SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 33, No. 6, pp. 1211–1224, 2014

Printed in the USA

Global Mercury Partnership

INTERACTIONS BETWEEN MERCURY AND PHYTOPLANKTON: SPECIATION, BIOAVAILABILITY, AND INTERNAL HANDLING

Séverine Le Faucheur,*† Peter G.C. Campbell,‡ Claude Fortin,‡ and Vera I. Slaveykova†
†Institute F.-A. Forel, Earth and Environmental Sciences, Faculty of Sciences, University of Geneva, Versoix, Switzerland
‡Institut National de la Recherche Scientifique, INRS Eau Terre et Environnement, Québec, Canada

(Submitted 18 March 2013; Returned for Revision 11 June 2013; Accepted 3 October 2013)

Abstract: The present review describes and discusses key interactions between mercury (Hg) and phytoplankton to highlight the role of phytoplankton in the biogeochemical cycle of Hg and to understand direct or indirect Hg effects on phytoplankton. Phytoplankton are exposed to various Hg species in surface waters. Through Hg uptake, phytoplankton affect the concentration, speciation, and fate of Hg in aquatic systems. The mechanisms by which phytoplankton take up Hg are still not well known, but several studies have suggested that both facilitated transport and passive diffusion could be involved. Once internalized, Hg will impact several physiological processes, including photosynthesis. To counteract these negative effects, phytoplankton have developed several detoxification strategies, such as the reduction of Hg to elemental Hg or its sequestration by intracellular ligands. Based on the toxicological studies performed so far in the laboratory, Hg is unlikely to be toxic to phytoplankton when they are exposed to environmentally relevant Hg concentrations. However, this statement should be taken with caution because questions remain as to which Hg species control Hg bioavailability and about Hg uptake mechanisms. Finally, phytoplankton are primary producers, and accumulated Hg will be transferred to higher consumers. Phytoplankton are a key component in aquatic systems, and their interactions with Hg need to be further studied to fully comprehend the biogeochemical cycle of Hg and the impact of this ubiquitous metal on ecosystems. *Environ Toxicol Chem* 2014;33:1211–1224. © 2013 SETAC

Keywords: Mercury Methylmercury Phytoplankton Algae Biogeochemistry

INTRODUCTION

Phytoplankton comprise an assemblage of free-floating photosynthetic species (eukaryotic algae and cyanobacteria) of micrometer size, which grow in the top layer of natural waters when conditions of light, temperature, and nutrients are favorable. The phytoplanktonic species composition of a particular aquatic system depends on the characteristics of the ambient waters, eutrophic lakes being dominated by cyanobacteria and diatoms being relatively more abundant in oligotrophic waters [1]. With their conversion of solar energy into chemical energy and the formation of their organic matter, microalgae form the base of aquatic food chains and thereby influence the composition and productivity of higher biological communities living in an aquatic ecosystem.

To perform their photosynthesis, algae take up nutrients, including trace metals, from ambient waters. Phytoplankton thus have a considerable impact on the natural cycle of these elements and, as such, are involved in some of the main processes determining metal behavior in aquatic ecosystems, such as the decrease of their concentration in the photic zones during warmer seasons, their sedimentation with sinking phytoplankton, and their partial release into the water column by remineralization [2,3]. Metals accumulated by phytoplankton will be further transferred to grazers and microbial communities. Although mercury (Hg) is not an essential element, phytoplankton do take up Hg and thus influence its behavior and fate in natural waters. Once accumulated, Hg taken up within the algal cell can induce physiological and cellular modifications in algae and affect phytoplankton health at a certain dose. The present review examines each aspect of these Hg–phytoplankton interactions in order to draw attention to their role in the overall Hg cycle in aquatic environments and to identify potential knowledge gaps and future areas of research.

EFFECT OF HG–PHYTOPLANKTON INTERACTIONS ON HG WATER COLUMN CHEMISTRY

Hg species present in algal ambient environments

In surface waters, living phytoplankton are exposed to an array of Hg species: elemental Hg (Hg^0); monovalent mercurous Hg (Hg^I), which is highly unstable under typical aqueous environmental conditions; and divalent mercuric Hg, present in both inorganic (Hg^{II}) and organic (methylated, CH_3Hg) forms. All of these Hg species are interrelated by physical and chemical reactions, often biologically mediated and in which Hg^{II} plays a central role [4–7]. Its reduction through abiotic (e.g., photoreduction) and biotic (e.g., microbial) reactions forms gaseous Hg^0, whereas its methylation produces monomethylmercury (CH_3HgX, with $X = Cl^-$, OH) and dimethylmercury ($[CH_3]_2Hg$) [8,9]. Although abiotic methylation has also been reported, Hg methylation is predominantly controlled biotically, notably by its production by sulfate-reducing bacteria [10,11]. Concurrently, oxidation of Hg^0 and demethylation of CH_3Hg also occur in surface waters, regenerating Hg^{II}. Dimethylmercury is the predominant methylated species in deep ocean waters, whereas it is quickly evaporated from freshwaters and estuarine waters [10]. In freshwaters, the overall outcome of these reactions results in a distribution that is usually in the order of $Hg^{II} > Hg^0 \sim CH_3Hg$. In seawater, the proportion of CH_3Hg is typically lower than in freshwaters, and the Hg distribution follows $Hg^{II} > Hg^0 > CH_3Hg$ [12].

All Supplemental Data may be found in the online version of this article.
* Address correspondence to severine.lefaucheur@unige.ch.
Published online 11 October 2013 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.2424

These Hg species are further distributed in natural waters among operationally defined particulate (>0.45 μm) and dissolved fractions (<0.45 μm). Mercury partitioning between these 2 fractions is variable and depends on the aquatic system properties [13,14]. Indeed, in a recent study, particulate Hg and CH_3Hg were found to range from 3% to 93% and from 17% to 97% of total Hg and CH_3Hg, respectively, in 8 streams differing in climate, landscape characteristics, atmospheric Hg deposition, and water chemistry [15]. Nevertheless, systems with high quantities of suspended particulate matter tend to have high concentrations of particulate Hg [16]. The dissolved Hg fraction can be further divided into colloidal (0.45 μm–1 kDa) and truly dissolved (<1 kDa) fractions. Colloidal material comprises inorganic colloids (e.g., iron, aluminum hydroxides), large macromolecules (e.g., natural organic matter), and their aggregates and represents a significant pool of Hg complexing capacity [16–19]. A thorough study on the colloidal distribution of Hg in Texas estuaries demonstrated that in the colloidal fraction Hg preferentially binds to colloidal organic matter with molecular weight <10 kDa rather than to the larger colloidal fraction (10 kDa–0.45 μm), which was mostly composed of iron hydroxides [20]. This Hg binding by the < 10-kDa fraction is presumably caused by the high affinity of Hg for sulfhydryl functional groups present in this fraction. In the dissolved fraction, Hg strongly binds with dissolved organic matter (DOM). Reported values for the conditional Hg-DOM binding constant are high ($\log K_{Hg\text{-}DOM} = 22–28$), provided the Hg/DOM ratio is statistically low, but at Hg/DOM concentrations higher than those encountered in natural waters, the $\log K_{Hg\text{-}DOM}$ values are much lower (the calculated molar ratio in natural waters between reduced DOM sulfur and total Hg is between 1.2×10^4 and 1.2×10^5) [21]. Each Hg ion would be expected to bind to 2 or more reduced sulfur atoms in the DOM; but when these high-affinity binding sites become saturated, amine functional groups as well as carboxylic acids or phenols may also participate in Hg complexation with mixed or single-type ligation [22–24]. Stability constants describing this latter complexation are much lower than those involving sulfur atoms [25]. Complexation of Hg by DOM collected from the Florida Everglades (USA) could be described using 2 conditional stability distributions, depending on the Hg/DOM ratios [26]. At ratios below 5 nmol Hg mg^{-1} DOM, $\log K'_{DOM}$ was determined to be 23.2 ± 1.0 L kg^{-1}, whereas at ratios above 50 nmol of Hg mg^{-1}, $\log K'_{DOM}$ dropped to 10.7 ± 1.0 L kg^{-1}. Natural DOM is thus a key component that controls dissolved Hg^{II} and CH_3Hg speciation and thus affects the bioavailability of these Hg forms toward aquatic organisms in surface waters [21]. In the Hg-contaminated waters of East Fork Poplar Creek (Oak Ridge, TN, USA), modeling with the equilibrium geochemical speciation program PHREEQC estimated that 3 mg L^{-1} of DOM would bind nearly 100% of Hg^{II} ($\log K_{Hg\text{-}DOM} = 28.7$ with bicoordinate complexation) and 60% of CH_3Hg ($\log K_{CH3Hg\text{-}DOM} = 14$ with monocoordinate complexation) [27].

Mercury that is not bound to DOM will form complexes with inorganic ligands such as the hydroxide ($Hg(OH)^+$, $Hg(OH)_2$, $Hg(OH)_3^-$, CH_3HgOH) and chloride ($HgCl^+$, $HgClOH$, $HgCl_2$, $HgCl^{3-}$, $HgCl_4^{2-}$, CH_3HgCl) anions [28]. The distribution among these various forms will depend on the pH and the chloride concentration in the ambient water. In freshwaters with circumneutral pHs, the dominant inorganic forms are usually $Hg(OH)_2$, $HgClOH$, and $HgCl_2$. Note that one cannot exclude the presence of additional Hg complexes, including those with monomeric sulfur-containing ligands other than DOM, that might strongly complex Hg and thus modify its bioavailability [29]. Given the very strong tendency of Hg^{II} to form complexes, the estimated free Hg^{2+} concentration, which is the species that is usually of main interest in metal bioavailability studies, is extremely low. For example, the calculations by Dong et al. [27] yielded [Hg^{2+}] estimates of 10^{-27} M to 10^{-28} M.

Effects of phytoplankton on Hg chemistry in natural waters

Phytoplankton accumulate Hg from their ambient environment, which will influence Hg cycling in natural waters by affecting its concentration, by favoring its transport from surface waters to sediments, and by modifying its speciation (Figure 1). In the San Francisco Bay estuary (USA), dissolved CH_3Hg concentrations, but not total Hg, were shown to decrease during an algal bloom (and particulate CH_3Hg increased) as a result of algal uptake, whereas algal decay was accompanied by an increase in dissolved CH_3Hg concentrations, this increase being attributed both to the release of CH_3Hg from the algae and to the formation of CH_3Hg by bacterially mediated methylation in the sediments [30]. Recently, net Hg methylation in open oceans has been shown to be promoted by the presence of small-sized nano- and picophytoplankton, whose Hg content was efficiently remineralized by bacteria [31]. Higher organisms will graze natural algal communities, leading to the transfer of Hg to the consumers and possibly to Hg recycling to the dissolved phase by "sloppy feeding." As far as we know, this latter phenomenon has not yet been demonstrated for Hg, but it has been described for dissolved organic matter and nutrients as well as for other trace elements such as Cd and Zn [32–35]. In the latter study concerning metals, the metals accumulated by the cyanobacterium *Synechococcus* were regenerated into the dissolved fraction by the grazing of natural microzooplankton. Because accumulated metals bind intracellularly to a variety of ligands, such as enzymes or phytochelatins, this release by grazing is expected to change their speciation in solution. Note that even in the absence of grazing, excretion of exudates by living phytoplankton will tend to modify Hg speciation in the dissolved phase [36,37].

Phytoplankton as biogenic particles will settle toward the bottom sediments, where benthic organisms will further degrade them. During the transport from the surface waters to the sediments, remineralization processes may biologically recycle Hg into the water column [38]. There is currently an active debate about the role of phytoplankton in Hg sedimentation in Arctic lakes in the context of global warming [39–41]. Climate warming has increased phytoplankton biomass production in the Arctic region and has led to increased Hg sedimentation [42]. These findings have a tremendous impact on the estimation of atmospheric Hg deposition in the Arctic region as most of the evidence for temporal changes in atmospheric Hg deposition has been derived from sediment cores [43]. However, these results are still controversial [41,44].

Besides these expected processes, additional Hg-specific reactions can be observed in natural waters. In the Arctic region, dissolved gaseous Hg production has been reported to be strongly linked to phytoplankton dynamics, whereas biogenic organic materials produced by algae favored the oxidation of Hg^0 to Hg^{II} [45,46]. Several mechanisms can explain these observations, including the direct reduction of Hg^{II} to Hg^0 by algae, which use this redox process as a detoxification mechanism [47].

