

Appendix 2B-2: Status Report on the Effect of Water Quantity and Quality on Methylmercury Production

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THE EFFECT OF THE SULFUR CYCLE ON METHYLMERCURY PRODUCTION

In 1994 a consortium of agencies led by the United States Geological Survey (USGS) began a multi-investigator study of the factors contributing to the high levels of mercury (Hg) in Everglades biota. Named the Aquatic Cycling of Mercury in the Everglades (ACME) Project, the study focused on the processes that lead from Hg deposition to methyl mercury (MeHg) formation and bioaccumulation. Initial hypotheses that MeHg production is the key control on MeHg bioaccumulation, and that MeHg production is generally favored in wetlands, were born out (Hurley et al., 1998; Krabbenhoft et al., 1998; Gilmour et al., 1998a,b; Marvin-DiPasquale et al., 1998; 2000). Among the additional findings, the ACME Project identified the important influence of sulfur (S) inputs to the Everglades on Hg methylation within the ecosystem (Gilmour et al., 1998a,b; Cleckner et al., 1999; Bates et al., in press; Orem et al., in review).

Surface water sulfate concentrations in northern Water Conservation Area 2A (WCA-2A) average roughly 50 mg/L, more than 100 to 1,000 times higher than sulfate levels in Everglades National Park (ENP or Park) and in southern WCA-3A (Orem et al., in review; Gilmour et al., 1998b; EPA/REMAP). Sulfur stable isotope data and the depth-based historical record suggest that sulfur concentrations in Everglades peats are elevated above historical ambient concentrations over most of the ecosystem (Bates et al., 2001, and in press). Sulfur may enter the Everglades from a number of potential sources, including canal water discharge, rainfall and groundwater. Another important source is sulfur amendments to agricultural fields in the Everglades Agricultural Area (EAA), used to enhance phosphorus (P) uptake by sugarcane and other crops through modification of soil acidity. This sulfur has a stable sulfur isotopic signal distinct from most other sources. This signal has been observed in Everglades Agricultural Area (EAA) soils and in the sulfate in canal water draining the EAA. The high levels of sulfate observed in canal water in the EAA and in areas of the Everglades that receive canal water discharge is thought to arise from runoff of this agricultural sulfur (Bates et al., 2001 and in press; Orem et al., in review).

Sulfur enrichment in the Everglades is a stressor, with specific impacts on MeHg production, as well as wider impacts on the ecosystem. In the Everglades, MeHg is generally produced in the top few cm of unconsolidated detritus and consolidated peat soil (Gilmour et al., 1998b; Cleckner et al., 1999) by obligately anaerobic bacteria, primarily sulfate-reducing bacteria (SRB)

(Compeau and Bartha, 1985; Gilmour et al., 1991; Gilmour et al., 1998a). Sulfate-reducing bacteria degrade organic matter in sediments, using sulfate as the electron acceptor. The end product of microbial sulfate-reduction activity is sulfide. Anaerobic microbial processes, including sulfate reduction, are key components of microbial organic carbon decomposition in Everglades peats and surficial flocs. The primary factors that control the metabolic activity of SRB are temperature, dissolved oxygen (DO), organic carbon supply and sulfate supply.

Aside from its impact on MeHg production in the Everglades, sulfur contamination of the ecosystem may have other deleterious effects. Sulfur appears to be a major control on redox conditions in the sediments. Everglades areas contaminated with sulfur from canal water discharge (e.g. WCA-2A and northern WCA-3A) have significantly more negative (more reducing) redox conditions compared to sites with background levels of sulfur (e.g. WCA-1, southern WCA-3A and much of ENP; Orem et al., in prep.). The lower redox conditions in the sediment may negatively affect rooted macrophytes not adapted to these highly reducing conditions by reducing oxygen flow to the roots. Tree islands may also be negatively impacted by the more reducing soil conditions resulting from sulfur contamination. Further study is needed to evaluate the extent of these impacts.

Microbial sulfate reduction appears to be the most important mechanism for reduced S storage in Everglades peat. Stable isotope signatures show that the majority of reduced S stored in Everglades sediments, at both eutrophic and more pristine sites, arises from dissimilatory sulfate reduction rather than assimilation by plants (Bates et al., 1998 and 2001; Orem et al., in review; Kendall et al., in prep.). Sulfate reduction was readily measured in surface sediments across the Everglades using isotopic tracers (Gilmour et al., 1998; Heyes et al., 1998).

Net MeHg accumulation in surface sediments represents the balance of microbial methylation and photochemical and microbial demethylation processes. Because Everglades food webs are generally benthic, the concentration of MeHg in surface sediments is an excellent predictor of MeHg in biota (Krabbenhof et al., 1998). Instantaneous methylation rates are also good predictors of MeHg concentrations in surface sediments (Gilmour et al., 2001; Heyes et al., 2001). Methylmercury concentrations in the top 0 to 4 cm of sediment are now used as primary indicators of MeHg production in the Everglades.

