHg at low EDTA concentrations. The remaining MeHg was bound by other ligands (Fig. 3). At higher EDTA concentrations, the algal sites were not saturated, and MeHg was bound mainly to EDTA. The calculated concentration of the $MeHg^+$-free ion was less than 1% of the total MeHg concentration for all treatments. Because the MeHg–algae complex corresponded to as much as 7% of the total MeHg concentration, MeHg apparently was taken up as complexed species or by disassociation of species, such as the chloride and hydroxide complexes in the no-EDTA treatment, demonstrating that MeHg in these relatively weak complexes is, at least in part, bioavailable.

Influence of natural ligands on MeHg bioavailability to algae

Results from our bioassay work were applied to natural ligands by modeling, using stability constants for binding of MeHg reported from other investigations. One important natural ligand pool is dissolved organic matter (DOM). Equilibrium binding constants for three DOM-associated thiol func-

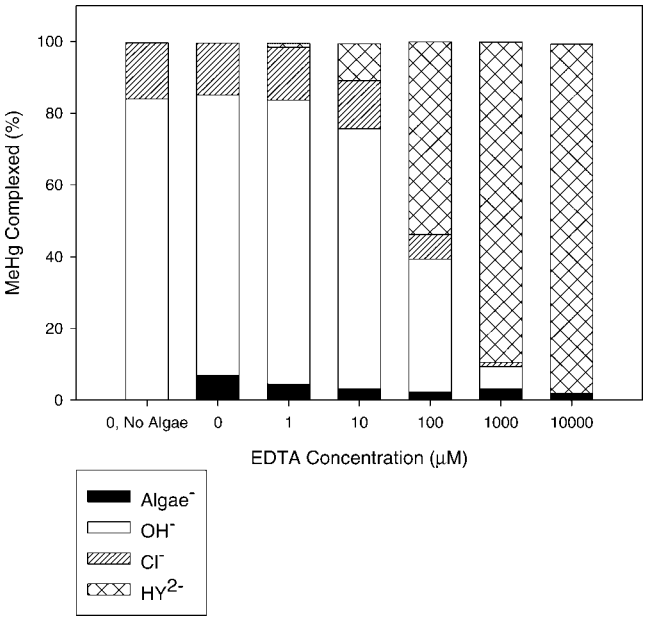

Fig. 3. Speciation of methylmercury (MeHg) changes with disodium ethylenediaminetetra-acetate (EDTA) concentration. The first bar represents the treatment in which neither algae nor EDTA was present. Free MeHg ($MeHg^+$) was much less than 1% of the total species. The MeHg–algae complex was a small percentage of the total MeHg because of the low abundance of algae and algal binding sites relative to the total MeHg concentration. Y = EDTA.

tional groups (RS1, RS2, and RS3) were added to the model based on stability constants reported by Amirbaham et al. [15] for Baker Brooks humic acid (SETAC Supplemental Data Archive, Item ETC-25-02-02; http://etc.allenpress.com). The authors also estimated the binding capacities for RS1, RS2, and RS3 to be 0.25 nmol/mg, 0.10 nmol/mg, and 0.76 nmol/mg, respectively, at 1 mg/L of humic acid. In our calculations, these binding capacities were increased or decreased proportionally for a series of DOM concentrations and entered into MINEQL+ as molar concentrations. The concentration of algal binding sites was maintained at the same level of our previous control (no-EDTA) treatment (6.67×10^{-13} mol/L of MeHg–algae binding sites in 15,000 cells/ml of algae).

The model results show the expected decrease in MeHg bound to algae with increasing DOM concentrations (Fig. 4A), corresponding to decreasing bioavailability. Predicted MeHg–algae concentrations decreased with increasing DOM concentrations up to 10 mg/L, indicating that MeHg would be most bioavailable at DOM concentrations less than 10 mg/L. At DOM concentrations greater than 15 mg/L, predicted MeHg–algae concentrations were less than 1% of the total bound MeHg species. For comparison, Playle et al. [32] found that DOC concentrations greater than 4.8 mg/L prevented Cu from binding to fathead minnow gills. These findings are similar, because our modeled humic acid was approximately 50% carbon [15]. Therefore, our DOM concentration would be equivalent to half the DOC concentration.