HG UPTAKE BY PHYTOPLANKTON

Uptake by phytoplankton under controlled conditions

In natural waters, cationic metals such as Cu, Zn, or Cd may bind both to inorganic anions (hydroxide, carbonate, chloride,

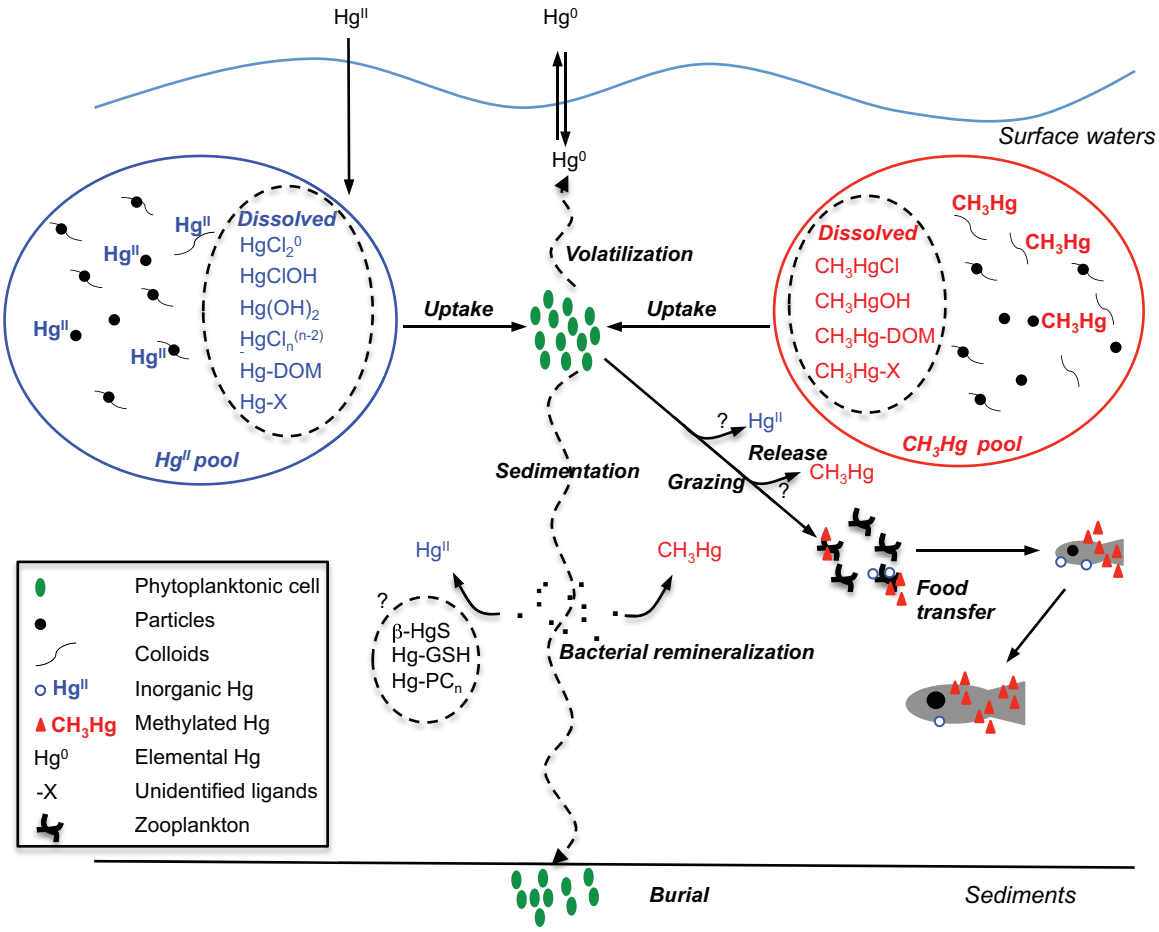

Figure 1. Mercury–phytoplankton interactions in natural waters (see text for details). DOM = dissolved organic matter. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

phosphate, etc.) and to organic ligands, including low–molecular weight metabolites, fulvic acids, and synthetic ligands such as ethylenediamine tetraacetic acid (EDTA) or nitrilotriacetate. The vast majority of the resulting metal complexes are polar and hydrophilic in nature. However, the hydrophobic lipid bilayers constituting algal membranes are virtually impermeable to charged and polar molecules. Algae thus possess a large set of transport proteins embedded in their membranes to allow the entry of metals into their cells [48,49]. For example, algae in the *Chlamydomonas* genus are known to express 4 families of metal transporters in their plasma membrane, 2 of which are ZIPs (Zrt-, Irt-like proteins, involved in Zn uptake) and NRAMPs (natural resistance–associated macrophage proteins, involved in Mn[II] and Fe[II] uptake) [50]. Thus, to be taken up, metals first have to diffuse through an unstirred boundary layer from the bulk solution to the plasma membrane, then bind to the specific transporter, and finally cross the membrane. Once inside the cells, metals will bind to a series of intracellular ligands involved in normal algal metabolism or in the metal detoxification processes [51].

Numerous studies have demonstrated the importance of taking metal speciation into account when trying to predict the response of aquatic organisms, including phytoplankton, to metal exposure. The free metal ion is recognized as a useful predictor of metal bioavailability (e.g., the free metal ion activity model [52]), and in many cases, uptake of metals such as Cd and Pb has been shown to be directly proportional to the free ion concentration in solution. In such cases, the addition of metal complexing ligands such as DOM or EDTA decreases metal bioavailability [53–55]. The presence of other cations can also decrease metal internalization when they compete with metals at transport binding sites, as predicted by the biotic ligand model [56,57].

In contrast to other trace metals, the hydrated free Hg ion, Hg^{2+}, is present in solution at vanishingly low concentrations (e.g., $< 10^{-26}$ M) at environmental pH values. Mercury is instead bound to various ligands, such as chloride ions, with which Hg^{II} forms a neutral lipophilic complex ($HgCl_2^0$, octanol–water partitioning constant, K_{OW} of 3.3); methylmercury also forms a neutral lipophilic complex with chloride (CH_3HgCl^0, K_{OW} of 1.7) [58]. Mercury-neutral complexes with other small inorganic ligands such as hydroxide also exist, but with K_{OW} values of 0.05 and 0.07 for $Hg(OH)_2^0$ and CH_3HgOH^0, respectively, these species are not lipophilic. The possible involvement of Hg^{2+} binding to membrane transporters, as postulated in the free metal ion activity model/biotic ligand model construct, is thus not as straightforward as for other metals.

Early experiments performed with artificial membranes demonstrated that the lipophilic $HgCl_2^0$ complex could diffuse passively across such lipid bilayers [59,60] (Figure 2). Very high membrane permeability coefficients were measured for $HgCl_2^0$ (approximately 10^{-3} cm s^{-1}), which led to the control of Hg uptake by the mass transport of Hg from the bulk solution to the membrane as a result of the lower permeability coefficient in solution (approximately 10^{-4} cm s^{-1}). Uptake of $HgCl_2^0$ has also been postulated for phytoplankton (see below), but in such cases the diffusional flux from the bulk solution to the algal

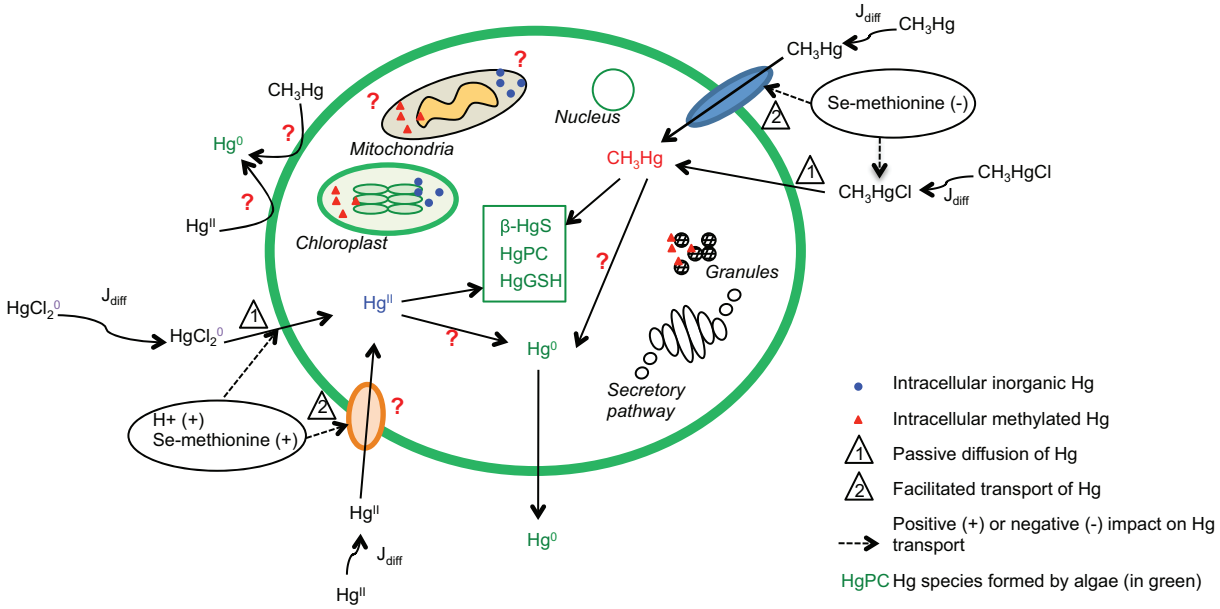

Figure 2. Mercury–phytoplankton interactions at the algal cellular level (see text for details). J_{diff} = diffusional flux for a metal through the unstirred boundary layer in the bulk solution. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

surface should always be calculated and compared with Hg uptake. This is especially true in natural waters when Hg concentrations are typically very low. The diffusional flux for a metal through the unstirred boundary layer in the bulk solution (J_{diff}, expressed in pmol $cm^{-2}\,s^{-1}$) is described with the following equation [54,61]:

$$J_{diff} = \frac{4\pi D(C_b - C_s)\left(\frac{r_c r_d}{r_d - r_c}\right)}{A}$$

where r_c is the radius of the cell (cm); r_d is the thickness of the unstirred boundary layer added to the radius of the cell (cm); D is the diffusion coefficient of the metal ($cm^2\,s^{-1}$); C_b and C_s are the metal concentrations in the bulk solution and at the algal surface ($mol\,cm^{-3}$), respectively; and A is the algal surface area (cm^2) (see calculation example in Supplemental Data). Based on this equation, the diffusional flux is related to metal concentrations in solution. Low metal concentrations, such as those for Hg encountered in natural waters, could lead to a low diffusional flux and thus to the limitation of Hg uptake by algae. Studies with synthetic membrane interfaces provide a simplified illustration of Hg uptake by organisms, notably because of the lack of membrane proteins; but nevertheless, they illustrate the importance of considering both diffusion fluxes, in solution as well as through membranes, to fully understand Hg uptake.

Mason et al. demonstrated the role of dichloro-Hg complexes in algal Hg uptake with the marine diatom *Thalassiosira weissflogii* [58]. In their study, Hg^{II} and CH_3Hg uptake rates were linearly correlated with the overall K_{OW} of Hg in the exposure solutions, and the algal growth inhibition by Hg^{II} and CH_3Hg was related to the concentrations of $HgCl_2^0$ and CH_3HgCl^0 but not to the total Hg or free ionic Hg^{2+} concentrations. The passive diffusion of $HgCl_2^0$ and CH_3HgCl^0 through algal membranes was thus deduced to be the main mechanism of Hg uptake by the diatom, bypassing ion transporters, and with similar permeability coefficients for both Hg species ($7.4 \times 10^{-4}\,cm\,s^{-1}$ for $HgCl_2^0$ and $7.2 \times 10^{-4}\,cm\,s^{-1}$ for CH_3HgCl^0). Note that the studied Hg concentrations varied from 0.3 pM to 240 pM for $HgCl_2^0$ and 150 pM for CH_3HgCl^0, which are higher than concentrations encountered in natural waters and may favor the passive diffusion pathway. Additional experiments performed with dimethylmercury (K_{OW} of 182) and Hg^0 (K_{OW} of 4.15) showed that lipophilicity was nevertheless not the only condition for bioaccumulation as no detectable Hg accumulation or toxicity was observed upon algal exposure to these 2 Hg species. The low affinity of dimethylmercury and Hg^0 with intracellular components was then shown to be responsible for their lower toxicity toward algae. Indeed, once inside cells, lipophilic Hg complexes will encounter a series of intracellular ligands that can induce dissociation of the original Hg complex. For example, in experiments conducted with the freshwater alga *Chlamydomonas reinhardtii*, lipophilic $HgCl_2^0$, once assimilated, remained within the algal cells even when the cells were resuspended in clean, Hg-free media [62]. Under similar conditions, however, "normal" organic lipophilic molecules are eliminated by algae and diffuse back into the external medium [63]. This phenomenon is explained by the relatively weak affinity between Hg^{II} and Cl^-, such that $HgCl_2$ can easily dissociate in algal cells in the presence of intracellular thiols [64]. Thus, the accumulation of lipophilic Hg complexes will depend not only on their lipophilicity but also on Hg affinity with intracellular ligands and the strength of the metal–ligand bonds in the lipophilic Hg complexes.