Because SRB are important Hg methylators, sulfate influences methylmercury production. However, the interaction between the Hg and S cycles is complex. While sulfur has a significant impact on MeHg production, the magnitude and even the direction of the impact varies with the sulfate and sulfide concentration. Sulfate stimulates the activity of Hg-methylating microorganisms and can therefore increase MeHg concentrations (Gilmour et al., 1991; Watras et al., 1994; Branfireun et al., 1999; King et al., 1999, 2000). However, the bioavailability of Hg to methylating bacteria also affects MeHg production rates by bacteria. Sulfide has a very strong impact on the bioavailability of inorganic mercury to methylating bacteria (Compeau et al., 1983; Craig et al., 1983; Choi et al., 1994). There is an optimal sulfide concentration for Hg uptake that is quite low. Above that concentration, sulfide inhibits bioavailability. These two forms of sulfur, sulfate and sulfide, whose concentrations are often correlated across the Everglades (Orem et al., in prep; Gilmour et al., 1998b), have opposing effects on Hg methylation. Understanding the quantitative relationships between sulfate stimulation of methylation and sulfide inhibition of methylation across the large sulfur gradient that exists in the Everglades is necessary for understanding how sulfur in agricultural runoff affects MeHg in Everglades fish.

All the available data point to a MeHg production maximum in the central Everglades. Largemouth bass (Lange, pers. comm., 2002) and *Gambusia* (Krabbenhof et al., 1998, in prep.; EPA/REMAP) concentrations are highest in WCA-3A. ACME data show maxima in both MeHg

concentration and production in surface sediments in central WCA-3A (Gilmour et al., 1998b; in prep.). Based on both the ACME data set and geochemical modeling, the ACME team proposed the following model for control of MeHg production by sulfur in the Everglades: (1) high concentrations of sulfide inhibit MeHg production in the northern part of the ecosystem, (2) intermediate sulfate (~ 50 to 100 μM) and low sulfide (< 10 μM) concentrations in the central part of the system (e.g. WCA-3A) are optimal for methylation, and (3) sulfate concentrations in the most pristine areas (< 25 μM) are suboptimal for sulfate reduction and MeHg production. This scenario implies that the excess load of sulfur to the Everglades minimizes MeHg production in WCA-2A but stimulates MeHg production in WCA-2B and WCA-3A.

ACME field data and research on the impact of sulfide on methylation strongly support the hypothesis that sulfide inhibits methylation in the northern Everglades. The ACME dataset consists of triennial samples collected in from March 1995 through July 1999 from 10 sites along a generally north-to-south transect through the Everglades. In these data, a strong inverse relationship was observed between the sulfide concentration in sediment pore waters and the concentration of methylmercury in surface sediments (Gilmour et al., 1998a,b, in prep.). Of the many variables examined, pore water sulfide was the single-best predictor of sediment MeHg concentrations.

During the ACME study the chemical speciation of Hg in sulfidic waters was analyzed and the mechanism of sulfide inhibition of methylation was examined. Substantial changes in existing models for this process were derived and are leading to better modeling of Hg methylation in ecosystems (TetraTech, 2002). Benoit et al. proposed that sulfide controls Hg bioavailability to SRB by controlling the concentration of neutral dissolved Hg species that diffuse across cell membranes. This model is different from older models in that (1) sulfide inhibition of Hg methylation is not caused simply by decreased concentration of dissolved inorganic Hg (Hg_D), due to precipitation of $\text{HgS}_{(s)}$, (2) the model includes the existence of small, dissolved, neutral Hg-S complexes, and (3) the model assumes Hg uptake into cells via passive diffusion rather than through active uptake of divalent metal cations. The existence of neutral Hg-sulfide complexes, probably HgS^0 , is supported by geochemical models (Benoit et al., 1999a) and experimental partitioning studies (Benoit et al., 1999b). Benoit's model for Hg partitioning and speciation in sulfidic pore waters shows a strong relationship between predicted HgS^0 concentration in the dissolved phase and MeHg concentration in bulk sediment in two ecosystems: the Everglades and a temperate estuary.

The uptake of HgS^0 was also demonstrated in pure cultures of the methylating SRB *Desulfobulbus propionicus* (Benoit et al., 2001a). Methylmercury production by cultures was not related to the absolute solid phase concentration of Hg in the ores, and it was only weakly related to the dissolved inorganic Hg concentration in the medium. However, MeHg production was linearly related to HgS^0 , the calculated concentration of the dominant neutral complex in solution. Furthermore, the diffusive membrane permeability of HgS^0 , as estimated from its octanol water partitioning coefficient, was found to be sufficient to support MeHg production by cells (Benoit et al., 2001b). Slightly large, neutral Hg polysulfide complexes may also be found in sediment pore waters (Jay et al., 2000); however, these appear to be too large to diffuse across cell membranes at a rate sufficient to support significant Hg methylation (Jay et al., in prep.).

