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

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SUMMARY

This is a summary of key findings regarding the effect of water quantity and quality on net methylmercury production that have been reported over the last year.

• At sites immediately downstream of District structures, when the structures are open, the loading per unit area (flux) of inorganic mercury and methylmercury from Lake Okeechobee releases and EAA runoff is likely to be greater than that from wet and dry atmospheric deposition, suggesting that removal of these species by the STAs could have a beneficial impact on the Northern Everglades.

• Methylmercury is likely to be synthesized primarily from the new inorganic mercury being supplied by runoff and wet and dry atmospheric deposition, not from soil release, even following a dryout event.

• Methylmercury production is much more temperature sensitive than methylmercury decomposition, and this temperature sensitivity changes with location, suggesting that different microbial communities are involved in methylation in these locations.

• Once methylmercury is synthesized in surficial peat soils, enhanced transport from soil to water occurs at night, which is probably mediated by nighttime movement of micro- and macroorganisms living on and in the surficial peat soils, but may also be due in part to thermal destratification of the water column.

• Once methylmercury is present in the water column, in areas of low vegetation density and productivity and low-DOC waters, decomposition by sunlight (photodegradation) is likely to compete with sorption to settling organic particles as the most significant removal pathway from the water column.

• The concentrations of sulfide in porewater and soil appear to influence methylmercury production by increasing the bioavailability of inorganic mercury to methylating bacteria, but whether the uptake of the inorganic mercury-sulfide complex occurs via a passive (diffusion) or active (facilitated) transport mechanism remains open to debate.
• The addition of sodium sulfate, sodium sulfide and a slurry of ferrous sulfide (pyrite) inhibited net methylmercury production in laboratory microcosms.

• Iron appears to play a role in mediating sulfur and mercury speciation, and stimulation of methylmercury production at WCA-3A-15 was observed when ferrous chloride was added to a soil slurry, but the mechanism(s) by which this occurs have yet to be elucidated.

• New biosensors are being developed that can detect inorganic mercury available for methylation in soil and water.

THE EFFECT OF THE SULFUR CYCLE ON METHYLMERCURY PRODUCTION

In the Everglades, methylmercury is not produced in its shallow water column (Gilmour et al., 1998a), but is produced primarily in the top four cm of unconsolidated detritus and consolidated peat soil (Gilmour et al., 1998b). Methylmercury is synthesized from inorganic mercury, $\text{Hg}^{(II)}{}^{2+}$, supplied by stormwater runoff, wet and dry atmospheric deposition, and release from the soil reservoir (Krabbenhoft et al., 2001). Methylation of inorganic mercury in periphyton mats and the roots of some floating macrophyte species (Mauro and Guimares, 1999; Hurley et al., 1999; Mauro et al., 2001) has also been observed in primarily eutrophic waters (Cleckner et al., 1998; 1999). The transformation of inorganic mercury to methylmercury is carried out by obligate anaerobic bacteria, generally chemolithotrophic bacteria, including iron-reducing bacteria (Gilmour et al., 1996), but primarily sulfate-reducing bacteria (SRB) (Compeau and Bartha, 1985; Gilmour and Henry, 1991; Choi and Bartha, 1994a,b; Pak and Bartha, 1998a,b; Gilmour et al., 1998a,b). The primary factors that control the metabolic activity of SRB are temperature, dissolved oxygen (DO), short-chain aliphatic acids ($C_{n=1,3}\text{COOH}$) and bioavailable sulfate ion ($\text{SO}_4^{2-}$), but other factors control the relationship between SRB metabolic activity and methylmercury production (Gilmour and Capone, 1987; Compeau and Bartha, 1987; Gilmour and Henry, 1991; Gilmour et al., 1992; Pak and Bartha, 1998a,b; King et al., 1999; Heyes et al., 1999). Those factors are the subject of this section, with a focus on the influence of the hydrological and sulfur cycles.