Since the pioneering work of Mason and collaborators, several studies have suggested that multiple uptake mechanisms may be involved and that Hg can possibly be transported into algal cells via facilitated transport (Figure 2). One of these studies showed that the CH_3Hg uptake rate in *Selenastrum capricornutum* (also known as *Pseudokirchneriella subcapitata*) as a function of the CH_3HgCl concentration in solution appeared to involve 2 different transporters (Figure 3) [65]. At low CH_3Hg concentrations (<10 nM), the CH_3Hg uptake curve showed a steeper linear increase (slope 4.8) with CH_3Hg concentrations than at the highest concentrations (slope 0.49). In the same study, *S. capricornutum* was also shown to take up less CH_3Hg when exposed to a range of chemical uncouplers of

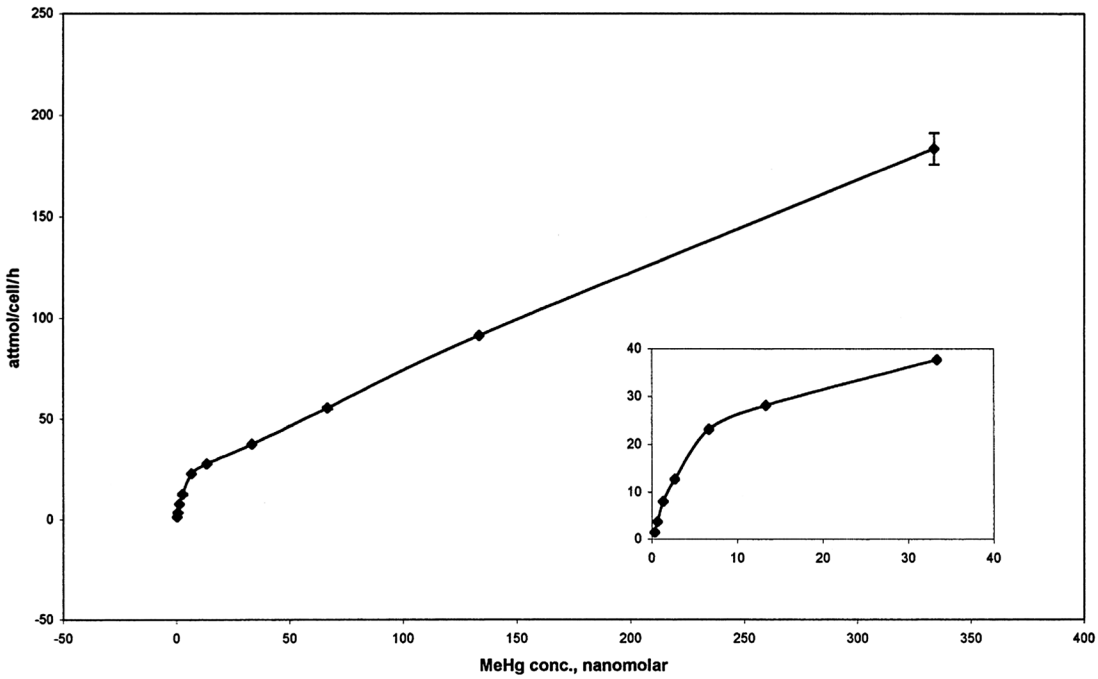

Figure 3. Organic methylated Hg (CH_3Hg) uptake rate (attmoles per cell per hour = 10^{-18} mol cell^{-1} h^{-1}) of *Selenastrum capricornutum* as a function of CH_3HgCl concentration (nanomoles) in solution. Reprinted with permission from Moye et al. [65]. Copyright 2013 American Chemical Society.

phosphorylation (carbonyl cyanide *m*-chlorophenylhydrazone, 2–4 dinitrophenol, 3-[3,4]–dichlorophenyl-1,1-dimethylurea [diuron], and paraquat), implying that facilitated transport could be involved in Hg uptake. Note, however, that to perform the exposure experiments, algae were exposed first to the inhibitors for 2 h and then to the inhibitors and CH_3Hg for 5 min; thus, complexation of CH_3Hg by the inhibitors, which would be expected to decrease CH_3Hg bioavailability, cannot be excluded. Facilitated transport of Hg was further supported by additional experiments with algae exposed to CH_3Hg in the dark or exposed to γ irradiation, which led to a decrease of CH_3Hg uptake in both cases. However, the value of 0.7 for Q_{10} (Q_{10} being the change in the rate of an activity resulting from a 10 °C increase in temperature [66]), obtained in experiments measuring CH_3Hg uptake as a function of temperature, was suggestive of passive diffusion because uptake involving transporters usually has a Q_{10} between 2 and 3. In another study, heat-killed diatoms were shown to contain less CH_3Hg and Hg^{II} in their cytoplasm, decreasing from 64% to 4% and from 15.5% to 0.2% of total cellular Hg, respectively, compared with living cells, suggesting a metabolically controlled uptake of both species by the diatoms [67]. Additionally, algal physiology has been reported to affect Hg uptake. Indeed, partition constants (K_{pl}, nmol of CH_3Hg per kg of algal cell divided by nmol of CH_3Hg per liter of exposure solution) between CH_3Hg and *S. capricornutum* were lower in phosphorus-limited algae compared with optimally grown algae [68], whereas aging *Cosmarium botrytis* contained more CH_3Hg than did cells in the exponential phase [65,68]. In contrast to CH_3Hg, no unequivocal evidence exists for facilitated transport of inorganic Hg^{II} into algae. However, recent studies performed with bacterial *mer-lux* bioreporters have suggested that uptake of Hg^{II} may involve facilitated transport at trace-level concentrations (<50 pM), suggesting that facilitated Hg^{II} uptake might also occur in eukaryotic cells [69–71].

To compare Hg uptake rates with those of lipophilic metal complexes and cationic metals reported in the literature, upper and lower values of Hg uptake rates presented in Table 1 were expressed as membrane permeability coefficients in the same units (cm s^{-1}) by dividing the uptake rates (in amol cell^{-1} h^{-1} nM^{-1}; normalized to the exposure concentration [(nM]) by the algal cell surface (cm^2 cell^{-1}) and 3600 (s). Inorganic Hg^{II} uptake coefficients vary from $1.3 \pm 0.1 \times 10^{-4}$ cm s^{-1} in *C. reinhardtii* to 8.5×10^{-4} cm s^{-1} in *Thalassiosira weissflogii*, whereas for CH_3Hg they vary from 3.2×10^{-6} cm s^{-1} in *S. capricornutum* to 5.9×10^{-4} cm s^{-1} in *Cosmarium botrytis*. This range of values is very wide, notably for CH_3Hg, and does not allow us to draw conclusions about possible mechanisms of Hg uptake by algae. Indeed, reported uptake rates of lipophilic metal complexes range from 0.55×10^{-4} cm s^{-1} to 19×10^{-4} cm s^{-1} (see Table S–4 in Boullemant et al. [63]), whereas those for cationic metals range from 10^{-6} cm s^{-1} (Pb^{2+} in *Chlorella vulgaris*) to 10^{-2} cm s^{-1} for Ag^+ in *C. reinhardtii* [72].

Influence of DOM on Hg uptake by phytoplankton

The influence of DOM on Hg uptake by phytoplankton has been studied in several laboratories, in part to help in the extrapolation of laboratory results to the field but also because of the observed natural phenomenon that concentrations of Hg are often high in fish from waters containing high concentrations of DOM [73,74]. The presence of DOM in the exposure solution generally decreases Hg uptake by algae, due to the strong binding of Hg by reduced sulfur sites on the DOM [65,75–77]. Nevertheless, the concentration and quality (sources) of DOM can affect the degree to which it inhibits algal Hg uptake. Gorski et al. [78] modeled CH_3Hg uptake by *S. capricornutum* at various DOM concentrations and concluded that accumulation of CH_3Hg would decrease substantially at DOM concentrations above 10 mg L^{-1}. Also, the diatom *Cyclotella meneghiniana* has been reported to accumulate less CH_3Hg in the presence of the hydrophobic fraction of DOM than in the presence of the transphilic fractions (i.e., those with intermediate polarity, between the hydrophilic and hydrophobic fractions), which was explained by the authors as a result of possible higher

Table 1. Normalized uptake rates of inorganic Hg (Hg^{II}) and organic methylated Hg (CH_3Hg) reported for several algal species

Algal species	Exposure medium	$[Hg^{II}]_{expo}$	$[CH_3Hg]_{expo}$	Normalized uptake rate (amol $cell^{-1}$ h^{-1} nM^{-1}) Hg^{II}	Normalized uptake rate (amol $cell^{-1}$ h^{-1} nM^{-1}) CH_3Hg	Reference
Thalassiosira weissflogii	FRAQUIL $[Cl^-] = 10^{-5}$–0.32 M, pH 4–8, $t_{expo} = 4$ h	0.3–240 pM	0.3–150 pM	2 (pH 7.3, $Hg(OH)_2$)[a] 12 (pH 4.8, $HgCl_2$)[a]	6 (pH 6.6, pCl 3.3)[b] 12 (pH 4.9, pCl 3.3)[b]	[58]
Selenastrum capricornutum	5 mM phosphate buffer, 5.6 mM KCl, pH 7, $t_{expo} = 5$ min		1.9 nM		2.8 ± 0.2 (CH_3HgCl)	[65]
Thalassiosira sp.					2.5 ± 0.2 (CH_3HgCl)	
Cosmarium botrytis					240 ± 18 (CH_3HgCl)	
S. capricornutum	Deficient FRAQUIL, pH 7.3, $t_{expo} = 1$ h		10 pM		6.95 (CH_3HgOH)	[78]
Cyclotella meneghiniana	Field-collected freshwaters from San Francisco Bay delta system enriched with N, S, and P, and containing low (L; 177 ± 15 μM C, pH 6.8) and high (H; 280 ± 40 μM C, pH 7.9) DOC content, $t_{expo} = 48$ h	0.74–1.62 nM	0.64–0.74 nM	L: 0.07 ± 0.01[c] H: 0.028 ± 0.02[c]	L: 0.45 ± 0.03[d] H: 0.97 ± 0.10[d]	[67]
Chlamydomonas reinhardtii				L: 0.12 ± 0.01[c] H: 0.113 ± 0.001[c]	L: 1.59 ± 0.31[d] H: 3.02 ± 0.63[d]	
Cryptomonas ozolini				L: 0.75 ± 0.06[c] H: 0.85 ± 0.03[c]	L: 1.54 ± 0.14[d] H: 3.94 ± 0.44[d]	
Synechocystis sp.	(*C. meneghiniana*), $t_{expo} = 24$ h			L: 0.007 ± 0.001[c] H: 0.009 ± 0.002[c]	L: 0.068 ± 0.005[d] H: 0.046 ± 0.003[d]	
S. capricornutum	Deficient FRAQUIL, pH 7.3, $t_{expo} = 24$ h	10 pM	10 pM	1[e]	0.008[e]	[75]
C. reinhardtii	Deficient MHSM, $[Cl^-] = 10$ mM, pH 5.5–7, $t_{expo} < 40$ min	54 nM		0.15 ± 0.1 (pH 7, $Hg(OH)_2$) 0.9 ± 0.1 (pH 5.5, $HgCl_2$)		[62]

[a,b]Read on Figures 3 and 4, respectively.
[c,d]Calculated from Hg accumulated, expressed in nanomoles per gram and averaged exposure Hg concentrations of 1.18 nM and 0.69 nM for Hg^{II} and CH_3Hg, respectively.
[e]Calculated from bioconcentration factor (of 2.4×10^4 and 2×10^2 for Hg^{II} and CH_3Hg, respectively, in the multisite experiment).
$[Hg^{II}]_{expo}$ = dissolved Hg^{II} concentration in exposure medium; $[CH_3Hg]_{expo}$ = dissolved CH_3Hg concentration in exposure medium; t_{expo} = Exposure time; FRAQUIL = artificial freshwater medium; DOC = dissolved organic carbon; MHSM = modified high salt medium.

complexation of CH_3Hg to the hydrophobic fraction because of its higher aromaticity [76].