The idea that sulfate stimulates methylation in much of the central Everglades is less strongly supported by correlations in the ACME field data, possibly because of lower data density in the central and southern ecosystem. Sulfate does stimulate MeHg production when added to sediment cores taken from sites with lower sulfate concentrations (< 5 mg/L) (Bell et al., 1999; Gilmour et al., 2000; Gilmour et al., in prep.). In these studies, methylation rates increased linearly with sulfate addition, up to a point, and then decreased with increasing pore water sulfide

concentrations. The optimal sulfate concentration appears to vary somewhat among Everglades regions because of the way sediment chemistry affects the solubility of sulfide produced by SRB. These experimental studies support the idea that the very low sulfate concentrations found in Taylor Slough and the central Arthur R. Marshall Loxahatchee National Wildlife Refuge (Refuge) limit the activity of SRB, and therefore limit methylation rates. Sulfate concentrations in the freshwater marsh within ENP and the central Refuge probably best reflect historical sulfate concentrations, which would have been derived primarily from rain in the freshwater Everglades.

To improve the understanding of the relationships between sulfate and MeHg in the Everglades, ACME initiated sulfate-addition mesocosm studies in late 2001. The objective of these studies is to specifically test for sulfate stimulation of methylation and sulfate reduction at "lower-sulfate" sites in the Everglades. Mesocosms are being amended at two sites: one in the central Refuge and in one in central WCA-3A. Sulfate concentrations of 5, 10 and 20 mg/L are being added to the mesocosms over the course of two months. The effect of sulfate on methylation of existing Hg pools and newly deposited Hg will be tested using Hg stable isotope additions. Additionally, the dose response to Hg additions is being tested, as is the influence of dissolved organic carbon (DOC) on Hg cycling and methylation.

DOC directly affects the complexation of Hg in pore waters, and it also affects the solubility of sulfide and of Hg-S complexes. Cinnabar precipitation can be retarded or reversed in the presence of certain fractions of Everglades DOC (Ravichandran et al., 1998). Understanding the relative strength of DOC and sulfide as ligands for Hg is important to modeling Hg behavior, including methylation. Recently, Benoit et al. (2001) characterized conditional stability constants for Hg with two DOC isolates from WCA-2A. These are similar to previously published stability constants for Hg binding to low molecular weight thiols, and show a pH dependency that is consistent with models of Hg complexation, with thiol groups as the dominant Hg binding sites in dissolved organic matter (DOM). These experiments demonstrate that the DOM isolates are stronger ligands for Hg than chloride ion or EDTA. Speciation calculations indicate that at the DOM concentrations frequently measured in the Everglades (20 to 40 μM), significant complexation of Hg by DOM would be expected in aerobic (sulfide-free) surface waters. However, even nanomolar sulfide concentrations would be expected to out-compete DOM for Hg binding at any realistic ($\mu\text{l mM}$) DOM concentration (Benoit et al., 1999a). These results are consistent with the dominance of Hg-sulfide complexes in pore waters in the Everglades. However, it should be noted that interactions other than simple ligand exchange may influence the reactivity of natural DOM toward Hg in sulfidic environments. For example, Ravichandran et al. (1998, 1999) showed that F1-HPoA enhanced dissolution and inhibited precipitation of $\text{HgS}_{(s)}$ to a greater degree (by an order of magnitude) than either 2BS-HPiA or thioglycolate, although these ligands appear to have similar stability constants. Furthermore, a chemical-equilibrium approach does not address how the kinetics of Hg-S-DOM interactions might affect the complexation of Hg in sediments with dynamic sulfide and DOM pools.

The influence of sulfate and sulfide on demethylation is less well characterized. Demethylation may occur by microbial or photochemical pathways. Using 14-C-labeled methylmercury, microbial demethylation has been observed by obligate anaerobes via two different pathways (reductive or oxidative), leading to methane or carbon dioxide formation (Oremland et al., 1993; Marvin-DiPasquale and Oremland, 1998; Marvin-DiPasquale et al., 2000; Marvin-DiPasquale et al., 2001).

Methylmercury can also be decomposed to Hg(II) or Hg(0) by the action of sunlight in water or soil (Krabbenhoft et al., 2001; Krabbenhoft et al., 2002). The rate at which this occurs is determined by the concentration and character of DOC. DOC-complexed Hg(II) can be reduced to Hg(0) by the action of sunlight on DOC (Zhang and Lindberg, 2001a). Fe(III) stimulates Hg(0)

production (Zhang et al., 2001). Once produced, Hg(0) will evade directly to the atmosphere (Vandal et al., 1994; Lindberg et al., 1999; Lindberg and Zhang, 2000; Zhang and Lindberg, 2001b). Rooted macrophytes are also believed to pump Hg(0) from the root zone through stems to leaves, and thence to the air (Lindberg et al., 1999). The influence of sulfur species on these processes is unknown.

