Appendix 7-4: Status of Methylmercury Production Studies

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BACKGROUND

In 1994, a consortium of agencies began a multi-investigator study of the factors contributing to the high levels of Hg in Everglades biota, the Aquatic Cycling of Mercury in the Everglades (ACME) project. The overall objective has been to understand Hg cycling well enough to create management strategies that will minimize MeHg bioaccumulation in the Everglades, while fulfilling other management objectives such as nutrient reduction and hydroperiod restoration. Methylmercury production appears to be favored in wetlands and impounded wetlands, which can produce and export MeHg (e.g. St. Louis et al., 1994; Krabbenhoft et al., 1995; Branfireun et al., 1996, Gilmour et al., 1998; Heyes et al., in prep.) in quantities that may ultimately lead to elevated fish MeHg concentrations (Driscoll et al., 1994; Cleckner et al., 1998; Krabbenhoft et al., in prep). High rates of microbial MeHg production, driven by high organic matter inputs and advective flows of nutrient bearing water, are the probable cause. The ACME project has focused on the processes that lead to from Hg deposition to MeHg formation and bioaccumulation.

What drives the high levels of MeHg in fish and other biota in the Florida Everglades? Is increased loading of Hg the more important factor, or are biogeochemical controls on Hg cycling more critical? The Everglades have been subject to many stresses of which high levels of Hg deposition (Dvonich et al., 1995; Guentzel et al., 1995) is just one. Hydroperiod alterations and eutrophication are key stressors and are the foci of restoration efforts under the Restudy. Sulfur enrichment in the Everglades is a stress with specific impacts on MeHg production. The source is sulfur amendments to agricultural fields in the EAA, used to achieve soil pH reductions after burning. Soil sulfur amendments help to reduce the amount of phosphorous fertilizer required for sugar cane production, and therefore reduce phosphorous runoff (Orem et al., 2000). However, the impact of oxidized sulfur, released as sulfate, on the Everglades Hg cycle is dramatic. Sulfate-reducing bacteria mediate Hg methylation and therefore we believe that agricultural S enhances MeHg production and bioaccumulation in much of the Everglades. Conversely, the end product of sulfate respiration, sulfide, inhibits Hg uptake by methylating bacteria, and hence MeHg production. In the most eutrophic portions of the Everglades, sulfide accumulation inhibits MeHg production. The balance between

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sulfate load and sulfide accumulation is a crucial factor in the control of MeHg production across the Everglades.

From 1995 to 1998, the ACME team studied the biogeochemical cycling of Hg in detail at a suite of sites across the Everglades. Sites spanned the roughly north to south trophic gradient generated from agricultural runoff, from the Everglades Nutrient Removal (ENR) Area, which is a re-constructed wetland, in the north, through heavily impacted Water Conservation Area (WCA) 2A, and south through more pristine WCA 2B and WCA 3A. The southern end of the ACME transect is in Taylor Slough in Everglades National Park. A site in Loxahatchee NWR was also examined as a potential analog for the historical Everglades in terms of hydroperiod and nutrient status. ACME process studies revealed a complex and dynamic Hg cycle in the Everglades. The study identified key processes that we were then able to examine across sites and across chemical gradients in the ecosystem. By combining this targeted distributional data with a variety of experimental studies, the ACME study was able to identify the key variables that influence MeHg production and bioaccumulation in the Everglades.

KEY FINDINGS OF THE ACME PROJECT:

- MeHg bioaccumulation is driven by internal MeHg production, mainly in Everglades surface sediments.
  - Gross Hg methylation rates (as measured using tracer 203Hginjected into intact sediment cores) are strongly correlated with MeHg concentrations (Gilmour et al., 1998; Gilmour et al., in prep.).
  - Methylation rate and MeHg concentration are almost always maximal at the surface of sediments (Gilmour et al., 1998; Gilmour et al., in prep.).
  - Microbial MeHg production and destruction are both very rapid in surface sediments (Gilmour et al., 1998; Marvin-DiPasquale et al 1998). However, net MeHg production appears to easily account for MeHg accumulation in sediments and in the food web.
  - MeHg concentrations in Gambusia are well correlated with MeHg concentrations in surface sediments (Krabbenhoft et al., in prep.).
  - MeHg concentrations in wet deposition are not sufficient to account for MeHg pools in the Everglades.
- MeHg concentrations in all matrices (surface sediment, sediment pore water, water, biota) are maximal in the central Everglades (WCA 2B and 3). MeHg is somewhat lower in more pristine areas like ENP and LNWR, and much lower in the most eutrophic areas, WCA 2A and ENR (Hurley et al., 1998; Clecker et al., 1998; Gilmour et al., 1998; Heyes et. al in prep.; Krabbenhoft et al., in prep.).
- The spatial MeHg pattern is not driven primarily by total Hg concentration, although there is weak but significant relationship between Hg and MeHg concentrations in surface sediments. The slope of the relationship between Hg and MeHg varies among sites, and is steepest in the central Everglades. Therefore, this part of the ecosystem is most sensitive to Hg deposition.

- Sulfur has a large impact on MeHg production, but the magnitude and even direction of the impact varies with the sulfate and sulfide concentration.
  - Of the parameters measured by our group, pore water sulfide best predicts MeHg concentration in surficial sediments. Sulfide and MeHg concentration are inversely correlated across the northern Everglades (Gilmour et al., 1998; Heyes et al., in prep).
  - Sulfate concentrations and sulfate reduction rates in ENR and WCA 2A are very high, similar to the oligohaline region of an estuary (Hurleyetal. 1998; Gilmour et al., 1998).
  - Experimental studies show inhibition of methylation by sulfide, and stimulation by sulfate, especially in the more pristine areas of the Everglades (Benoit et al., in prep.).
- Phosphate and nitrate generally had no direct effect on MeHg production rates in sediment cores.
- Anaerobic microbial processes, including sulfate reduction, are key components of microbial organic carbon decomposition in Everglades peats and surficial flocs.
  - Microbial sulfate reduction appears to be the most important mechanism for reduced S storage in Everglades peats. Stable isotope signatures show that the majority of reduced S stored in Everglades sediments, at both eutrophic and more pristine sites, arises from Assimilatory sulfate reduction rather than assimilation by plants (Orem et al., in prep).
  - Sulfate reduction was readily measured in surface sediments from all sites using isotopic tracers (Gilmour et al., 1998; Gilmour et al., in prep.).
- MeHg may leave sediments and enter food chains through solute efflux from sediment porewaters, by movement of benthic invertebrates into the water column, and by direct grazing on surface sediments and benthic invertebrates.
  - MeHg efflux from sediments has a strong diel component. Solute efflux occurs mainly at night when the sediment water interface becomes suboxic. Migration of benthic zooplankton into the water column also occurs at night.
- The mechanism of sulfide inhibition of methylation is not the expected one (precipitation of HgS)
  - Hg concentrations in Everglades sediment porewaters are controlled by sorption of Hg to the solid phase rather than precipitation of cinnabar (HgS) (Benoit et al., 1 999a).
  - Dissolved Hg complexation changes across a sulfide gradient. Neutral complexes dominate at lower (low ~M) sulfide concentrations, while charged Hg-S complexes dominate at higher sulfide concentrations (Benoit et al., 1 999b).
  - Hg is taken up by methylating bacteria through diffusion of neutral complexes across cell membranes (Benoit et al., 2000); therefore sulfide

inhibits methylation through the formation of charged complexes that are not bioavailable for uptake.

- Hg methylation rates in cell cultures, and MeHg concentrations in Everglades surface sediments can be predicted from the modeled HgS° concentration in solution (Benoit et al., 1 999a; 2000).