Using $^{14}$C-labeled methylmercury, 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; Pak and Bartha, 1998a,b; Marvin-DiPasquale and Oremland, 1998; Marvin-DiPasquale et al., 2000; Marvin-DiPasquale et al., 2001). Methylmercury is decomposed to $\text{Hg}^{(II)}$ or $\text{Hg}(0)$ by the action of sunlight in water or soil (Sellers et al., 1996; Krabbenhoft et al., 2001). The rate at which this occurs is determined by the concentration and light-absorbing character of dissolved organic carbon (DOC). DOC-complexed $\text{Hg}^{(II)}$ can be reduced to $\text{Hg}(0)$ by the action of sunlight on DOC (Zhang and Lindberg, 2001a). The relative proportions of $\text{Hg}(0)$ produced by methylmercury photo-oxidation or $\text{Hg}^{(II)}$ photo-reduction are determined by the character of the DOC in water (Zhang and Lindberg, 2001a) and the water depth. $\text{Fe}^{(III)}$ stimulates $\text{Hg}(0)$ production (Zhang et al., 2001). Once produced, $\text{Hg}(0)$ 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 $\text{Hg}(0)$ from the root zone through stems to leaves, and thence to the air (Lindberg and Meyers, 1998). Methylmercury is broken down to inorganic mercury or elemental mercury, $\text{Hg}(0)$, by the action of sunlight (photolysis) and soil bacteria (microbial degradation). The quantity of methylmercury transported from the surficial peat soil to the overlying water per unit area of soil surface (flux) is determined
by the difference between the gross rates of production and decomposition (net production) in the surficial soil and the difference between net production and physical, chemical and biological processes in the water column.

In northern temperate lakes, low DO, sulfidic waters are generally associated with high methylmercury production (Gilmour et al., 1992). Sulfide (S\textsuperscript{2-}) is the product of SRB electron transfer to SO\textsubscript{4}\textsuperscript{2-}. In microcosm experiments carried out on intact Everglades soil cores, methylation rates increased linearly with sulfate addition up to a point (Gilmour et al., 1998a), then decreased with increasing pore water sulfide concentrations (Gilmour et al., 1998b; Benoit et al., 1999a,b). Based on triannual samples collected in March 1995 through July 1999 from 10 sites along a generally north-south transect through the Everglades, an inverse relationship was observed between pore water sulfide and the concentration of methylmercury in soil, as a surrogate for the net methylmercury production rate (Gilmour et al., 1998b). To explain this complex relationship between sulfate, sulfide and methylmercury production, Benoit et al. (1999a,b; 2001) and Jay et al. (2000) have hypothesized that methylmercury is formed when SRB inadvertently absorb a neutral mercury-sulfide complex of the form [Hg\textsubscript{x}S\textsubscript{y}]\textsuperscript{0}. Methylmercury production is retarded by the presence of excess sulfide, which shifts the equilibrium to favor a negatively charged complex of the form [Hg\textsubscript{x}S\textsubscript{y}]\textsuperscript{-n}. This complex may be either dissolved or sorbed to a ferrous sulfide-polysulfide precipitate (C. Gilmour, ANSERC, personal communication, 1998). Inorganic mercury uptake and methylmercury production by *Desulfobulbus propionicus* (1pr3), a species that methylates under both sulfate-reducing and fermentative conditions, has been demonstrated to produce methylmercury under fermentative conditions in the absence of sulfate ion when the only source of inorganic mercury in the culture was precipitated mercuric sulfide (cinnabar) in natural ores, but the rate of methylmercury production was not proportional to the concentration of filterable inorganic mercury in the culture but to the concentration of a soluble form of inorganic mercury that first increases, then decreases, with increasing sulfide concentration (Benoit et al., 2001). Cinnabar precipitation can be retarded or reversed in the presence of high Everglades dissolved organic carbon (DOC) levels (Ravichadran et al., 1998; Ravichadran, 1999).

