MR. TELLIARD: Our next speaker is Bob Hudson. Bob is with the Department of Natural Resources and Environmental Science, University of Illinois. Bob is going to talk about Mercury and Methylmercury uptake in freshwater plankton.

METHYLMERCURY UPTAKE BY FRESHWATER PHYTOPLANKTON: ANALYTICAL AND MODELING METHODS

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In this talk (Slide 1), I will summarize the studies of methylmercury uptake by freshwater phytoplankton that Carl Watras and I have been conducting over the past few years. These investigations have helped motivate the development both of new analytical methods by Dr. Watras and his mercury biogeochemistry group located at Trout Lake Station in northern Wisconsin and new modeling approaches by myself in collaboration with Carl and Steve Gherini of Tetra Tech, Inc. A major goal of our studies has been to identify and model the mechanism of methylmercury uptake by phytoplankton (Slide 1). This process is important because most of the mercury in fish is accumulated by transfer through aquatic food webs after first being absorbed from the water at the lowest trophic levels. Lake water quality appears to have a strong influence on this step. Thus, our efforts are directed towards enabling us to better predict the levels of mercury in aquatic organisms from diverse aquatic systems and provide a sound scientific basis for resource management and regulatory decisions.

The importance of methylmercury uptake in particular is apparent from a comparison of the relative abundance of the two dominant forms of mercury? methylmercury (meHg) and mercury(II) (Hg$^{II}$)? in different compartments of freshwater systems (Slide 2). The proportion of meHg increases from sediment to water and on up through the higher trophic levels of the aquatic food web. At the base of the pelagic food web, mercury in seston, which comprises all the particulate matter retained by filters of 0.4- to 0.8-mm pore size, has a higher fraction Hg$^{II}$ than meHg. The proportion of meHg increases in zooplankton and reaches >95% in fish. Mason et al. (1996) showed that the efficiency of meHg transfer between phytoplankton and zooplankton was about 60 percent for meHg as compared to about 15 percent for Hg$^{II}$. This combined with other evidence shows that the increases in percentage of meHg at higher trophic levels does not reflect any further uptake of meHg from water at higher trophic levels. Thus, our study has been focussed on meHg uptake at the base of the food web.
Slide 3 depicts a conceptual model for meHg bioaccumulation in lakes taken from Hudson et al. (1994). It begins from the concept of metal speciation—the fact that metals in solution exist in a variety of chemical species. Like other metals in natural waters, the methylmercury ion can be bound by ligands, including inorganic ligands such as chloride and hydroxide as well as binding sites in natural dissolved organic matter (DOM). One or more of the inorganic species are taken up by the seston via the processes of adsorption on surfaces and absorption into cells. Methylmercury has a rather low octanol-water partition coefficient of 1.7 for its neutral chloride complex. So, unlike hydrophobic pollutants that partition into cellular lipids, methylmercury bioaccumulates because of its strong binding by ligands inside cells. While these ligands have not been characterized yet, it’s quite likely that they involve thiols moieties of small compounds or of proteins. Subsequent transfer of mercury up the food chain depends on the nature of the organisms and, as discussed above, the form of the mercury involved.

Depending on which mechanism(s) of meHg uptake in seston predominate, different meHg species may control the rates of uptake (Hudson, 1998). Since lake water quality influences meHg speciation, knowing the mechanism is crucial for establishing relationships between water quality parameters, such as pH, DOC, and Cl, and meHg concentrations in the seston and ultimately fish. Of course, empirical correlations between lake water chemical parameters and mercury in fish have been widely observed and provide some basis for predicting which lakes will have problems with mercury in fish. For example (Slide 4), data from Wisconsin lakes (Watras et al., 1998) exhibit a good correlation of fish mercury with pH, as has been observed in lake surveys throughout North America and Europe. A weaker correlation with dissolved organic carbon is also observed in the Wisconsin lakes. Although similar observations are fairly widespread, because regression parameters from different studies vary significantly it is difficult to use them predictively. However, such correlations suggest strongly that pH and dissolved organic carbon must frequently influence either the amount of methylmercury in lakes or its bioavailability to aquatic organisms. This is consistent with models of methylmercury speciation in lakes (Hudson et al., 1994) and gives us reason to suspect that the complex interrelationships can be successfully probed further.

The remainder of this paper is divided between an examination of the analytical methods used to obtain the sestonic mercury concentrations and the modeling methods used to analyze the data.

FIELD METHODS

The primary data for this study are the seston-water partition coefficients for methylmercury measured in a set of lakes in northern Wisconsin (Watras et al., 1998). The principal challenge in obtaining methylmercury partitioning data has been to accurately measure both its particulate (or sestonic) and dissolved concentrations when the total meHg itself occurs at concentrations of only 0.05-0.5 ng meHg-Hg/L. Making these measurements requires scrupulously clean sampling methods, sensitive analytical methods, and a suitable method for distinguishing the particulate and dissolved methylmercury. Our focus will be on
filtration as the sampling and analysis methods have been described in other work. The data we discuss here are from samples collected and filtered by the Trout Lake group and analyzed by Frontier Geosciences using methods summarized in Slide 5. As an aside, I would like to point out that Dr. Watras’ group was heavily involved in developing the protocols in EPA Method 1669, which several previous speakers have mentioned. Of course, these protocols were applied for sampling the lakes in Wisconsin. The analytical methods employed were EPA method 1631 for total mercury and an adaptation of Nick Bloom’s ethylation technique for methylmercury (Horvat et al., 1993).