In summary, sulfur exerts one of the strongest controls on net MeHg production in the Everglades. The inhibitory response of methylation to sulfide is fairly well understood mechanistically, and a simple equation relating pore water sulfide to sediment MeHg across the Everglades can be derived. Sulfide accumulating in sediment surface pore waters appears to limit MeHg production and accumulation in northern WCA-2A and in some of the Stormwater Treatment Areas (STAs), especially the Everglades Nutrient Removal (ENR) Project. It has been proposed that the high MeHg levels found across much of the central Everglades are driven by sulfate enrichment through stimulation of the bacteria that produce MeHg. Small-scale experimental studies support this hypothesis, and larger-scale mesocosm studies are underway.

THE EFFECT OF SOIL DRYOUT ON METHYLMERCURY PRODUCTION: APPLICATIONS TO THE STORMWATER TREATMENT AREAS

Results of a joint USGS-South Florida Water Management District (SFWMD or District) study of an Everglades dryout and burn that occurred in the spring of 1999 demonstrated a pronounced methylmercury production period when the dried/burned soils across the northern Everglades were rewetted (Krabbenhoft and Fink, 2000; Krabbenhoft et al., 2000; Krabbenhoft et al., 2001a,b). Understanding the causal factors that gave rise to the elevated levels of methylmercury in the dried/burned areas of the Everglades in 1999 has a direct bearing on STA construction and management and on how the effectiveness and efficacy of the STAs are viewed within the overall Everglades restoration strategy.

The 1999 dry/burn study showed a pulse of methylmercury production in surface soils after inundation in June and July of 1999. The pulse was relatively short-lived (a few days to a few weeks), with maximal sediment MeHg concentrations in July. However, the elevated pools of methylmercury in sediments and pore waters became part of the actively cycling mass in the environment. The pulse in methylmercury production was followed by substantial increases in methylmercury in *Gambusia* and in young-of-year sunfish and largemouth bass (Lange et al., 2000). Details of this study are provided in Appendix 7-8 of the *2000 Everglades Consolidated Report* (2000 ECR).

Krabbenhoft et al. concluded that the large pulse of MeHg production was most likely due to increased sulfate after drying and rewetting, and to concomitant increases in the activity of methylating microorganisms that respire sulfate. The hydrated peat soils of the Everglades are normally anoxic within mm to cm of the soil surface, and most of the sulfur is stored in reduced forms (Bates et al., 1998; Orem et al., in review; Krabbenhoft and Fink, 2000; Fink, 2001). Drying and burning result in oxidation of soils, including oxidation of reduced sulfur stored in peat. Reinundation of oxidized soils is usually accompanied by a “first-flush” release of oxidized nutrients (Newman and Pietro, 2000) and sulfur (Krabbenhoft et al., 2000). After reinundation the peat soils quickly return to an anoxic state (Kelly et al., 1997), and anaerobic sulfate reduction is stimulated by the presence of the newly oxidized sulfur. It is important to note that in the 1999 study the concentration of inorganic mercury in surface waters and sediment pore waters was not elevated in the months following rewetting, suggesting that soil oxidation did not

release Hg, and that changes in Hg concentration are not driving the pulse of methylation. However, the study began a few weeks after inundation so some initial response might have been missed. Sulfate, sulfide and methylmercury concentrations were significantly increased in dried or burned and rewetted soils. The relatively rapid decline from peak methylmercury concentrations in pore water and soils was coincident with the rapid depletion of the elevated sulfate pool. This coincidence would suggest that the decline in methylmercury levels was a result of a sulfate depletion. However, one cannot rule out two alternative hypotheses. The first alternative hypothesis is that methylation declined after an initial pulse because of the relatively rapid buildup of sulfide after rewetting. The second alternative hypothesis is that the pulse in methylation after rewetting is driven by highly bioavailable Hg in rain.

Another gap in this puzzle is the source of mercury for the newly produced methylmercury. Inorganic mercury to feed the methylation process could be contributed by “old” mercury pools existing in the peat soils, by rain and canal water that gives rise to the reinundation, or by both. Resolution of this gap in understanding is important for prescribing the proper management response regarding how to minimize future methylmercury production periods following future drying and wetting cycles. Mercury-addition studies in mesocosms conducted under phase II of the ACME Project should provide key insights into which pools of mercury are most likely responsible for fueling this response (Krabbenhoft et al., 2001; Gilmour et al., 2001).

The effect of first flooding of dry land on inorganic mercury release, methylmercury production, and methylmercury bioaccumulation has also been observed numerous times during the construction of reservoirs (e.g. Bodaly et al., 1984; Scruton et al., 1994; Rodgers et al., 1995). This “reservoir effect” has been examined in detail in an experimentally flooded boreal peatland (St. Louis et al., 1994; St. Louis et al., 1996; Kelly et al., 1997; Paterson et al., 1998). In some settings the production of new methylmercury and bioaccumulation is a short-term phenomenon, while in other settings it can persist for decades. Long-term elevated methylmercury production in reservoirs has generally been attributed to the slow degradation of organic matter in newly flooded soils, fueling anaerobic microbial activity. Short-term responses in newly flooded wetlands may be fueled by oxidized sulfur, but these responses are not as well documented. In the extreme case, if sulfate and organic matter are present in substantial excess, sulfide can build up to the point of inhibiting methylmercury production (see above).