Although humic substances normally dominate the DOM fraction in natural waters, small organic acids such as amino acids also contribute to DOM; recent work has demonstrated that such constituents can modify Hg uptake by bacteria [79,80]. For example, the presence of cysteine in bacterial exposure media increased Hg^{II} and CH_3Hg uptake by the microorganisms, revealing an additional plausible effect of DOM components on Hg bioaccumulation. Note, however, that no uptake of Hg-cysteine by the alga *C. reinhardtii* has been observed in short-term exposure experiments [62].

Influence of pH and selenium

Besides DOM, pH and selenium are 2 additional parameters that have been observed to affect Hg accumulation in aquatic organisms [38,81–85]. Acidification of ambient waters increased Hg bioaccumulation, whereas the presence of selenium was reported to have a protective effect against Hg uptake. Both the H^+ ion and selenium were also found to modify Hg uptake by algae (Figure 2). Uptake of $HgCl_2^0$ by *C. reinhardtii* was observed to increase by 40% when the pH of the exposure solutions was decreased from 6.5 to 5.5 [62]. This result was quite unexpected, given that acidification is known to decrease algal uptake of cations, as a result of competition at transporter binding sites, and of lipophilic metal complexes, as a result of the stronger packing of algal membranes that occurs with protonation of the phospholipid head groups [63,86]. This result thus raised the possibility that Hg^{II} uptake could be partly proton-mediated (e.g., H^+ cotransport).

The effects of several forms of selenium on Hg^{II} and CH_3Hg uptake were studied in the diatom *Thalassiosira pseudonana* [87]. Selenite and selenate did not induce any modification in Hg^{II} and CH_3Hg uptake by the alga. However, rather high concentrations of seleno-L-methionine (2.5 μM) provoked a decrease in CH_3Hg uptake within the first hour of exposure and an increase in Hg^{II} uptake. One of the mechanisms suggested by Wang et al. [87] was uptake of the methionine–Hg^{II} complex at a faster rate than Hg^{II} alone. Further investigations are clearly needed to understand the mechanisms behind the proton and selenomethionine effects.

Hg accumulation by natural algal communities

Despite the very low Hg concentrations in natural waters (1–100 pM), phytoplankton have been reported to accumulate 4.1 pmol to 4150 pmol of Hg per g of algal dry weight and 10 pmol to 150 pmol of CH_3Hg per g of algal dry weight (see Table 2 for freshwater and Table 3 for seawater). High phytoplanktonic Hg content can be found in contaminated areas, notably those impacted by metal mining [88,89]. In the Carson River–Lahontan Reservoir (NV, USA), classified as a

Table 2. Mercury (Hg) concentrations in phytoplankton collected in natural freshwaters, including ambient dissolved Hg concentrations and calculated bioaccumulation factors (BAFs; [Hg] in seston [in nmol kg^{-1} wet wt] divided by [Hg] in water [nmol L^{-1}]), as measured in several ecosystems

Assemblage (size fraction, definition)	Ecosytem	[Hg] water (pM)	Total algal Hg content (pmol g^{-1} dry wt)	BAF	Reference
Phytoplankton (>0.8 μm)	Onondaga Lake, NY, USA (Hypereutrophic)	$[CH_3Hg] = 1.5$	$[CH_3Hg] = 159.5$[a]	$CH_3Hg = 5$	[121]
Seston (phytoplankton)	Lake Michigan, USA	$[Hg] = 1.15–1.90$; $[CH_3Hg] = 0.063 \pm 0.022$	$[Hg] = 820 \pm 280$; $[CH_3Hg] = 19$		[13]
Microseston (0.45–64 μm)	15 northern Wisconsin lakes, USA (Mesotrophic)	$[Hg^{II}] = 1.8–20$; $[CH_3Hg] = 0.1–3.6$	$[Hg^{II}] = 847 \pm 219$; $[CH_3Hg] = 164 \pm 70$	$Hg^{II} = 4.7–5.9$[b] $CH_3Hg = 4.8–6.2$[b]	[38]
Phytoplankton (<63 μm)	Mouse (M) and Ranger (R) Lakes, ON, Canada (Dystrophic)	$[Hg] = 7.5–20$	$[Hg] = 1057 \pm 184$ (M) $[Hg] = 1037 \pm 159$ (R)		[38]
Phytoplankton (50 μm)	James Bay, QC, Canada (La Grande hydroelectric reservoir)	$[Hg] = 4.8–7.1$; $[CH_3Hg] = 0.2–0.3$	$[Hg] = 434 \pm 100$; $[CH_3Hg] = 135 \pm 15$		[129]
Phytoplankton (<63 μm, microscopically determined)	Tapajos River, Brazil (Clear water river [floodplain])	$[Hg] = 2.5–9$; $[CH_3Hg] = 0.05–0.20$	$[Hg] = 85–623$; $[CH_3Hg] = 10–125$		[130]
Phytoplankton (<64 μm)	Grande Marsh, Colombia (Gold production area)	$[Hg] = 1645 \pm 150$	$[Hg] = 2592 \pm 150$		[89]
Picoseston (0.2–2 μm)	9 lakes in the Adirondack Mountains, NY, USA	$[Hg] = 5.5–21.4$	$[Hg] = 50–4148$		[88]
Nanoseston (2–20 μm)			$[Hg] = 154–1331$		
Microseston (20–200 μm)			$[Hg] = 234–1241$		
Phytoplankton	Carson River-Lahontan Reservoir system, NV, USA (Superfund site)	ND $[Hg]_p$ up to 49 850	$[Hg] = 29\,910–254\,237$ $[CH_3Hg] = <997–28\,415$		[90]
Seston	Lake Champlain (12 sampling sites), USA (From oligotrophic to eutrophic)	ND	$[Hg] = 150–1079$; $[CH_3Hg] = 12–151$		[125]

[a] Accumulated Hg in picomoles per gram wet weight.
[b] The BAF was calculated based on dry weight and assuming that 50% of the microseston is living biomass.
$[Hg]_p$ = Hg concentration in the particulate form; Hg^{II} = inorganic Hg; CH_3Hg = organic methylated Hg; ND = not determined.

Superfund site, phytoplankton were found to contain from 30 nmol to 254 nmol Hg g^{-1} dry weight and 1 nmol to 28 nmol CH_3Hg g^{-1} dry weight [90].

Although these accumulation values are useful for assessing the extent of Hg contamination in natural waters, they should be interpreted with care. For example, it is practically impossible to determine the Hg content in natural algal communities accurately because of the way in which microalgae are sampled. Phytoplankton are collected from natural waters by the use of specific nets or filters, which can also capture other microorganisms, abiotic particles, or cellular debris. Determinations of Hg concentrations in natural algal assemblages can thus be affected by the presence of nonspecific biotic and abiotic fractions, which can adsorb or absorb Hg. In principle, the presence of this nonalgal component could lead to an under- or overestimation of the quantity of Hg accumulated by natural phytoplankton. The term "microseston" is sometimes used instead of "phytoplankton" to recognize this bias in algal Hg determinations [38,91,92]. However, in the case of an algal bloom, the contribution from abiotic particles may become

Table 3. Mercury (Hg) concentrations in phytoplankton collected in coastal and seawaters, including ambient dissolved Hg concentrations and calculated bioaccumulation factors (BAFs; [Hg] in seston [in nanomoles per kilogram wet wt] divided by [Hg] in water [nanomoles per liter]), as measured in several ecosystems

Assemblage (size fraction, definition)	Ecosystem	[Hg] water (pM)	Total algal Hg content (pmol g dry wt^{-1})	Log BAF	Reference
Phytoplankton	Terra Nova Bay (Ross Sea, Antarctica)	ND	$[Hg] = 194 \pm 35$		[131]
Microseston	Long Island Sound, near-shore marine systems, USA	$[CH_3Hg] = 0.15$	$[CH_3Hg] = 2.5$[a]	$CH_3Hg = 4.2$	[92]
Phytoplankton	Baltic Sea	ND	$[Hg] = 10 \pm 5$[a]		[132]
Plankton (20–40 μm)	Seine estuary, France	$[Hg] = 0.8–4.7$ $[CH_3Hg] = 40 \times 10^{-3} - 98 \times 10^{-3}$	$[Hg] = 690 \pm 100$ $[CH_3Hg] = 26$		[134]
Plankton (40–150 μm)			$[Hg] = 590 \pm 130$		
Seston (1.2–70 μm)	Guanabara Bay, Brazil (Eutrophic system)	ND	$[Hg] = 289–1806$ $[CH_3Hg] = 115–1022$		[135]
Microplankton (70–290 μm)			$[Hg] = 75–199$ $[CH_3Hg] = 45–115$		
Phytoplankton (25 μm)	Northern Rio de Janeiro State, southeastern Brazil	ND	$[Hg] = 21$		[133]
Phytoplankton	13 different locations	$[CH_3Hg] = 0.09–0.58$	$[CH_3Hg] = 0.3–0.8$[a]	3.3–4.2	[136]

[a] Accumulated Hg concentrations in picomoles per gram wet weight.
CH_3Hg = organic methylated Hg; ND = not determined.

negligible. This was successfully demonstrated recently by Luengen and Flegal in the San Francisco Bay estuary [30]. They expressed phytoplankton Hg accumulation by dividing the change in the amount of dissolved Hg by the increase in algal biomass, to tease out the role of phytoplankton in Hg cycling.

Algal Hg accumulation data for field experiments are sometimes reported as bioaccumulation factors (BAFs; nmol of Hg per kg dry [or wet] wt of algae divided by nmol of Hg per L of ambient water), with $\log_{10}$ BAF values varying from 4.7 to 5.9 for Hg^{II} and from 4.8 to 6.2 for CH_3Hg (Tables 2 and 3). Although BAFs have proved valuable in the examination of the fate of Hg in relation to particle behavior in aquatic systems, their use to express algal accumulation capacities is inherently flawed. Indeed, BAF calculations do not take into account metal speciation in ambient waters but rather use total dissolved metal concentrations. However, inorganic and organic ligands are present in natural waters and complex metals such as Hg, to a certain degree, and as such will affect its bioavailability [52]. For example, values of $\log_{10}$ BAF for Cd accumulated by freshwater phytoplankton collected in Lake Orta (Switzerland) were 4.4 and 6.4 when calculated with total dissolved Cd and free Cd^{2+} concentrations, respectively ($\log_{10}$ BAF calculated from accumulated Cd values read in Figure 3c in the original article) [93]. The use of the total dissolved Cd concentration in this latter study would have underestimated the capacity of phytoplankton to accumulate Cd from ambient waters by a factor of 100. Such underestimations would be expected to be even more important for Hg since Hg^{II} and CH_3Hg are predominantly bound to DOM in natural waters, and this complexation decreases Hg uptake by natural algal communities with few exceptions (see *Influence of DOM on Hg uptake by phytoplankton*).