Laboratory microcosm studies have demonstrated that the methylation of inorganic mercury in the absence of sulfide occurs slowly, if at all, most likely because the inorganic mercury is sorbed to cell surfaces without being readily absorbed across the cell membrane (Benoit et al., 1999a,b; Benoit et al., 2001). Conversely, the measured methylation rate in a microcosm is at a maximum when the concentration of the neutral inorganic mercury-sulfide complex is calculated to be at a maximum (Benoit et al., 1999a,b; Jay et al., 2000; Benoit et al., 2001). With the exception of some limited abiotic methylmercury production, only live cells methylate inorganic mercury (Wood et al., 1968; Gilmour et al., 1998b). However, it is not yet clear whether uptake occurs by the mechanism of passive diffusion or active uptake and whether the species to be taken up must be dissolved or sorbed to a solid surface. Most metals are taken up by bacteria via active transport. However, passive uptake by a neutral chloride complex of silver by algae has been observed by Reinfelder and Chang (1999), and of a neutral inorganic mercury-chloride complex by Mason et al. (1996), although the efficiency of uptake of the neutral MeHg-chloride complex was four-fold greater. According to Benoit et al. (1999a,b), the case for passive diffusion is strengthened by the observation that the neutral inorganic mercury-sulfide complex is partitioned preferentially from water into n-octanol (K\textsubscript{ow} = 25) under conditions that maximize in situ methylmercury production. Confounding observations exist, however, so this hypothesis must be considered tentative.
THE EFFECT OF SOIL DRYOUT ON METHYLMERCURY PRODUCTION

The hydrated peat soils of the Everglades are generally anoxic below a few centimeters depth, even in waters with dissolved oxygen > 2 ppm (P. McCormick, SFWMD, personal communication, 2000; W. Orem, USGS-Reston, personal communication, 2000). Under these conditions anaerobic bacteria are active and most of the sulfur, iron and manganese ions present are in a reduced chemical state. However, following soil dryout it can be confidently predicted that carbon, sulfur and iron species in surficial soils are oxidized, albeit to different depths and degrees (Dmitriw et al., 1995; Yin et al., 1997; Lamers et al., 1998; Gun et al., 2000; Taillfert et al., 2000; W. Orem, USGS, personal communication, 2000; Krabbenhoft and Fink, 2000; Fink, 2001). Reindundation of oxidized soils is usually accompanied by a “first-flush” release of nutrients (Newman and Pietro, 2000) and trace metals, including inorganic mercury (Dmytriw et al., 1995; Rawlik, 2001b). Following the first-flush release of inorganic mercury, some of it can be converted to dissolved elemental mercury Hg(0), which may be lost to the overlying air via evasion. Alternatively, in competition with this process the inorganic Hg(II) can be reabsorbed by bacteria microfilms (Hintelman et al., 1993), algae (Hurley et al., 1998; Miles and Moye, 2000), floating and rooted macrophytes (SFWMD, 1995-1999; Hurley et al., 1998; Fink and Rawlik, 2000) and/or surficial peat soil (Ambrose and Araujo, 1998). It has been hypothesized that the presence of high concentrations of oxidized carbon, sulfur and iron, along with readily bioavailable Hg(II), accelerates methylmercury production until the oxidized species are reduced by biotic or abiotic processes (Morel et al., 1998; Krabbenhoft and Fink, 2000; Krabbenhoft et al., 2000; Krabbenhoft et al., 2001a,b). Following the pulse of methylmercury production, it absorbs in a similar fashion to inorganic mercury (see above discussion), and can be subsequently decomposed to inorganic mercury or elemental mercury by sunlight (Sellers et al., 1996; Krabbenhoft et al., 1998; D. Krabbenhoft, USGS, personal communication, 2000), or demethylated by bacteria under both aerobic and anaerobic conditions (Oremland et al., 1991; Marvin-DiPasquale and Oremland, 1998; Pak and Bartha, 1998; Marvin-DiPasquale et al., 2000; Marvin-DiPasquale et al., 2001)