The ENR Project and the STAs have exhibited different behaviors with regard to methylmercury production and bioaccumulation. Throughout its lifetime, the ENR Project has exhibited very low methylmercury production and correspondingly low concentrations in fish at all trophic levels (Cleckner et al., 1998; Lange et al., 1998, 1999; Loftus et al., 1998; Rumbold et al., 2000; Rawlik, 2001a; Rumbold et al., 2001). Sulfur levels in soils and sulfate in incoming waters are both high in ENR, and it has been hypothesized that sulfide inhibition has limited the production of methylmercury there (Gilmour et al., 1998b). A pulse of methylmercury production and accumulation of mosquitofish in STA-1W cell 5 occurred within four weeks of flooding in May 1999. However, this cell relaxed back to ENR-like conditions in water and mosquitofish by January 2000. The mercury behavior of STA-1W cell 5 must be contrasted with that of STA-2 cell 1. Within about eight weeks of reflooding, the concentration of unfiltered methylmercury rose in water to an unprecedented 4.8 ng/L, which is considered anomalously high relative to the ENR Project, which averaged about 0.1 ng/L. Water column concentrations oscillated up and down thereafter to concentrations as low as 0.1 ng/L until cell 1 again began to dry out in mid-April 2001, when methylmercury concentrations rose to 4.2 ng/L. However, total mercury concentrations in mosquitofish increased throughout this same period, averaging about 350 µg/Kg wet weight when the last sample was collected in mid-March 2001. This value exceeds the

average value of about 200 µg/Kg wet weight at WCA-3A-15, the Everglades “hot spot” for mercury.

Results of the 1999 dry/burn study and of the ACME study suggest that the differences in STA behavior are most likely related to differences in wetting and drying regimes, differences in historical sulfur accumulation in soils, or differences in sulfate concentrations in treatment water. Examination of the STA sulfur regime is warranted, as are studies of mercury and sulfur cycling following rewetting in the STAs. As was noted in the *2000 Everglades Consolidated Report*, Comprehensive Everglades Restoration Plan (CERP)-related changes in drying and rewetting cycles might affect methylmercury production in the Everglades, and these processes should be considered in risk-management strategies.

NEW FINDINGS IN 2001 AND 2002

MODELING Hg METHYLATION

The equations that model Hg methylation and demethylation, and the equations that determine Hg speciation in sediment pore waters are key routines in the Everglades Mercury Cycling Model (E-MCM). Work is currently underway to revise these routines based on developments in understanding Hg complexation and uptake by methylating organisms. Revisions are being considered that would allow the calculation of HgS⁰ concentrations in sediment pore waters, which would model HgS uptake via diffusion by methylating bacteria. Work published by Benoit et al. in 2001 showed that the calculated rate of diffusion of HgS⁰ into cells of a Hg-methylating SRB, *D. propionicus*, was more than sufficient to support measured rates of Hg methylation in pure culture. Further, geochemical equilibrium models that include HgS⁰ formation and its uptake into methylating bacteria via passive diffusion explain the patterns of MeHg production found in ecosystems with sulfur gradients (Benoit et al., 1999; 2001). Nonetheless, passive diffusion has been examined only in one organism. In two studies with non-methylating microorganisms, active Hg uptake seems to be a more important accumulation pathway than diffusion. Using laboratory cultures of *Selenastrum capricornutum*, *Cosmarium botrytis* and *Schizothrix calcicola*, Moye et al. (2001) concluded that uptake of methylmercury could not be explained by the passive diffusion of a neutral methylmercury-chloride complex as had been observed by others in marine algal species and conditions (Mason et al., 1996). Golding et al. have been studying Hg uptake by a “Hg bioreporter,” *Vibrio anguillarum*, which was engineered to include the mer operon, and a reporter gene that manufactures luciferase, a light-emitting protein, when the bacterium takes up inorganic mercury (Golding et al., 2001). Hg uptake by this organism appears to occur via facilitated transport.

There is ongoing scientific debate regarding the pathways by which mercury might enter cells (passive versus active uptake, for example). Some efforts to model algal uptake of MeHg to match observations in lake environments of the E-MCM models has suggested active, rather than passive transport as the dominant mechanism (Hudson et al., 1994; R. Harris, Tetra Tech, Inc., pers. comm.). It should be noted that the relative importance of active and passive transport could be very different for bacteria taking up Hg(II) in anaerobic conditions versus algae taking up methylmercury in surface waters. Differing cell sizes, water chemistry, and mercury forms would all influence the rates of active and passive uptake.