HG TOXICITY TOWARD PHYTOPLANKTON

Once inside algal cells, accumulated Hg will bind to cytosolic ligands and distribute into organelles. The binding to Hg could in principle induce toxicity by blocking the functional groups of essential biomolecules (e.g., enzymes), by displacing essential ions from such sites, or by modifying their conformation. If the binding occurs instead to molecules involved in detoxification processes, then toxicity could well be limited [51]. To diagnose which of these subcellular fates predominates, it would be useful to determine the intracellular distribution of Hg. By disrupting the algal cells and subjecting the resulting suspension to differential centrifugation and heat treatment, one can define various operationally defined fractions: cellular debris (nuclei, membranes, cell walls), granules, organelles (mitochondria and chloroplast), heat-denaturated proteins (e.g., enzymes), and heat-stable proteins and peptides (e.g., glutathione and phytochelatins) [94,95]. The fractions "organelles" and "heat-denaturated proteins" represent the Hg-sensitive fractions; Hg binding to these physiologically important fractions will tend to induce a stress for the algae, whereas the fractions "granules" and "heat-stable proteins" are involved in cellular protection against Hg. Thus, the intracellular distribution of Hg among these fractions could determine its impacts on the studied alga. In a recent study with 3 phytoplanktonic species [95], the diatom *T. pseudonana* was observed to be the species that was the most sensitive to Hg^{II} (median inhibitory concentration [IC50] = 285 $\pm$ 1 nM) and that contained the highest concentrations of intracellular Hg^{II}. Moreover, the diatom had the highest proportion of Hg^{II} in the metal-sensitive fractions, especially in organelles, whereas *Chlorella autotrophica*, the species least sensitive to Hg^{II}, had the highest proportion of Hg^{II} in the detoxified fraction, mainly in heat-stable proteins. When exposed to CH_3Hg, the 3 algae also showed different sensitivities, with *T. pseudonana* being the least sensitive (IC50 = 4.4 nM) and *C. autotrophica* the most sensitive (IC50 = 2.4 $\pm$ 0.1 nM). In the 3 algae, intracellular CH_3Hg was mainly bound to heat-stable proteins (50%–85% of intracellular CH_3Hg), whereas among the sensitive metal fractions, the organelles were the major target sites. This organelle fraction contains mitochondria and chloroplasts, the latter being the site of photosynthetic activities inside algal cells.

These findings are in agreement with earlier studies that showed the strong impact of Hg on the photosynthetic systems of phytoplankton [96,97]. The mechanisms underlying Hg effects on algae are dependent on the Hg exposure concentrations, although virtually all of the reported work has been done at environmentally unrealistic concentrations. For example, at concentrations of 6 μM, Hg impacted electron transport activity in the cyanobacterium *Synechococcus* by interfering at the level of the intersystem electron transport carrier, whereas at even higher concentrations (24 μM and 36 μM), Hg also affected electron transport activity in photosystems II and I [98]. Such Hg interactions with photosynthetic systems have been observed to lead to a decrease in the quantum yield of photosynthesis in *Spirulina platensis* exposed to up to 20 μM Hg for 2 h and to a change in photosystem II photochemistry [99]. Lower Hg concentrations (at the nanomolar level) were also found to impact the photosystem of 6 algal species [100]. For example, exposure of *Microcystis aeruginosa* to 3.7 nM Hg for 5 h decreased the dark-adapted maximum fluorescence (F_m) and the light-adapted maximum fluorescence (F'_m), as measured with pulse-amplitude modulation fluorometry, by 25% and 30% compared with the control, respectively. However, a recent study has demonstrated that CH_3Hg (up to 27 nM) did not have any effect on the electron transfer chain in *T. weissflogii* [101].

Mercury has also been reported to increase reactive oxygen species concentrations in the green alga *C. reinhardtii* exposed to Hg at concentrations up to 8 μM, which led to cellular lipid peroxidation and decreased growth by 56% at a Hg concentration of 4 μM [102]. Reactive oxygen species, which are normal by-products of oxidative reactions occurring in cells, can become harmful when their concentrations are not tightly controlled [103]. Some metals can increase reactive oxygen species concentrations by several mechanisms, such as by damaging photosynthetic systems, impacting iron-mediated processes, or decreasing the antioxidant pool. In the case of Hg, impacts on algal photosynthetic systems might be a plausible mechanism.

The observed effects of Hg at the algal cellular level might be expected to lead to inhibition of algal population growth (Table 4). Because the bioavailable forms of Hg^{II} and CH_3Hg are still unknown, the median effective concentration (EC50) values in the table are expressed using total Hg^{II} and CH_3Hg concentrations; EC50 values expressed in this manner are inherently sensitive to variation in the composition of the exposure solutions. Nevertheless, when considering only the lipophilic metal complexes, both CH_3HgCl^0 and $HgCl_2{}^0$ were found to induce 50% growth inhibition for the diatom *T. weissflogii* at 500 pM, suggesting that Hg^{II} and CH_3Hg might have roughly the same toxic potential. However, a recent study showed that even though both Hg^{II} and CH_3Hg impacted algal growth, their modes of toxic action were different [101]; CH_3Hg reduced the algal growth rate, whereas Hg^{II} induced strong damage to the cells, though the remaining live cells divided at normal rates.

Table 4. Concentrations of inorganic Hg (Hg^{II}) and organic methylated Hg (CH_3Hg) at which algal growth or maximal photosystem II quantum yield is reduced by half (EC50)

Algal species	Medium	Measured end points	EC50 (Hg^{II}) (nM)	EC50 (CH_3Hg) (nM)	Reference
Thalassiosira pseudonana	Mediterranean surface water,	Cell densities	3.2		[137]
Dunaliella tertiolecta	spiked with N, P, vitamins		5000		
Emiliania huxleyi	(at f/2 concentrations), and		800		
Oscillatoria woronichinii	Si (at f/10 concentrations);		2.0		
	no added metals, no EDTA				
Thalassiosira weissflogii	FRAQUIL, $[Cl^-] = 10^{-5} - 0.32$ M, pH = 4–8, $t_{expo} = 4$ h	Cell densities	0.5 ($HgCl_2^0$)[a]	0.5 (CH_3HgCl)[b]	[58]
Microcystis aeruginosa	C medium (for *M. aeruginosa*,	Maximal photosystem	65		[100]
Selenastrum capricornutum	*A. falcatus*, and *Nannoplankton*)	II quantum yield	1282		
Ankistrodesmus falcatus	and AAP medium (for *S. capricornutum*)		1412		
Nannoplankton	without EDTA, [Fe]/100, and [metal]/2		1700		
T. pseudonana	Hong Kong shore water, spiked with N, P,	Cell densities	258 ± 1	4.39	[95]
Chlorella autotrophica	vitamins, and Si (at f/2 concentrations)		482 ± 3	2.44 ± 0.1	
Isochrysis galbana			281 ± 5	3.29	
T. weissflogii	f/2 medium without trace metal stock solutions	Cell densities	250		[101]

[a,b]Read on Figure 6c and d, respectively.
FRAQUIL = artificial freshwater medium.

In conclusion, given that growth inhibition in algae has only been demonstrated at Hg^{II} and CH_3Hg concentrations that are rarely, if ever, encountered in natural waters, phytoplankton will most likely not be impacted by ambient Hg concentrations at the population level in the environment.

HG DETOXIFICATION BY PHYTOPLANKTON

To prevent Hg toxicity, phytoplankton use 2 strategies: limiting Hg accumulation and controlling its intracellular speciation (Figure 2). Excretion of accumulated Hg does not seem to be a significant detoxification mechanism, because of its strong intracellular binding (see *Hg Toxicity Toward Phytoplankton*). To avoid Hg accumulation inside their cells, some algae take advantage of the redox properties of Hg and transform intracellular Hg into its gaseous form, Hg^0 [47,104,105]. This reduction is very rapid (onset within the first hour of exposure) and depends on the duration of the algal exposure to Hg. Higher volatilization rates were measured in *Euglena gracilis* exposed to 5 μM Hg^{II} for 1 h (2.2 fmol Hg $cell^{-1}\,h^{-1}$) than for 3 h (0.7 ± 0.2 fmol Hg $cell^{-1}\,h^{-1}$) [106]. Algal Hg reduction rates also depend on Hg exposure concentrations but not on the presence of light [64,106]. The diatom *T. weissflogii* exposed to 5 nM Hg^{II} produced 92 ± 16 zmol Hg $cell^{-1}\,h^{-1}$ (zmol = 10^{-21} mol) in the light and 90 ± 18 zmol Hg $cell^{-1}\,h^{-1}$ in the dark [64]. These values are in good agreement with those measured with various phytoplankton species, the Hg^0 production rates of which ranged from 4.2 zmol Hg $cell^{-1}\,h^{-1}$ to 21 zmol Hg $cell^{-1}\,h^{-1}$ when exposed to 0.5 nM Hg^{II} [47].

The mechanism by which algae reduce Hg is still unknown. Reduction of Cu(II) to Cu(I) has been reported to occur at algal cell surfaces, whereas intracellular Hg reduction in bacteria is known to be performed enzymatically by the so-called Hg^{II} reductase [11,107]. Algal Hg methylation has also been investigated, stimulated largely because of the known ability of algae to methylate other metals such as As^{III} [108], but no evidence has yet been found for Hg methylation in algae [109].

Algal Hg volatilization has often been observed to be accompanied by another mechanism of detoxification—namely, an increase in reduced glutathione (GSH) content, induction of phytochelatin PCn synthesis, and formation of *meta*cinnabar, β-HgS [64,106,109]. All of these components have the capacity to bind intracellular Hg and to minimize its nonspecific binding to physiologically important biomolecules. Glutathione (γ–glutamylcysteinylglycine; γGluCysGly) is the predominant thiol in algal cells, with concentrations up to the millimolar level, and it is involved in various metabolic processes, including the storage and transport of reduced sulfur, the control of oxidative stress, and the detoxification of some xenobiotics and metals [110]. Its concentration has been reported to increase in *C. reinhardtii*, *E. gracilis*, and *T. weissflogii* cells exposed to Hg^{II} [64,106,111].

Glutathione plays another indirect role in Hg detoxification, as the precursor for phytochelatin synthesis. Phytochelatins are small polypeptides with an amino acid composition of $(\gamma GluCys)_nGly$, with $n = 2$ to 11; they have a higher capacity to bind Hg than does GSH [112]. Although earlier studies reported little or no induction of phytochelatins in algae exposed to Hg, concentrations of phytochelatins with degrees of polymerisation 2 and 3 have recently been observed to increase in *T. weissflogii* exposed to [Hg^{II}] between 5 and 150 nM [64,111,113]. In contrast, CH_3Hg seems to be a poor inducer of phytochelatins [114]. Finally, algae can also detoxify Hg by sequestering it in the form of β-HgS [105,109]. Its formation is also very rapid, within minutes to a few hours, and it is present in a variety of algal species, from chlorophytes to diatoms. This detoxification strategy is very complementary to algal Hg^{II} reduction. In the

same study, the manipulation of the intracellular thiol concentration in *Chlorella fusca* with dimethylfuramate led to an increase in Hg volatilization. The formation of CdS crystallite has also been reported in *C. reinhardtii* [115]. Such metal–sulfide complexes form high–molecular weight compounds in microorganisms, the solubility of which is enhanced by complexation with phytochelatins [116].

INFLUENCE OF PHYTOPLANKTON ON HG TROPHIC TRANSFER

Photosynthetic primary producers are a key entry point of Hg into aquatic food chains. Mercury that has been accumulated is transferred to primary consumers, whose Hg content will be available for higher organisms [38] (Figure 1). However, this transfer depends on Hg speciation and on algal quality and density.

The extent to which Hg passes from phytoplankton to grazers depends on the form of Hg present in the algal cells, Hg^{II} having a lower assimilation efficiency than CH_3Hg [38,91,117,118]. This metal form–specific behavior in trophic transfer is determined by the intracellular metal distribution in algae [119]; metals binding preferentially to algal cell membranes, such as Hg^{II}, have been shown to be less efficiently assimilated than metals incorporated in the cytoplasm, such as CH_3Hg. Copepods feeding on the marine diatom *T. weissflogii* laden with CH_3Hg and Hg^{II} were observed to contain 62% of CH_3Hg but only 15% of Hg^{II}; the preferential assimilation of CH_3Hg was attributed to the very low proportion of Hg^{II} in the algal cytosol (9%) compared with CH_3Hg (63%) [58]. The very low concentrations of CH_3Hg in natural waters and its high propensity to transfer between phytoplankton and grazers make phytoplankton the primary source of CH_3Hg for zooplankton [120]. Field data indicate that CH_3Hg concentrations increase between phytoplankton and zooplankton by a factor of about 2 [81,92,121]. A recent study in a simulated trophic chain showed that the trophic transfer factor (which expresses the ratio between the assimilation efficiency times the weight-specific ingestion rate divided by the efflux rate constant [k_e] of CH_3Hg for a mixture of microalgae [*C. reinhardtii* and *C. meneghiniana*] and *Daphnia pulex*) was between 26 and 40, whereas the trophic transfer factor between *D. pulex* and *Fundulus heteroclitus* (a killifish) ranged between 1 and 8 [122], demonstrating the importance of food transfer between algae and *D. pulex*. The tight relation between Hg concentrations in phytoplankton and in consumers such as *Daphnia* can also be observed in the field. During the mixing of stratified waters in Devil's Lake (WI, USA), dissolved CH_3Hg concentrations in the former hypolimnion were observed to decrease. This decrease was accompanied by an increase in CH_3Hg concentrations in particles (sizes 2.2–35 μm, mostly phytoplankton) and *Daphnia* species present in the former epilimnion, suggesting the transfer of CH_3Hg along the food chain during the autumn water turnover [123].