The effect of first flooding of dry land on inorganic mercury release, methylmercury production and methylmercury bioaccumulation has also been observed in hydroelectric reservoirs created by flooding forested glacial till soils in northern temperate regions (Bodaly et al., 1984; Scruton et al., 1994; Rodgers et al., 1995). In some reservoirs, the “first-flush” of inorganic mercury and its effect on methylmercury production and bioaccumulation are a short-term phenomenon, while in others it can persist for decades (Rodgers et al., 1995). In reservoirs exhibiting a short-term anomalous mercury condition, one might speculate that this is because the soil pools of labile, bioavailable sulfate, carbon and inorganic mercury are small and rapidly consumed. Under such circumstances, the total mass of “first-flush” methylmercury produced will be small and the magnitude and duration of subsequent excessive bioaccumulation and recycling of methylmercury in top predator fish and their predators will be short-lived. Conversely, if these pools are large or there is an external source of the limiting factor capable of sustaining a high, first-flush methylmercury production rate for a long time, one can reasonably conjecture that the first-flush mass of methylmercury produced will be large, resulting in excessive bioaccumulation at the top of the food web, which will clear only slowly from the ecosystem as it is efficiently recycled from detritus to detritivores, then back up the food chain. This “reservoir effect” has also been observed in natural, created or expanded wetlands (St. Louis et al., 1994; St. Louis et al., 1996; Kelly et al., 1997; Paterson et al., 1998).
However, if sulfate is present in substantial excess, surficial sediments remain anaerobic and no other factor limits microbial metabolism or affects sulfur speciation, then sulfide, a byproduct of the life processes of sulfate-reducing bacteria, can accumulate to concentrations that inhibit methylmercury production (Craig and Bartlett, 1978; Compeau and Bartha, 1984; Berman and Bartha, 1986; Gilmour et al., 1998b; Benoit, 1999a,b; Jay et al, 2000; Benoit et al., 2001; Marvin-DiPasquale et al., 2001). It has been hypothesized with moderate confidence (Gilmour et al, 1998b) that sulfide inhibition is causing eutrophic Everglades regions with conditions otherwise deemed ideal for methylmercury production (e.g., ENR Project and WCA-2A-F1) to exhibit low methylmercury production and correspondingly low concentrations in fish at all trophic levels (Cleckner et al., 1998; Lange et al., 1998, 1999; Rumbold et al., 2000; Rawlik, 2001a; Rumbold et al., 2001). Conversely, unimpacted or virtually pristine areas in the Everglades exhibit much higher methylmercury production rates (e.g., WCA-2A-U3 and WCA-3A-15) and correspondingly higher concentrations in fish at all trophic levels. An alternative hypothesis is that there is an inverse relationship between the degree of eutrophication and methylmercury bioaccumulation in fish at all trophic levels based primarily on the work of Hakanson (1980). This hypothesis has been addressed previously (1999 EIR; 2000 ECR; 2001 ECR). More recently, this relationship has not been found to hold throughout the Everglades (USEPA 2001) or in mesocosms dosed with stable mercury isotopes at sites down the phosphorus gradient (C. Gilmour, ANSERC, personal communication, 2001).

Results of a joint USGS-District study of an Everglades dryout and burn that occurred in the spring of 1999 suggest that the relatively rapid decline from peak methylmercury concentrations in pore water and soils was brought about by the rapid depletion of the excess sulfate pool created by the oxidation of inorganic and organic sulfides. However, one cannot rule out the alternative hypothesis that this was caused by the relatively rapid onset of sulfide inhibition in soils containing excess sulfate (Krabbenhoft and Fink, 2000; Krabbenhoft et al., 2000). The relatively rapid onset of sulfide inhibition in sulfur-amended agricultural soils could also explain the methylmercury trajectory followed by STA-1W Cell 5. After exhibiting a first-flush effect in water within four weeks of flooding in May 1999, and in mosquitofish a month later, STA-1W Cell 5 relaxed back to ENR-like conditions in water and mosquitofish by January 2000 (Rawlik 2000a,b).