ACME MESOCOSMS STUDIES

As a followup to the ACME ecosystem-scale study of Hg biogeochemistry in the Florida Everglades, the impacts of four key biogeochemical parameters are being studied individually using *in situ* mesocosms. ACME identified sulfur, Hg, and potentially DOC as key variables that influence MeHg production and bioaccumulation in the Everglades. A fourth biogeochemical parameter, nutrients, appeared to exert a lesser effect on MeHg production. Everglades restoration efforts are underway, beginning with STA construction and including changes in flow path, rates in water storage and, possibly, controls on Hg emissions. These changes are intended primarily to reduce nutrient loads to the Everglades, to restore a more natural flow and hydroperiod, and to provide water storage and flood control to South Florida. STAs are designed to reduce phosphate loading to the northern Everglades, but so far the STAs do not appear to have had a substantial impact on sulfate loading (Miles and Fink, 1998). However, other restoration efforts that change the timing, path and rate of flow may substantially alter delivery of nutrients, sulfur and Hg to the Everglades.

To model the potential effects of Everglades restoration efforts on Hg cycling, understanding of the relationships between sulfur, nutrient and Hg loading, and MeHg production and bioaccumulation must improve and should be separately quantified. Sulfur, nutrients and DOC generally co-vary across the ecosystem because they arise from similar sources, making it difficult to quantify the individual relationships between of the each variables and MeHg production and bioaccumulation.

In 2000 ACME began a series of experiments to better quantify these individual relationships and the interactions among these key parameters through amendments of Hg, S, DOC, and nutrients, individually and in combination, to *in situ* mesocosms. Although ACME has conducted short-term additional experiments to examine these relationships, it may not predict long-term responses for several reasons. Response of plant growth to nutrients is the obvious example, but other changes, such as changes in Hg speciation and bioavailability over time, or development of microbial communities, are also important. An understanding of the relationship between Hg, S, DOC or nutrient loading and MeHg production and bioaccumulation requires a long-term, large-scale approach because there are many steps between the entry of inorganic Hg to the ecosystem, its conversion to the methylated form, and its bioaccumulation in fish.

ACME phase-II mesocosm studies began with Hg dose-response studies at four sites across the Everglades. Mercury loading experiments were conducted in replicate in 1.0-m diameter enclosures at WCAs 2A, 2B and 3A. In May and September 2000, three mesocosms at each site were dosed with a Hg-stable isotope. Use of Hg-stable isotopes has allowed determination of MeHg production and accumulation from the new stable isotope Hg spike separately from the existing Hg pools. For comparison, short-term methylation rates were also assessed using a different isotope. Using ICP-MS methylation of Hg-stable isotopes added at levels of about 5 percent of the existing pool in sediments was detectable.

The preliminary results from phase II mesocosm studies indicated that inorganic mercury dosed to the water's surface was rapidly deposited in the unconsolidated detritus layer atop the consolidated peat soil, with a half-life of about one or two days. Methylmercury produced from the stable isotope spike began to appear within 24 to 48 hours of dosing. Most methylmercury production occurred within three days of dosing. MeHg produced from the spike accumulated rapidly in mosquitofish (*Gambusia holbrooki*), with maxima probably a few weeks after the spike. The increase in MeHg in surface sediments and in fish showed a linear response to the Hg

dose. However, the slope of the response was highly variable among sites. Sites that support high levels of *in situ* MeHg production and bioaccumulation were also most sensitive to Hg additions.

The extremely rapid response in MeHg production to the Hg spikes suggests that newly deposited Hg is much more available for methylation and bioaccumulation than existing Hg in surface soils. However, the bioavailability of “new” Hg for methylation and bioaccumulation decreased rapidly through time.

During 2001 and 2002, sulfate, Hg, and DOC additions will be made to the ACME mesocosms, with a focus on responses to sulfate additions at low-sulfate sites. In late 2000, a fifth set of mesocosms was placed in the Refuge to provide a very low sulfate site at which to conduct these experiments. The Refuge site also provides a contrast in calcium and DOC concentrations with the intermediate-level sulfate site at WCA-3A-15.

Hg-DOC Complexation

Understanding and modeling Hg complexation chemistry is a key to modeling bioavailability. A number of efforts are underway to understand Hg and MeHg complexation with DOC, including interaction between Hg, DOC, and solid surfaces, particularly cinnabar and iron oxides.

Dissolved organic matter (DOM) has been implicated as an important complexing agent for Hg that can affect its mobility and bioavailability in aquatic ecosystems. However, binding constants for natural Hg-DOM complexes are not well known. Benoit et al. (in press) employed a competitive ligand approach to estimate conditional stability constants for Hg complexes with DOM isolates collected from Everglades surface waters. The isolates examined were the hydrophobic fraction of DOM from a eutrophic, sulfidic site (F1-HPoA), and the hydrophilic fraction from an oligotrophic, low-sulfide site (2BS-HPiA). Experimental determinations utilized overall octanol water partitioning coefficients (D_{ow}) for ^{203}Hg at 0.01 M chloride and across pH and DOM concentration gradients. Use of this radioisotope allowed rapid determinations of Hg concentrations in both water and octanol phases without problems that result from matrix interference.