- If surface sediments in ENR and other STAs are sulfidic, methylation rates should remain low in re-constructed Everglades wetlands. The pool of sulfur accumulated within the agricultural soils used for ENR is large.
- Methylation is very rapid in certain types of periphyton (Cleckner et al., 1999). Methylation occurs only in "mats" where microbial sulfur cycling occurs, and is most common in the less-calcareous periphyton found in eutrophic areas. MeHg production in periphyton may provide a very direct entry point for MeHg into the aquatic food web. However, for most of the Everglades, methylation in surface sediments appears to be the dominant source of new MeHg.
- Photochemical redox processes are key components of the Hg and MeHg budgets for the Everglades. Photochemical Hg(II) reduction and evasion are key loss mechanisms for Hg from surface waters. Photodemethylation of MeHg is also rapid. Daytime MeHg photodemethylation must be balanced against nighttime sediment efflux to create a mass balance for MeHg.
- 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 (Orem et al., 2000).
- Drying events and fire can trigger massive new MeHg production, apparently triggered by increased sulfate supplied by reoxidation of reduced sulfur pools within sediments. These events may provide another mechanism to quantify how the sulfate/sulfide balance controls methylation - by monitoring the sequence of sulfur oxidation, sulfate reduction and the re-accumulation of sulfide, along with MeHg production.

We conclude that sulfur and Hg loading are key parameters that control MeHg production and bioaccumulation in the Everglades. Our model for control of MeHg production by sulfur in the Everglades is that 1) high concentrations of sulfide inhibit MeHg production in the northern part of the ecosystem, 2) intermediate sulfate (~50-100 ~M) and low sulfide (<1 0~M) concentrations in the central part of the system are optimal for methylation, and 3) sulfate concentrations in the most pristine areas (<25 ,uM) are sub-optimal 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 WCAs 2B and 3A.

Within the ACME data set, the relationships between sulfate, sulfide and MeHg in the northern (ENR to site 3A15 in WCA3) part of the system are significant. This correlative relationship is supported by experimental sulfide addition studies and by our biogeochemical models. The relationship between sulfur and methylation in the southern part of the system is less clear, mainly because of lower data density. However, data in hand show a positive relationship between sulfate and MeHg south of 3A15, and experimental studies show sulfate stimulation of methylation in surface sediments. This information supports the idea that the very low sulfate concentrations found in Taylor

Slough and central LNWR limit the activity of SRB and therefore limit methylation rates. Sulfate concentrations in the freshwater marsh within ENP, and in central LNWR probably best reflect historical sulfate concentrations, which would have been derived mainly from rain.

The relationship between Hg and MeHg concentrations in sediments across the Everglades is weak but significant. Total Hg concentrations in surface sediments varied by about a factor of three across the gradient studied, while MeHg concentrations varied by more than two orders of magnitude. As noted above, the slope of the MeHg:Hg relationship varies among sites, and is steepest in the central Everglades. Therefore, this part of the ecosystem is most sensitive to changes in Hg deposition. Concentration dependence experiments to date have also shown a nonlinear response of methylation rate to added Hg, with the magnitude of the response strongly affected by sediment chemistry. Because the gradient in Hg concentration across the study sites is fairly small, and because of the overriding influence of sulfur chemistry, careful quantification of the relationship between Hg loading and MeHg production using the Everglades field data set is difficult.

Nutrients (phosphate and nitrate) generally had no direct effect on MeHg production rates in sediment cores. However, nutrients probably DO have indirect effects on MeHg production through plant growth and organic matter supply. These remain poorly understood. The organic matter supply to sediments affects microbial activity in sediments, and will control sulfate reduction and sulfide production rates at locations where sulfate is not limiting. Further, dissolved organic carbon acts as a strong ligand for Hg (Ravichandra et al., 1999; Benoit et al., 2000) and for MeHg (Hintelmann et al., 1995) and may inhibit the uptake of MeHg into biota. Strong correlations between MeHg in surface sediments and MeHg in biota suggest that DOC does not severely impact the bioavailability of MeHg for uptake. However, the impact of DOC on Hg availability for methylation bears more attention. Nutrients co-vary with sulfate in a general sense across the ecosystem, having the same agricultural source, and decreasing in concentration with distance from that source. Therefore, the effects are somewhat difficult to separate using the field data and correlative approaches. While the effects of sulfate addition are rapid, through microbial sulfate reduction, and can be tested in short-term experiments, nutrient effects on Hg cycling that are mediated through plant growth need to be examined over the longer term.

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