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, considered anomalously high relative to the Everglades Nutrient Removal (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 and averaged about 350 ug/Kg wet weight when the last sample was collected in mid-March 2001. This value must be compared to an average value of about 200 ug/Kg wet weight at WCA-3A-15, the Everglades “hot spot,” which is generally considered to be the location where concentrations of methylmercury in fish are sufficient to cause toxic effects in sensitive members of some fish-eating populations feeding there preferentially.
NEW FINDINGS IN 2001

The United States Environmental Protection Agency (USEPA) sponsored a workshop on mercury biogeochemistry in aquatic and terrestrial ecosystems in West Palm Beach, Florida, May 8 through 10, 2001. Presentations were made on a variety of subjects, but the focus of this summary is on factors controlling methylmercury production in aquatic ecosystems.

Cynthia Gilmour and co-workers at the National Academy of Sciences are compiling an inventory of the bacteria that are known to methylate inorganic mercury (Gilmour et al., 2001). These include most species of sulfate-reducing bacteria (Gilmour and Henry, 1991), most species of iron-reducing bacteria (Gilmour et al., 1996) and some species of methanogenic bacteria (Gilmour et al., 2001).

The genetic material of the bacterium *vibrio anguillarum*, a facultative anaerobe, has been engineered to include the *mer* operon and a reporter gene that manufactures luciferase, a light-emitting protein, when the bacterium is exposed to methylmercury (Golding et al., 2001). This bacterium exhibits active uptake of inorganic mercury under aerobic conditions and no uptake under anaerobic conditions, but it is not yet clear whether active or passive uptake of inorganic mercury is at work in the sulfate-reducing species that are the primary methylators and obligate anaerobes. The case for passive uptake rests primarily on the observation that methylmercury production peaks at a sulfide concentration where the neutral inorganic mercury-sulfide complex is calculated to be at a maximum (Benoit et al., 1999a). This complex has been shown to partition readily into n-octanol (Benoit et al., 1999b), which is used as a surrogate for membrane permeability in the pharmaceutical industry (Leo et al., 1971) and in predicting bioaccumulation potential (Lyman et al., 1990).

Conversely, Robb Mason and co-workers at the University of Maryland found that the rate of uptake of neutral inorganic mercury-sulfide complexes by sulfate-reducing bacteria was slower than the rate predicted by passive diffusion and the thickness of the bacterial membrane, based on flux measurements over a wide range of sulfide concentrations (Mason et al., 2001). Using laboratory cultures of *selenastrum*, *cosmarium* and *schizothrix*, 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). Nor was the mechanistic mathematical model, E-MCM, able to calibrate when passive uptake of methylmercury by algae was assumed (Reed Harris, TetraTech, personal communication, 2001). While these discrepancies may be an artifact of the choice of diffusion model and/or molecular species and/or the uncertainty in the magnitude of its diffusion coefficient, the magnitudes of the discrepancies for both the inorganic mercury-sulfide complex and the methylmercury-chloride complex suggests otherwise.

The METAALICUS project is led by Reed Harris of TetraTech, Inc., with principal investigators Mark Amyot (University of Quebec), Cynthia Gilmour (Academy of Natural Sciences Environmental Research Laboratory), Holger Hintelman, James Hurley, David Krabbenhoft, John Rudd and Vincent St. Louis. It involves a whole lake mercury amendment in the Experimental Lakes Area (ELA) in Ontario, Canada. In this study, a different stable mercury isotope was added at the same time and loading rate to a 1000-m$^2$ area in an upland forest and a wetland in the watershed, and to four 10-m diameter enclosures in the lake itself. In the second year a different set of isotopes was added to the upland forest and wetland, and another isotope was added to the lake surface itself. The addition rate per unit area in all cases was four-to-five times the natural atmospheric deposition flux of about five-to-six micrograms per square meter.
per year. The preliminary results suggest that virtually none of the inorganic mercury dosed in the upland forest and wetland were transported to the lake in the first year, while the inorganic mercury dosed directly to the enclosures was converted to elemental mercury and evaded (probably an artifact of the dosing regimen), but the remaining inorganic mercury in the enclosure was deposited to the sediments, with a half-life on the order of a month. This is much slower than the sediment deposition velocity in the Everglades mesocosms, but this is to be expected in deeper, less densely vegetated waters. The subsequent appearance of labeled methylmercury in the water column followed the buildup of inorganic mercury in the surficial sediment. In the second year of the study, a different mercury isotope was added directly to the lake mesocosms. The preliminary data suggest that more of the inorganic mercury dosed to the upland forest and wetland areas in year one found its way to the lake than in year one, but the quantities were still insignificant, so the vast majority of the year-two methylmercury was produced in the lake. However, more importantly and perhaps most surprisingly, the methylmercury in the water in year two was produced predominantly from the stable mercury isotope added in year two. This suggests that the year-one inorganic mercury was rapidly bound up in a form that was unavailable for methylation. In subsequent years, fish from several trophic levels will be sampled to monitor the rate and efficiency with which methylmercury from each source bioaccumulates at each successive step in the aquatic food chain.