The biomagnification of Hg along the food chain also depends on algal quality. *Daphnia* fed with high-quality food (algae with C:P = 139) were shown to accumulate one-third less CH_3Hg than those fed with low-quality food (algae with C:P = 1320), because of the higher growth and slightly lower ingestion rates for the animals fed with high-quality food [124]. However, this quality-related phenomenon depends on the consumers. For example, the copepod *Eurytemora affinis* assimilated less CH_3Hg when fed with degenerating algae than when fed with living algae, whereas the amphipod *Hyallela azteca* accumulated CH_3Hg to the same extent in both cases [117]. Lawson and Mason [117] explained this species-specific difference on the basis of the gut passage time of CH_3Hg in the studied organisms, which is shorter and thus less efficient in the copepod than in the amphipod.

Ambient phytoplankton density also has a direct impact on Hg accumulation by grazers and subsequent consumers. A mesocosm experiment showed that the trophic transfer of CH_3Hg, but not Hg^{II}, between phytoplankton and 3 zooplankton species (2 copepods and 1 cladoceran) was reduced when phytoplankton growth was stimulated with nutrient enrichment [91,118]. This result was explained by a biodilution effect—that is, by a higher algal growth rate, which led to lower accumulated Hg per algal cell and, thus, lower Hg transfer to grazers. The "phytoplankton density" effect has been confirmed in the field. High seston density was found to correlate with a decrease in Hg accumulation in the pico-, nano-, and macro-seston size fractions [88]. It follows that primary consumers living in systems with elevated productivity will tend to have lower Hg^{II} and CH_3Hg concentrations than those living in systems with lower productivity [125]. The phenomenon was found to be more important than the influence of other well-known water chemistry factors, such as pH or the DOM concentration, in predictions of zooplankton Hg concentrations in 20 lakes in the northeastern United States [126]. High algal concentrations have also been shown to decrease Cd, Zn, and Se assimilation by copepods from their diet [127]. In this case, biodilution was excluded as an explanation and the decrease of the gut passage time with higher algal densities was found to be responsible for the lower metal assimilation efficiency at higher algal densities. Exceptions to the Hg biodilution effect also exist. In 2 dystrophic lakes on the Canadian Shield, Hg accumulated by seston (expressed as picomoles of Hg per liter of filtered water) was found to be positively correlated with algal biomass proxies such as dry weight ($r^2 = 0.64$) and chlorophyll-*a* ($r^2 = 0.85$) [128].

CONCLUSION AND PERSPECTIVE

Phytoplankton are involved in key processes controlling the fate of Hg in surface waters (Figure 1). Algal Hg uptake directly affects dissolved Hg^{II} and CH_3Hg concentrations, and phytoplankton can also affect Hg speciation in the water column, with the production of Hg^0 and the possible release of Hg bound to reduced sulfur species (e.g., as complexes with glutathione and phytochelatins and as *meta*cinnabar) during their microbial degradation. The extent to which these phenomena affect Hg cycling in natural waters is still partly unknown, but field studies described in the present review suggest that they cannot be neglected if one wishes to understand Hg fate, especially in eutrophic lakes during algal bloom periods. Field data are thus still needed to better define Hg interactions with phytoplankton, with as much seasonal and geographical difference as possible. Information about the phytoplankton community will be necessary, such as algal density, species composition, or quantity of chlorophyll-*a* on a dry-weight basis, as well as information about Hg speciation in the ambient water column, which is very challenging. To address the speciation question, basic water-quality parameters should be measured—including pH, chloride, and DOM concentrations—so as to be able to calculate Hg speciation with equilibrium chemical models.

Based on the measured dissolved Hg concentrations in natural waters (1–100 pM), phytoplankton should not be inhibited by the presence of Hg in their ambient environment.

However, this statement could justifiably be criticized, given that we still do not know precisely which Hg species control Hg bioavailability and how Hg enters algal cells under natural environmental conditions. Regarding algal uptake of Hg, there are some indications that CH_3Hg may be taken up by facilitated transport and passive diffusion (as its chloro complex), whereas Hg^{II} could diffuse passively through biological membranes, also as a (di)chloro complex. No evidence for facilitated transport of Hg^{II} has yet been reported for phytoplankton (Figure 2).

From experiments run at much higher Hg concentrations, we do know that intracellular Hg binds to physiologically important organelles such as chloroplasts, affects photosynthesis, and induces an imbalance in intracellular reactive oxygen species concentrations. In such cases, phytoplankton use different detoxification mechanisms such as the volatilization of intracellular Hg in the form of Hg^0 or its sequestration in the form of *meta*cinnabar. The trophic transfer of Hg should be influenced by the detoxification form involved and still needs to be investigated.

SUPPLEMENTAL DATA

Section S1. (26 KB DOCX).

Acknowledgment—S. Le Faucheur would like to thank former and present colleagues and students (Y. Tremblay, A. Garcia Bravo, E. Portilla Castillo, and P. Dranguet). Helpful comments provided by 2 anonymous reviewers were appreciated. S. Le Faucheur and V.I. Slaveykova gratefully acknowledge the financial support provided by Swiss National Science Foundation project IZERZ0–142228. C. Fortin and P.G.C. Campbell are supported by the Canada Research Chair program.

REFERENCES

1. Wetzel RG. 2001. *Limnology: Lake and River Ecosystems*, 3rd ed. Academic, San Diego, CA, USA.
2. Stumm W, Morgan JJ. 1996. Trace metals: Cycling, regulation, and biological role. In Stumm W, Morgan JJ, eds, *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters*, 3rd ed. Environmental Science and Technology, Wiley, New York, NY, USA, pp 614–671.
3. Sunda WG. 2012. Feedback interactions between trace metal nutrients and phytoplankton in the ocean. *Front Microbiol* 3:1–22.
4. Stein ED, Cohen Y, Winer AM. 1996. Environmental distribution and transformation of mercury compounds. *Crit Rev Environ Sci Technol* 26:1–43.
5. Morel FMM, Kraepiel AML, Amyot M. 1998. The chemical cycle and bioaccumulation of mercury. *Annu Rev Ecol Syst* 29:543–566.
6. Fitzgerald WF, Lamborg CH, Hammerschmidt CR. 2007. Marine biogeochemical cycling of mercury. *Chem Rev* 107:641–662.
7. Douglas TA, Loseto LL, Macdonald RW, Outridge P, Dommergue A, Poulain A, Amyot M, Barkay T, Berg T, Chetelat J, Constant P, Evans M, Ferrari C, Gantner N, Johnson MS, Kirk J, Kroer N, Larose C, Lean D, Nielsen TG, Poissant L, Rognerud S, Skov H, Sorensen S, Wang FY, Wilson S, Zdanowicz CM. 2012. The fate of mercury in Arctic terrestrial and aquatic ecosystems: A review. *Environ Chem* 9:321–355.
8. Vost EE, Amyot M, O'Driscoll NJ. 2012. Photoreactions of mercury in aquatic systems. In Liu GL, Cai Y, O'Driscoll NJ, eds, *Environmental Chemistry and Toxicology of Mercury*. Wiley, Hoboken, NJ, USA, pp 193-218.
9. Lin CC, Yee N, Barkay T. 2012. Microbial transformations in the mercury cycle. In Liu G, Cai Y, O'Driscoll NJ, eds, *Environmental Chemistry and Toxicology of Mercury*. Wiley, Hoboken, NJ, USA, pp 155–192.
10. Ullrich SM, Tanton TW, Abdrashitova SA. 2001. Mercury in the aquatic environment: A review of factors affecting methylation. *Crit Rev Environ Sci Technol* 31:241–293.
11. Barkay T, Miller SM, Summers AO. 2003. Bacterial mercury resistance from atoms to ecosystems. *FEMS Microbiol Rev* 27:355–384.
12. Leopold K, Foulkes M, Worsfold P. 2010. Methods for the determination and speciation of mercury in natural waters—A review. *Anal Chim Acta* 663:127–138.
13. Mason RP, Sullivan KA. 1997. Mercury in Lake Michigan. *Environ Sci Technol* 31:942–947.
14. Lindstrom M. 2001. Distribution of particulate and reactive mercury in surface waters of Swedish forest lakes—An empirically based predictive model. *Ecol Model* 136:81–93.
15. Brigham ME, Wentz DA, Aiken GR, Krabbenhoft DP. 2009. Mercury cycling in stream ecosystems. 1. Water column chemistry and transport. *Environ Sci Technol* 43:2720–2725.
16. Choe KY, Gill GA, Lehman R. 2003. Distribution of particulate, colloidal, and dissolved mercury in San Francisco Bay estuary. 1. Total mercury. *Limnol Oceanogr* 48:1535–1546.
17. Choe KY, Gill GA. 2003. Distribution of particulate, colloidal, and dissolved mercury in San Francisco Bay estuary. 2. Monomethylmercury. *Limnol Oceanogr* 48:1547–1556.
18. Stordal MC, Santschi PH, Gill GA. 1996. Colloidal pumping: Evidence for the coagulation process using natural colloids tagged with Hg-203. *Environ Sci Technol* 30:3335–3340.
19. Babiarz CL, Hurley JP, Hoffmann SR, Andren AW, Shafer MM, Armstrong DE. 2001. Partitioning of total mercury and methylmercury to the colloidal phase in freshwaters. *Environ Sci Technol* 35:4773–4782.
20. Stordal MC, Gill GA, Wen LS, Santschi PH. 1996. Mercury phase speciation in the surface waters of three Texas estuaries: Importance of colloidal forms. *Limnol Oceanogr* 41:52–61.
21. Ravichandran M. 2004. Interactions between mercury and dissolved organic matter—A review. *Chemosphere* 55:319–331.
22. Skyllberg U, Bloom PR, Qian J, Lin C-M, Bleam WF. 2006. Complexation of mercury(II) in soil organic matter: EXAFS evidence for linear two-coordination with reduced sulfur groups. *Environ Sci Technol* 40:4174–4180.
23. Hesterberg D, Chou JW, Hutchinson KJ, Sayers DE. 2001. Bonding of Hg(II) to reduced organic sulfur in humic acid as affected by S/Hg ratio. *Environ Sci Technol* 35:2741–2745.
24. Yoon SJ, Diener LM, Bloom PR, Nater EA, Bleam WF. 2005. X-ray absorption studies of CH_3Hg^+-binding sites in humic substances. *Geochim Cosmochim Acta* 69:1111–1121.
25. Drexel RT, Haitzer M, Ryan JN, Aiken GR, Nagy KL. 2002. Mercury(II) sorption to two Florida Everglades peats: Evidence for strong and weak binding and competition by dissolved organic matter released from the peat. *Environ Sci Technol* 36:4058–4064.
26. Haitzer M, Aiken GR, Ryan JN. 2002. Binding of mercury(II) to dissolved organic matter: The role of the mercury-to-DOM concentration ratio. *Environ Sci Technol* 36:3564–3570.
27. Dong W, Liang L, Brooks S, Southworth G, Gu B. 2010. Roles of dissolved organic matter in the speciation of mercury and methylmercury in a contaminated ecosystem in Oak Ridge, Tennessee. *Environ Chem* 7:94–102.
28. Powell KJ, Brown PL, Byrne RH, Gadja T, Hefter G, Sjöberg S, Wanner H. 2005. Chemical speciation of environmentally significant heavy metals with inorganic ligands. Part 1: The Hg^{2+}-Cl^-, OH^-, CO_3^{2-}, SO_4^{2-}, and PO_4^{3-}aqueous systems (IUPAC report). *Pure Appl Chem* 77:739–800.
29. Hsu-Kim H, Sedlak DL. 2005. Similarities between inorganic sulfide and the strong Hg(II)-complexing ligands in municipal wastewater effluent. *Environ Sci Technol* 39:4035–4041.
30. Luengen AC, Flegal AR. 2009. Role of phytoplankton in mercury cycling in the San Francisco Bay estuary. *Limnol Oceanogr* 54:23–40.
31. Heimbürger L-E, Cossa D, Marty J-C, Migon C, Averty B, Dufour A, Ras J. 2010. Methylmercury distributions in relation to the presence of nano- and picophytoplankton in an oceanic water column (Ligurian Sea, north-western Mediterranean). *Geochim Cosmochim Acta* 2010: 5549–5559.
32. Twiss MR, Campbell PGC, Auclair JC. 1996. Regeneration, recycling, and trophic transfer of trace metals by microbial food-web organisms in the pelagic surface waters of Lake Erie. *Limnol Oceanogr* 41:1425–1437.
33. Bianchi TS, Lambert C. 1995. Plant pigments as biomarkers of high–molecular-weight dissolved organic carbon. *Limnol Oceanogr* 40:422–428.
34. Saba GK, Steinberg DK, Bronk DA. 2011. The relative importance of sloppy feeding, excretion, and fecal pellet leaching in the release of dissolved carbon and nitrogen by *Acartia tonsa* copepods. *J Exp Mar Biol Ecol* 404:47–56.