Conditional stability constants ($I = 0.06$, 23°C) were: $\log K' = 11.9$ for F1-HPoA and $\log K' = 10.6$ for 2BS-HPiA. These are similar to previously published stability constants for Hg binding to low molecular-weight thiols. Further, F1-HPoA showed a pH-dependent decline in D_{ow} consistent with models of Hg complexation, with thiol groups as the dominant Hg binding sites in DOM. These experiments demonstrate that the DOM isolates are stronger ligands for Hg than either chloride ion or EDTA. Speciation calculations indicate that at the DOM concentrations frequently measured in the Everglades (20 to 40 μM), significant complexation of Hg by DOM would be expected in aerobic (sulfide-free) surface waters. However, even nanomolar sulfide concentrations would be expected to out-compete DOM for Hg binding at any realistic (≤ 1 mM) DOM concentration, through the reaction $\text{Hg}^{2+} + \text{HS}^- + \text{HgS}_{(aq)} + \text{H}^+$ ($\log K = 26.5$; Benoit et al., 1999a), for instance.

These results are consistent with the dominance of Hg-sulfide complexes in Everglades pore waters, as predicted in the model previously put forward in Benoit et al. (1999a). However, it should be noted that interactions other than simple ligand exchange may influence the reactivity of natural DOM toward Hg in sulfidic environments. For example, Ravichandran et al. (1998, 1999) showed that F1-HPoA enhanced dissolution and inhibited precipitation of $\text{HgS}_{(s)}$ to a greater degree (by an order of magnitude) than either 2BS-HPiA or thioglycolate, though the work suggests that the Hg complexes with these ligands have similar stability constants.

Therefore, the ligand exchange reactions considered for the aerobic Hg-Cl-DOM chemical system might not adequately reflect the reactivity of DOM in the anaerobic Hg-sulfide-DOM system. Furthermore, a chemical equilibrium approach does not address how the kinetics of Hg-S-DOM interactions may affect the complexation of Hg in sediments with dynamic sulfide and DOM pools.

Miller and Mason (2001) are examining inorganic mercury binding to ferric oxide-hydroxide precipitate surfaces. It has been suggested that Fe redox cycling influences the distribution of Hg and MeHg between solid and aqueous phases in aquatic environments, in particular that Fe(III) oxyhydroxides at the sediment/water interface may block diffusive Hg and MeHg efflux from sediments through stronger complexation than that which occurs with reduced Fe complexes in anoxic sediment below. To examine the strength of Fe(II)/mercury interactions, binding experiments were conducted in which Hg and MeHg were mixed with freshly synthesized ferric oxide with or without the presence of DOM. The DOM isolates generated by George Aiken and used above by Benoit were also used in this work. This work showed that Hg(II) forms bonds with two Fe oxide surface hydroxyl groups through two reactions, while the binding of MeHg to Fe oxide occurs through two different mechanisms. The binding of Hg with Fe oxide was greater than the binding of MeHg. However, in the presence of DOM the binding of Hg and MeHg to Fe oxide is controlled by the interaction of the DOM with the Fe oxide surface, not the direct binding of Hg or MeHg to ferric oxide. At low concentrations, DOC enhanced the binding MeHg to Fe oxide through the formation of ternary complexes; in general, however, DOC acted as a competing ligand for Hg and MeHg and reduced their binding to Fe oxide.

With regard to Hg and MeHg efflux from sediments, this new information suggests that binding of Hg and MeHg to the iron oxyhydroxide layer at the surface of many sediments is generally weaker than in past models because DOC generally acts to reduce Hg and MeHg binding to the oxidized Fe surfaces. Nevertheless, reduction of iron oxyhydrides in the surface layers of sediments would result in the release of Hg and MeHg through the dissolution of the solid iron phase, whether Hg and MeHg are released as DOC complexes or otherwise. Aiken et al. are continuing their studies of the influence of DOC on cinnabar dissolution (Ravichandran, 1998, 1999).

Studies have been conducted regarding the influence of sulfate and iron using sediment slurries (Mark Marvin-DiPasquale). Marvin-DiPasquale and co-workers at USGS-Menlo Park examined the effects of iron, sulfate and sulfide on net methylmercury production using Everglades soil homogenates dosed with radioactive ^{203}Hg and ^{14}C -methylmercury. Under anaerobic conditions they found that the methylmercury production rate increased exponentially with temperature, while demethylation rates were virtually temperature independent. Further, the temperature sensitivity of methylmercury production varied across the Everglades' chemical and ecological gradients (Marvin-DiPasquale et al., 2001). This suggests either that different microbial communities are involved in methylmercury production along the agricultural chemical gradients, or that strong geochemical gradients of sulfur, carbon and/or iron mediate the availability of Hg(II) to methylating bacteria differently in various regions of the system.

Marvin-DiPasquale and co-workers also found that excess, higher concentrations of sulfate, sulfide and ferrous sulfide (pyrite) slurry inhibited net methylmercury production. Ferrous chloride stimulated net methylmercury production at one site only: WCA-3A-15. This suggests that the influence of the iron cycle on the mercury cycle is complex and is mediated by the bacterial communities, the sulfur cycle, and soil and pore water redox potentials and chemistries.