The Aquatic Cycling of Mercury in the Everglades (ACME) Project is led by Dave Krabbenhoft of the U.S. Geological Survey in Middleton, WI. Phase 1 involved a combination of monitoring along the nutrient and sulfate gradients in the Northern Everglades to characterize the system and its mercury cycle, as well as microcosm manipulations to verify the key controlling factors extracted from statistical analysis of the monitoring data. Phase 2 shifts the emphasis from monitoring and laboratory microcosm studies to the dosing of field mesocosms along those same nutrient and sulfate gradients with inorganic mercury salts and methylmercury produced from stable mercury isotopes. The preliminary results from Phase 2 mesocosm studies indicate 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 on the order of one or two days. The more rapid deposition of inorganic mercury in the Everglades relative to the dosed experimental lake was attributed to its much shallower waters (i.e., 0.5 versus 20 m). In addition, labeled methylmercury began to appear within 24 to 48 hours of dosing, but the methylmercury did not contain Hg (II) from undosed soil, but from dosed water, suggesting that soil Hg (II) is too strongly bound to be bioavailable for methylation. This also suggests that methylmercury production in the Everglades is being driven predominantly by the “new” Hg (II) supplied by wet and dry atmospheric deposition, not by the release of “old” Hg (II) bound to peat soil. Perhaps most surprisingly, this was also true even in situations where the soil was dried out and rewetted. The sulfate produced from the oxidation of inorganic and organic sulfides stimulated methylmercury production, but the Hg (II) from which methylmercury was being produced was predominantly the new inorganic mercury dosed to the water, not the soil (Krabbenhoft et al., 2001). This suggests the Everglades will respond even more quickly to a reduction in atmospheric deposition of inorganic mercury than previously believed, perhaps within years instead of decades.

To evaluate the influence of the sulfur cycle on the mercury cycle more directly, in Phase 2 the ACME mesocosms are being dosed with sulfate in water to quantify (1) the rate of sulfate transfer to the underlying soil pore water, (2) the latency period and rate of metabolic activity of SRB stimulated by that sulfate addition, (3) the efficiency with which sulfate is converted to sulfide, (4) the latency periods, rates, and efficiencies with which inorganic mercury species in surface water, pore water, and soil are methylated by those metabolically stimulated SRB, and (5) the ambient chemical factors controlling those rates and efficiencies. Preliminary findings
indicate that excess sulfate inhibits methylmercury production where conditions favor sulfide buildup in pore water, supporting the earlier empirical findings.

In evaluating the influence of the sulfur cycle on the mercury cycle, other factors that mediate sulfur and mercury speciation must also be taken into account. The mesocosms are placed along a nutrient as well as a sulfate gradient, and preliminary results suggest that phosphorus may play some role in methylmercury production, perhaps by influencing bacterial community composition, metabolic rates, or anaerobic conditions. In addition, the mesocosm are placed along gradients of other potentially influential factors, including calcium, magnesium, iron, and manganese, among others. Iron not only mediates sulfur speciation (Gun et al., 2001), but iron-reducing bacteria have been demonstrated to methylate inorganic mercury (Gilmour et al., 1996).