35. Keller DP, Hood RR. 2011. Modeling the seasonal autochthonous sources of dissolved organic carbon and nitrogen in the upper Chesapeake Bay. *Ecol Model* 222:1139–1162.
36. Lamborg CH, Fitzgerald WF, Skoog A, Visscher PT. 2004. The abundance and source of mercury-binding organic ligands in Long Island Sound. *Mar Chem* 90:151–163.
37. Han S, Gill GA, Lehman RD, Choe K-Y. 2006. Complexation of mercury by dissolved organic matter in surface waters of Galveston Bay, Texas. *Mar Chem* 98:156–166.
38. Watras CJ, Back RC, Halvorsen S, Hudson RJM, Morrison KA, Wente SP. 1998. Bioaccumulation of mercury in pelagic freshwater food webs. *Sci Total Environ* 219:183–208.
39. Kirk JL, Muir DCM, Antoniades D, Douglas MSV, Evans MS, Jackson TA, Kling H, Lamoureux S, Lim DSS, Pienitz R, Smol JP, Stewart K, Wang XW, Yang F. 2011. Climate change and mercury accumulation in Canadian high and subarctic lakes. *Environ Sci Technol* 45:964–970.
40. Outridge PM, Sanei H, Stern GA, Goodsite M, Hamilton PB, Carrie J, Goodarzi F, Macdonald RW. 2011. Comment on climate change and mercury accumulation in Canadian high and subarctic lakes. *Environ Sci Technol* 45:6703–6704.
41. Kirk JL, Muir DCG, Antoniades D, Douglas MSV, Evans MS, Jackson TA, Kling H, Lamoureux S, Lim DSS, Pienitz R, Smol JP, Stewart K, Wang XW, Yang F. 2011. Response to comment on climate change and mercury accumulation in Canadian high and subarctic lakes. *Environ Sci Technol* 45:6705–6706.
42. Outridge PM, Sanei H, Stern GA, Hamilton PB, Goodarzi F. 2007. Evidence for control of mercury accumulation rates in Canadian high arctic lake sediments by variations of aquatic primary productivity. *Environ Sci Technol* 41:5259–5265.
43. Stern GA, Sanei H, Roach P, Dalaronde J, Outridge PM. 2009. Historical interrelated variations of mercury and aquatic organic matter in lake sediment cores from a subarctic lake in Yukon, Canada: Further evidence toward the algal-mercury scavenging hypothesis. *Environ Sci Technol* 43:7684–7690.
44. Cooke CA, Wolfe AP, Michelutti N, Balcom PH, Briner JP. 2012. A Holocene perspective on algal mercury scavenging to sediments of an arctic lake. *Environ Sci Technol* 46:7135–7141.
45. Poulain AJ, Amyot M, Findlay D, Telor S, Barkay T, Hintelmann H. 2004. Biological and photochemical production of dissolved gaseous mercury in a boreal lake. *Limnol Oceanogr* 49:2265–2275.
46. Poulain AJ, Garcia E, Amyot M, Campbell PGC, Raofie F, Ariya PA. 2007. Biological and chemical redox transformations of mercury in fresh and salt waters of the high Arctic during spring and summer. *Environ Sci Technol* 41:1883–1888.
47. Mason RP, Morel FMM, Hemond HF. 1995. The role of micro-organisms in elemental mercury formation in natural waters. *Water Air Soil Pollut* 80:775–787.
48. Simkiss K, Taylor MG. 1995. Transport of metals across membranes, In Tessier A, Turner D, eds, *Metal Speciation and Bioavailability in Aquatic Systems*. IUPAC Series on Analytical and Physical Chemistry of Environmental Systems. Wiley, Chichester, UK, pp 1-44.
49. Hanikenne M, Merchant SS, Hamel P. 2009. Transition metal nutrition: A balance between deficiency and toxicity. In Harris EH. ed, *The Chlamydomonas Sourcebook*, Vol. 2: Elsevier, Oxford, UK, pp 333–400.
50. Blaby-Haas CE, Merchant SS. 2012. The ins and outs of algal metal transport. *Biochim Biophys Acta* 1823:1531–1552.
51. Mason AZ, Jenkins KD. 1995. Metal detoxification in aquatic organisms. In Tessier A, Turner D, eds, *Metal Speciation and Bioavailability in Aquatic Systems*. IUPAC Series on Analytical and Physical Chemistry of Environmental Systems. Wiley, Chichester, UK, pp 479–608.
52. Campbell PGC. 1995. Interactions between trace metals and organisms: A critique of the free-ion activity model. In Tessier A, Turner D, eds, *Metal Speciation and Bioavailability in Aquatic Systems*. IUPAC Series on Analytical and Physical Chemistry of Environmental Systems. Wiley, Chichester, UK, pp 45–102.
53. Vigneault B, Campbell PGC. 2005. Uptake of cadmium by freshwater green algae: Effects of pH and aquatic humic substances. *J Phycol* 41:55–61.
54. Slaveykova VI, Wilkinson KJ. 2002. Physicochemical aspects of lead bioaccumulation by *Chlorella vulgaris*. *Environ Sci Technol* 36:969–975.
55. Kola H, Wilkinson KJ. 2005. Cadmium uptake by a green alga can be predicted by equilibrium modelling. *Environ Sci Technol* 39:3040–3047.
56. Di Toro DM, Allen HE, Bergman HL, Meyer JS, Paquin P, Santore RC. 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environ Toxicol Chem* 20:2383–2396.
57. Campbell PGC, Errecalde O, Fortin C, Hiriart-Baer WR, Vigneault B. 2002. Metal bioavailability to phytoplankton—Applicability of the biotic ligand model. *Comp Biochem Phys Part C Toxicol Pharmacol* 133:189–206.
58. Mason RP, Reinfelder JR, Morel FMM. 1996. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. *Environ Sci Technol* 30:1835–1845.
59. Gutknecht J. 1981. Inorganic mercury (Hg^{2+}) transport through lipid bilayer membrane. *J Membr Biol* 61:61–66.
60. Bienvenue E, Boudou A, Desmazes JP, Gavach C, Georgescauld D, Sandeaux J, Sandeaux R, Seta P. 1984. Transport of mercury compounds across bimolecular lipid membranes: Effect of lipid composition, pH and chloride concentration. *Chem-Biol Interact* 48:91–101.
61. Fortin C, Campbell PGC. 2000. Silver uptake by the green alga *Chlamydomonas reinhardtii* in relation to chemical speciation: Influence of chloride. *Environ Toxicol Chem* 19:2769–2778.
62. Le Faucheur S, Fortin C, Campbell PGC. 2011. Acidification increases mercury uptake by a freshwater alga, *Chlamydomonas reinhardtii*. *Environ Chem* 8:612–622.
63. Boullemant A, Fortin C, Lavoie M, Campbell PGC. 2009. Uptake of hydrophobic metal complexes by three freshwater algae: Unexpected influence of pH. *Environ Sci Technol* 43:3308–3314.
64. Morelli E, Ferrara R, Bellini B, Dini F, Di Giuseppe G, Fantozzi L. 2009. Changes in the non-protein thiol pool and production of dissolved gaseous mercury in the marine diatom *Thalassiosira weissflogii* under mercury exposure. *Sci Total Environ* 408:286–293.
65. Moye HA, Miles CJ, Phlips EJ, Sargent B, Merritt KK. 2002. Kinetics and uptake mechanisms for monomethylmercury between freshwater algae and water. *Environ Sci Technol* 36:3550–3555.
66. Dhaka A, Viswanath V, Patapoutian A. 2006. TRP ion channels and temperature sensation. *Annu Rev Neurosci* 29:135–161.
67. Pickhardt PC, Fisher NS. 2007. Accumulation of inorganic and methylmercury by freshwater phytoplankton in two contrasting water bodies. *Environ Sci Technol* 41:125–131.
68. Miles CJ, Moye HA, Phlips EJ, Sargent B. 2001. Partitioning of monomethylmercury between freshwater algae and water. *Environ Sci Technol* 35:4277–4282.
69. Golding GR, Kelly CA, Sparling R, Loewen PC, Rudd JWM, Barkay T. 2002. Evidence for facilitated uptake of Hg(II) by *Vibrio anguillarum* and *Escherichia coli* under anaerobic and aerobic conditions. *Limnol Oceanogr* 47:967–975.
70. Kelly CA, Rudd JWM, Holoka MH. 2003. Effect of pH on mercury uptake by an aquatic bacterium: Implications for Hg cycling. *Environ Sci Technol* 37:2941–2946.
71. Golding GR, Kelly CA, Sparling R, Loewen PC, Barkay T. 2007. Evaluation of mercury toxicity as a predictor of mercury bioavailability. *Environ Sci Technol* 41:5685–5692.
72. Wilkinson KJ, Buffle J. 2004. Critical evaluation of physicochemical parameters and processes modelling the biological uptake of trace metals in environmental (aquatic) systems, In van Leeuwen HP, Koster W, eds, *Physicochemical Kinetics and Transport at Biointerfaces*. Wiley, Chichester, UK, pp 445-533.
73. Driscoll CT, Blette V, Yan C, Schofield CL, Munson R, Holsapple J. 1995. The role of dissolved organic-carbon in the chemistry and bioavailability of mercury in remote Adirondack lakes. *Water Air Soil Pollut* 80:499–508.
74. Sveinsdottir AY, Mason RP. 2005. Factors controlling mercury and methylmercury concentrations in largemouth bass (*Micropterus salmoides*) and other fish from Maryland reservoirs. *Arch Environ Contam Toxicol* 49:528–545.
75. Gorski PR, Armstrong DE, Hurley JP, Krabbenhoft DP. 2008. Influence of natural dissolved organic carbon on the bioavailability of mercury to a freshwater alga. *Environ Pollut* 154:116–123.
76. Luengen AC, Fisher NS, Bergamaschi BA. 2012. Dissolved organic matter reduces algal accumulation of methylmercury. *Environ Toxicol Chem* 31:1712–1719.
77. Zhong H, Wang W-X. 2009. Controls of dissolved organic matter and chloride on mercury uptake by a marine diatom. *Environ Sci Technol* 43:8998–9003.
78. Gorski PR, Armstrong DE, Hurley JP, Shafer MM. 2006. Speciation of aqueous methylmercury influences uptake by a freshwater alga (*Selenastrum capricornutum*). *Environ Toxicol Chem* 25:534–540.
79. Schaefer JK, Morel FMM. 2009. High methylation rates of mercury bound to cysteine by *Geobacter sulfurreducens*. *Nature Geoscience* 2:123–126.
80. Ndu U, Mason RP, Zhang H, Lin S, Visscher PT. 2012. Effect of inorganic and organic ligands on the bioavailability of methylmercury