The present results are indicative of the potential importance of humic materials, but the binding characteristics may vary among sources and types of humic materials contained in DOM. Humic acids have been incorporated into BLMs for some metals. One approach has involved calibration of the Windermere humic aqueous model for binding of the metal of interest to humic acid [12,33]. Natural DOM also contains

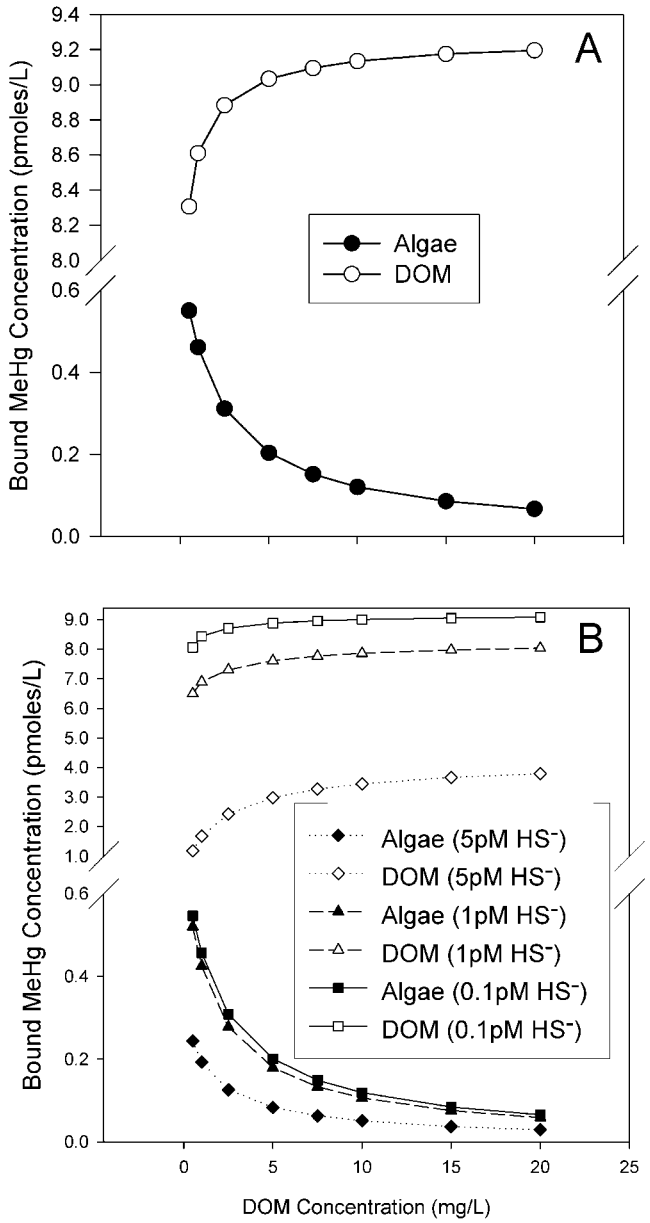

Fig. 4. Modeled influence of dissolved organic matter (DOM) and sulfide on methylmercury (MeHg) distribution between DOM and algae when MeHg concentration was 9.28 PM and algae concentration was 15,000 cells/ml (binding sites, 0.667 pmol/L). (**A**) The MeHg–algae concentration decreases as the DOM concentration increases and MeHg is preferentially bound to DOM. The algal density was held constant. Most of the decrease in MeHg–algae occurs at DOM greater than 10 mg/L. (**B**) Increasing sulfide (HS^-) decreases availability of MeHg for binding to DOM and algae. Concentrations of sulfide greater than 5 PM bind all available MeHg. Note the scale difference for bound DOM–bound MeHg between **A** and **B.**

algal-produced thiols [31], which likely are strong ligands for MeHg, potentially contributing to site-specific variations in MeHg bioavailability.

Sulfide clusters also are potentially important ligands for MeHg. Although sulfide is unstable in the presence of oxygen, sulfide clusters at nanomolar concentrations have been measured in oxic waters [34]. To examine the potential importance of sulfide clusters, we added bisulfide (HS^-) to our model (see *Supplemental Material*; http://XXX). Stability constants for complexes with HS^- were derived from reactions for association of MeHg with S^{2-} [35] and the deprotonation of HS^-.

The present results demonstrate the potential for sulfide (or HS^-) to reduce the binding of MeHg to both DOM and algae (Fig. 4B). As the sulfide concentration approaches half the MeHg concentration (9.28 PM), sulfide complexes, mainly $(MeHg)_2HS^+$, become dominant, decreasing the association of MeHg with algae or DOM. Whereas this example illustrates the potential importance of sulfide clusters, we expect sulfide clusters to be somewhat less reactive compared to free sulfide.

In summary, the present results demonstrate the potential for aqueous complexes of MeHg with strong natural ligands, such as humic material, organic thiol groups, and inorganic sulfides, to determine the bioavailability of MeHg and, therefore, the uptake of MeHg by phytoplankton. Bioavailability to algae may be an important factor in explaining differences among systems in subsequent bioaccumulation of MeHg in higher trophic levels. Our findings, including estimation of log $K_{MeHgAlgae}$, will aid in the modeling and predictability of MeHg bioavailability.

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