Effect of phosphate enrichment on MeHg production (ACME-SFWMD)

McCormick and others at the SFWMD conducted phosphate enrichment mesocosms studies at four sites in the Everglades during the last two to three years (McCormick et al., 1999). These experimental systems provided the opportunity to examine the influence of phosphate on MeHg production separately from other factors (such as sulfate) that co-vary with nutrients across the Everglades. Phosphate might influence net MeHg production directly either through effects on the growth of methylating and demethylating bacteria, or by affecting the complexation and therefore the bioavailability of Hg. However, experiments in which phosphate was added to sediment cores suggest that phosphate had no direct effect on net methylation (Gilmour et al., 2000). More likely, phosphate might indirectly effect net MeHg production through enhanced plant growth, leading to higher organic carbon supply to sediment microorganisms, and possibly to changed redox conditions in sediments. The organic matter supply to sediments affects microbial activity in sediments and would control sulfate reduction and sulfide production rates at locations where sulfate is not limiting. Further, DOC acts as a strong ligand for Hg (Ravichandra et al., 1999; Benoit et al., 2000) and for MeHg (Hintelmann et al., 1995; Miller et al., 2001) and may inhibit the uptake of MeHg into biota. Nutrient effects on Hg cycling that are mediated through plant growth should be examined over the longer term. During 2000, ACME scientists worked with Newman and others to measure MeHg concentrations in surface sediments in the mesocosms. At the time of sampling, the mesocosms were at or near steady state with respect to responses to phosphate additions. This provided an opportunity to observe any effects of enhanced plant growth on net MeHg production.

The SFWMD conducted phosphate-enrichment mesocosms experiments at four sites with a range of *in situ* phosphate enrichment, from the moderately enriched site U3 in WCA-2A, to more pristine sites in central WCA-3A, in the central Refuge, and in Taylor Slough in Everglades National Park (ENP). While phosphate enrichment significantly changed plant and periphyton communities in the mesocosms, phosphate enrichment changed MeHg concentrations in surface sediments by less than a factor of three at any site. Further, there was no trend across sites in the direction of any MeHg response to PO₄ loading (Gilmour et al., 2001). To put these responses in context, they should be compared with the more than 100-fold range in MeHg concentrations and production rates across the Everglades, from eutrophic northern WCA-2A to the MeHg maxima in central WCA-3A. These *in situ* mesocosms studies confirm and extend smaller-scale studies showing little direct or indirect effect of phosphate on MeHg production and accumulation in surface sediments.

RELATED RESEARCH

The METAALICUS project is a whole-ecosystem, mercury loading experiment, the findings of which will directly affect research and management of Hg in the Everglades. METAALICUS is being carried out at the Experimental Lakes Area (ELA) in northwestern Ontario, Canada. The ELA, set aside by the Canadian Federal Government, is a unique area where researchers can manipulate remote lakes and can also study lakes over a period of many decades. The METAALICUS study site consists of an entire catchment, including uplands, wetlands and a first-order drainage lake. Some major questions being addressed by METAALICUS are:

- What is the relationship between the amount of Hg deposited from the atmosphere and the amount of methylmercury in fish?
- How quickly will Hg in fish respond to a change in Hg deposition?

- How bioavailable is newly deposited Hg relative to existing Hg pools in sediments and soils?

These are key questions that must be considered with respect to making future regulatory decisions about controlling Hg emissions. The total amount of Hg stored in soils and sediments of an ecosystem is many times greater than the amount entering via annual deposition. If these “old” Hg pools are equally as mobile and bioavailable as newly deposited Hg, then it will take many decades for Hg emissions controls to have an effect on Hg levels in fish.

To address these questions, METAALICUS uses two powerful techniques that are new to the Hg research community: (1) the use of stable Hg isotopes, and (2) the manipulation of a whole watershed. By adding Hg to ecosystems as specific stable isotopes, new Hg deposition can be traced in separately existing Hg pools in sediments and soils. Use of stable Hg isotopes allows researchers to study the relative bioavailability of Hg through time and after Hg deposition. This was not possible in the past. An understanding of the relationship between Hg loading and MeHg production and bioaccumulation requires a whole-ecosystem approach because there are many steps between the entry of inorganic Hg to the ecosystem, its conversion to the methylated form, and its bioaccumulation in fish.

The METAALICUS team includes 17 principal investigators from institutions in Canada and the United States and nearly 60 scientists, some of whom also conduct Hg research in the Florida Everglades. The total cost for METAALICUS over a four-year period will be about \$10 million (U.S.). Initial pilot studies were conducted in 1999 and 2000; the full-scale experimental addition began in June 2001.

Many of the techniques and approaches being developed in METAALICUS are being developed in parallel in the Everglades. In particular, the ACME team is using Hg-stable isotopes to study the timing and magnitude of the Hg dose response in Everglades mesocosms (Krabbenhoft et al., 2001; Gilmour et al., 2001). Parallel modeling efforts are also being developed in both ecosystems (Harris et al., 2001).

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