as determined by using a *mer-lux* bioreporter. *Appl Environ Microbiol* 78:7276–7282.
81. Watras CJ, Bloom NS. 1992. Mercury and methylmercury in individual zooplankton: Implications for bioaccumulation. *Limnol Oceanogr* 37:1313–1318.
82. Andersson P, Borg H, Kärrhage P. 1995. Mercury in fish muscle in acidified and limed lakes. *Water Air Soil Pollut* 80:889–892.
83. Southworth GR, Peterson MJ, Ryon MG. 2000. Long-term increased bioaccumulation of mercury in largemouth bass follows reduction of waterborne selenium. *Chemosphere* 41:1101–1105.
84. Belzile N, Chen Y-W, Gunn JM, Tong J, Alarie Y, Delonchamp T, Lan C-Y. 2006. The effect of selenium on mercury assimilation by freshwater organisms. *Can J Fish Aquat Sci* 63:1–10.
85. Yang D-Y, Chen Y-W, Gunn JM, Belzile N. 2008. Selenium and mercury in organisms: Interactions and mechanisms. *Environ Rev* 16:71–92.
86. François L, Fortin C, Campbell PGC. 2007. pH modulates transport rates of manganese and cadmium in the green alga *Chlamydomonas reinhardtii* through non-competitive interactions: Implications for an algal BLM. *Aquat Toxicol* 84:123–132.
87. Wang W, Wong RSK, Wang J, Yen Y. 2004. Influences of different selenium species on the uptake and assimilation of Hg(II) and methylmercury by diatoms and green mussels. *Aquat Toxicol* 68:39–50.
88. Adams RM, Twiss MR, Driscoll CT. 2009. Patterns of mercury accumulation among seston in lakes of the Adirondack mountains, New York. *Environ Sci Technol* 43:4836–4842.
89. Marrugo-Negrete J, Benitez LN, Olivero-Verbel J. 2008. Distribution of mercury in several environmental compartments in an aquatic ecosystem impacted by gold mining in northern Colombia. *Arch Environ Contam Toxicol* 55:305–316.
90. Carroll RWH, Memmott J, Warwick JJ, Fritsen CH, Bonzongo JCJ, Acharya K. 2011. Seasonal variation of mercury associated with different phytoplankton size fractions in Lahontan Reservoir, Nevada. *Water Air Soil Pollut* 217:221–232.
91. Pickhardt PC, Folt CL, Chen CY, Klaue B, Blum JD. 2005. Impacts of zooplankton composition and algal enrichment on the accumulation of mercury in an experimental freshwater food web. *Sci Total Environ* 339:89–101.
92. Hammerschmidt CR, Fitzgerald WF. 2006. Bioaccumulation and trophic transfer of methylmercury in Long Island Sound. *Arch Environ Contam Toxicol* 51:416–424.
93. Knauer K, Ahner B, Xue HB, Sigg L. 1998. Metal and phytochelatin content in phytoplankton from freshwater lakes with different metal concentrations. *Environ Toxicol Chem* 17:2444–2452.
94. Lavoie M, Le Faucheur S, Fortin C, Campbell PGC. 2009. Cadmium detoxification strategies in two phytoplankton species: Metal binding by newly synthesized thiolated peptides and metal sequestration in granules. *Aquat Toxicol* 92:65–75.
95. Wu Y, Wang WX. 2011. Accumulation, subcellular distribution and toxicity of inorganic mercury and methylmercury in marine phytoplankton. *Environ Pollut* 159:3097–3105.
96. Kamp-Nielsen L. 1971. The effect of deleterious concentrations of mercury on the photosynthesis and growth of *Chlorella pyrenoidosa*. *Physiol Plant* 24:556–561.
97. De Filippis LF, Hampp R, Ziegler H. 1981. The effects of sublethal concentrations of zinc, cadmium and mercury on *Euglena. Arch Microbiol* 128:407–411.
98. Murthy SDS, Mohanty P. 1993. Mercury ions inhibit photosynthetic electron transport at multiple sites in the cyanobacterium *Synechococcus* 6301. *J Biosci* 18:355–360.
99. Lu CM, Chau CW, Zhang JH. 2000. Acute toxicity of excess mercury on the photosynthetic performance of cyanobacterium, *S. platensis*—Assessment by chlorophyll fluorescence analysis. *Chemosphere* 41:191–196.
100. Juneau P, Dewez D, Matsui S, Kim SG, Popovic R. 2001. Evaluation of different algal species sensitivity to mercury and metolachlor by PAM-fluorometry. *Chemosphere* 45:589–598.
101. Wu Y, Zeng Y, Qu JY, Wang W-X. 2012. Mercury effects on *Thalassiosira weissflogii*: Applications of two-photon excitation chlorophyll fluorescence lifetime imaging and flow cytometry. *Aquat Toxicol* 110-111:133–140.
102. Elbaz A, Wei YY, Meng Q, Zheng Q, Yang ZM. 2010. Mercury-induced oxidative stress and impact on antioxidant enzymes in *Chlamydomonas reinhardtii*. *Ecotoxicology* 19:1285–1293.
103. Pinto E, Sigaud-Kutner TCS, Leitao MAS, Okamoto OK, Morse D, Colepicolo P. 2003. Heavy metal–induced oxidative stress in algae. *J Phycol* 39:1008–1018.
104. Ben-Bassat D, Mayer AM. 1977. Reduction of mercury chloride by *Chlorella*: Evidence for a reducing factor. *Physiol Plant* 40:157–162.
105. Kelly D, Budd K, Lefebvre DD. 2006. Mercury analysis of acid- and alkaline-reduced biological samples: Identification of *meta*-cinnabar as the major biotransformed compound in algae. *Appl Environ Microbiol* 72:361–367.
106. Devars S, Avilés C, Cervantes C, Moreno-Sánchez R. 2000. Mercury uptake and removal by *Euglena gracilis*. *Arch Microbiol* 174:175–180.
107. Jones GJ, Palenik BP, Morel FMM. 1987. Trace metal reduction by phytoplankton: The role of plasmalemma redox enzymes. *J Phycol* 23:237–244.
108. Phillips D. 1990. Arsenic in aquatic organisms: A review, emphasizing chemical speciation. *Aquat Toxicol* 16:151–186.
109. Kelly DJA, Budd K, Lefebvre DD. 2007. Biotransformation of mercury in pH-stat cultures of eukaryotic freshwater algae. *Arch Microbiol* 187:45–53.
110. Rennenberg H. 1982. Glutathione metabolism and possible biological roles in higher plants. *Phytochemistry* 21:2771–2781.
111. Howe G, Merchant S. 1992. Heavy metal–activated synthesis of peptides in *Chlamydomonas reinhardtii*. *Plant Physiol* 98:127–136.
112. Mehra RK, Miclat J, Kodati R, Abdullah R, Hunter TC, Mulchandani P. 1996. Optical spectroscopic and revers-phase HPLC analysis of Hg^{2+} binding to phytochelatins. *Biochem J* 314:73–82.
113. Ahner BA, Morel FMM. 1995. Phytochelatin production in marine algae. 2. Induction by various metals. *Limnol Oceanogr* 40:658–665.
114. Wu Y, Wang W-X. 2012. Thiol compounds induction kinetics in marine phytoplankton during and after mercury exposure. *J Hazard Mater* 217-218:271–278.
115. Hu S, Lau KWK, Wu M. 2001. Cadmium sequestration in *Chlamydomonas reinhardtii*. *Plant Sci* 16:987–996.
116. Winge DR, Dameron CT, Mehra RK. 1992. Metal:sulfide quantum crystallites in yeast. In Stillman MJ, Shaw FC, Suzuki KT, eds, *Metallothioneins: Synthesis. Structure and Properties of Metallothioneins, Phytochelatins and Metal–Thiolate Complexes*. VCH, New York, NY, USA, pp 257-270.
117. Lawson NM, Mason RP. 1998. Accumulation of mercury in estuarine food chains. *Biogeochem* 40:235–247.
118. Pickhardt PC, Folt CL, Chen CY, Klaue B, Blum JD. 2002. Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. *Proc Natl Acad Sci USA* 99:4419–4423.
119. Reinfelder JR, Fisher NS. 1991. The assimilation of elements ingested by marine copepods. *Science* 251:794–796.
120. Tsui MTK, Wang WX. 2004. Uptake and elimination routes of inorganic mercury and methylmercury in *Daphnia magna*. *Environ Sci Technol* 38:808–816.
121. Becker DS, Bigham GN. 1995. Distribution of mercury in the aquatic food web of Onondaga Lake, New-York. *Water Air Soil Pollut* 80:563–571.
122. Mathews T, Fisher NS. 2008. Evaluating the trophic transfert of cadmium, polonium, and methylmercury in an estuarine food chain. *Environ Toxicol Chem* 27:1093–1101.
123. Herrin RT, Lathrop RC, Gorski PR, Andren AW. 1998. Hypolimnetic methylmercury and its uptake by plankton during fall destratification: A key entry point of mercury into lake food chains? *Limnol Oceanogr* 43:1476–1486.
124. Karimi R, Chen CY, Pickhardt PC, Fisher NS, Folt CL. 2007. Stoichiometric controls of mercury dilution by growth. *Proc Natl Acad Sci USA* 104:7477–7482.
125. Chen CL, Kamman N, Williams J, Bugge D, Taylor V, Jackson B, Miller E. 2012. Spatial and temporal variation in mercury bioaccumulation by zooplankton in Lake Champlain (North America). *Environ Pollut* 161:343–349.
126. Chen CY, Stemberger RS, Kamman N, Mayes BM, Folt CL. 2005. Patterns of Hg bioaccumulation and transfer in aquatic food webs across multi-lake studies in the northeast US. *Ecotoxicology* 14:135–147.
127. Xu Y, Wang WX. 2001. Individual responses of trace-element assimilation and physiological turnover by the marine copepod *Calanus sinicus* to changes in food quantity. *Mar Ecol Prog Ser* 218:227–238.
128. Kirkwood AE, Chow-Fraser P, Mierle G. 1999. Seasonal mercury levels in phytoplankton and their relationship with algal biomass in two dystrophic shield lakes. *Environ Toxicol Chem* 18:523–532.
129. Schetagne R, Doyon JF, Fournier JJ. 2000. Export of mercury downstream from reservoirs. *Sci Total Environ* 260:135–145.
130. Roulet M, Lucotte M, Guimaraes JRD, Rheault I. 2000. Methylmercury in water, seston, and epiphyton of an Amazonian river and its floodplain, Tapajos River, Brazil. *Sci Total Environ* 261:43–59.

131. Bargagli R, Monaci F, Sanchez-Hernandez JC, Cateni D. 1998. Biomagnification of mercury in an Antarctic marine coastal food web. *Mar Ecol Prog Ser* 169:65–76.
132. Nfon E, Cousins IT, Jarvinen O, Mukherjee AB, Verta M, Broman D. 2009. Trophodynamics of mercury and other trace elements in a pelagic food chain from the Baltic Sea. *Sci Total Environ* 407:6267–6274.
133. Di Beneditto APM, Bittar VT, Camargo PB, Rezende CE, Kehrig HA. 2012. Mercury and nitrogen isotope in a marine species from a tropical coastal food web. *Arch Environ Contam Toxicol* 62:264–271.
134. Laurier FJG, Cossa D, Gonzalez JL, Breviere E, Sarazin G. 2003. Mercury transformations and exchanges in a high turbidity estuary: The role of organic matter and amorphous oxyhydroxides. *Geochim Cosmochim Acta* 67:3329–3345.
135. Kehrig HA, Palermo EFA, Seixas TG, Branco CWC, Moreira I, Malm O. 2009. Trophic transfer of methylmercury and trace elements by tropical estuarine seston and plankton. *Estuar Coast Shelf Sci* 85:36–44.
136. Mason RP, Choi AL, Fitzgerald WF, Hammerschmidt CR, Lamborg CH, Soerensen AL, Sunderland EM. 2012. Mercury biogeochemical cycling in the ocean and policy implications. *Environ Res* 119:101–117.
137. Fisher NS, Bohe M, Teyssie JL. 1984. Accumulation and toxicity of Cd, Zn, Ag, and Hg in four marine phytoplankters. *Mar Ecol Prog Ser* 18:201–213.