To understand those potential influences, the results of previous work and the ACME Phase 1 studies must be considered. In a screening study conducted by Howard (D. Howard, University of Trent, personal communication, 1993), the acid volatile sulfide fraction of the sediment was not a good predictor of the bioavailable fraction of inorganic mercury for methylation, but enhanced methylmercury production in a shaken slurry of sediment was observed when ferrous chloride was added to an aquatic microcosm (D. Howard, University of Trent, personal communication, 1993). However, the mechanism was not elucidated, nor were taxonomic studies of the bacterial communities undertaken. Gilmour has speculated that iron influences inorganic mercury sulfide speciation via the formation of insoluble iron polysulfides, but the required studies have not been carried out (C. Gilmour, ANS, personal communication, 1998). More recent work by Mason et al. (2001) measured inorganic mercury binding to ferric oxide-hydroxide precipitate surfaces. This binding is influenced by DOC, which in theory should compete with the solid-phase surface for inorganic mercury. The observed isotherm did not match what would be expected for competitive complexation with DOC, however. Rather, they found that Everglades DOC from WCA-2A-F1 and –U3 complexes 1:1 with inorganic mercury, but DOC sorbs to the iron oxide-hydroxide precipitate surfaces. Correcting for DOC sorption does not correct the deviations from the expected sorption behavior. Clearly, more work is needed to elucidate the mechanism by which iron exerts its influence on inorganic mercury and methylmercury speciation.

While the mechanism by which iron influences methylmercury production remains uncertain, Marvin-DiPasquale and co-workers at USGS-Menlo Park were able to more carefully resolve the effect of iron and sulfide speciation on net methylmercury production. In studies of Everglades soil homogenates dosed with radioactive $^{203}$Hg and $^{14}$C-methylmercury under anaerobic conditions, they found the methylmercury production rate increases exponentially with temperature, while demethylation rates were virtually temperature independent and that the temperature sensitivity of methylmercury production varied across the chemical and ecological gradients in the Everglades (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. They also found that excess sulfate, sulfide and ferrous sulfide (pyrite) slurry inhibited net methylmercury production, while ferrous chloride stimulated net methylmercury production only at 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. It can be conjectured that where iron-reducing bacteria are present or Fe(II)$^{2+}$ is the limiting nutrient for SRB, the addition of Fe(II)$^{2+}$ could increase methylmercury production unless it is readily oxidized to Fe(III)$^{3+}$ or complexed or precipitated in the presence of excess sulfide. Once a sulfide precipitate of Fe(II)$^{2+}$ forms, inorganic mercury may complex with its surface, either decreasing or increasing the concentration of the neutral
[Hg(II),S₃]⁰ complex at the solid surface, with a subsequent decrease or increase in the rate of methylmercury production by SRB growing at the solid surface. To reduce the uncertainties in our understanding of the mechanistic influence of the iron cycle on the sulfur and mercury cycles, dosing of the mesocosms is now being planned.
LITERATURE CITED


Compeau and Bartha. 1984.


Appendix 2B-2


Harris, R.C. et al. 2001. METAALICUS: A study to determine the relationship between mercury deposition and MeHg concentrations in fish. Presentation at the Workshop on the Fate, Transport, and Transformation of Mercury in Aquatic and Terrestrial Ecosystems, sponsored by the U.S. Environmental Protection Agency, the U.S. Geological Survey, and the South Florida Mercury Science Program. May 8-10. Sheraton West Palm Beach, West Palm Beach, FL.


Appendix 2B-2


Appendix 2B-2 2002 Everglades Consolidated Report


SFWMD. 1999b. Final Report on the Effect of Best Management Practices on the Loading of Mercury Species to/from the Everglades Nutrient Removal Project: Monitoring Program (Project C-1). Submitted by the South Florida Water Management District, West Palm Beach, Florida, to the Florida Department of Environmental Protection, Tallahassee, Florida, to fulfill the requirements of a Section 319 Grant (SP335/C-6663/4) from U.S. Environmental Protection Agency Region 4, Atlanta, GA.