In their first major study of sestonic mercury concentrations, Dr. Watras’ group used 0.8-mm glass fiber filters and directly measured meHg retained on the filter. Although the method had reasonable filter blanks and sample throughput, the 0.8-mm pores were large enough that cyanobacteria and some bacteria could pass through the filter. In order to resolve this problem, they switched to 0.4-mm pore-size, pleated filter cartridges and calculated particulate meHg by difference between the unfiltered and filtered portions of samples. This approach worked well in most cases, but was subject to a new problem. The difference of two measurements that are similar in magnitude, even when each is measured with good precision (7-8 % coefficient of variation), is subject to significant error and can even take on negative values (Slide 6). The resulting negative partition coefficient is physically impossible, but perfectly understandable from a statistical perspective. So because of this problem, Dr. Watras’ group has continued to refine their filtration methodology.

The latest filtration technique they have developed (Morrison and Watras, in press) uses a dual, in-line filter apparatus with 0.4-mm cellulose nitrate filters. The first filter retains the particles while the second one serves as an integrated blank of both Hg contained in the filter material and of any Hg absorption on the filter. The method can be used to analyze for either total or methylmercury. This method seems to have resolved all of the issues raised above; it has low blanks (32 pg for total Hg and nearly zero for meHg), sufficient throughput, has the proper pore size, and allows direct measurement of particulate Hg. The method detection limit is 30 picograms for total Hg, 3 picograms for methyl (Slide 8). The conditions for digesting the filter permit good yields using certified reference materials. The precision of the method is quite good as well. As compared to obtaining particulate Hg from the difference between total and dissolved (Slide 9), the relative percent difference between replicates of the dual filtration method is a factor of two and a half better for total mercury and almost an order of magnitude better for methylmercury. A wider scale application of the method to field samples from the Wisconsin lakes will occur in the near future.

MODELING METHODS

These field methods have yielded high quality data on the seston-water partitioning of methylmercury in the Wisconsin lakes. Now our task is to model this data. A complicating factor is that seston contains both live cells – phytoplankton and bacteria – and detritus, as would be apparent in a simple microscopic observation. Our conceptual model (Slide 10) for seston-water partitioning accounts for the range of particle types and solutes which
methylmercury can interact with. The chemical forms of methylmercury in lake waters include dissolved forms such as complexes with chloride, hydroxide, and truly dissolved organic ligands. Association with dissolved organic matter, a very important process for meHg in these lakes, probably largely involves binding to colloids, i.e., particles too small to be retained by the 0.4-mm filter. Although some Fe and Mn oxides are found, DOM probably makes up the majority of the colloids in these systems. Association with phytoplankton (and bacterioplankton) is our primary interest and, we will argue, is a very significant form of meHg in the seston.

The field data can be mapped onto this model by remembering that the ’sestonic’ or particulate meHg corresponds to meHg contained in plankton and detritus, while the dissolved fraction corresponds to meHg in inorganic and organically-complexed plus colloidal forms. Our conceptual model indicates that there are several critical processes such as biotic uptake and mortality, aggregation and disaggregation of colloids, and disaggregation of larger particles that influence the seston-water partitioning of meHg. In addition, exudation, lysis, sloppy feeding of zooplankton on the plankton will create dissolved and colloidal particles. It is important to remember the dynamic situation that exists in lake waters as we proceed with the modeling.

Ideally, experimental studies would have already established everything we need to know to turn our conceptual model into a mathematical form. In particular, knowing the equilibrium constants for meHg binding to (inorganic and organic) ligands and to abiotic particles in the lakes would be most helpful. In addition it is essential to know the mechanisms of meHg uptake in order to model the dependence of meHg uptake into the plankton on water chemistry. Although the equilibrium constants for meHg complexation by hydroxide and chloride are well known, we don’t really know the strength of its complexation by natural organic matter or the pH dependence of this reaction. In addition, although it has long been known that methylmercury chloride, and to a lesser extent, methylmercury hydroxide will passively diffuse through membranes, we don’t know whether any other uptake mechanisms that could be accumulating mercury in the live cells is active in these lakes. So, the challenge in the modeling phase of our study has been to see if we can discriminate between alternative models for the unknown processes and reactions of the model using the field observations.

The simplest model of seston-water partitioning treats the process as simply an adsorption problem. In these seepage lakes with little input of inorganic particles, one could reasonably assume that plankton and detritus are chemically similar particles, i.e., one needn’t worry about the cytoplasm of the plankton as being different from the surfaces of detritus. Then, we model the equilibrium distribution of meHg between binding sites on particles and dissolved ligands. Now, the dissolved organic matter (especially colloidal DOM) and the particulate matter have the same sources. They are derived from sphagnum, phyto- and bacterioplankton, and detritus and are interconverted by the processes described above. So, the simplest approach is to assume that colloidal organic matter and particulate organic matter have similar chemical properties. In other words, the meHg reaction with strong binding sites in each was assumed to have similar equilibrium constants for meHg as well as H’ ions,
although an allowance for different site densities was made. Using this approach, it was possible to fit the average of the data fairly well (Slide 11) by optimizing the unknown equilibrium constants and site densities. But, the individual field data (shown as black dots with error bars) and the model predictions (shown as circles) do not agree as strongly as one would like ($r^2 = 0.53$).

So, we next attempted to formulate a model that separates uptake by phytoplankton from adsorption on detritus. In this approach, the biomass of phytoplankton was estimated using chlorophyll $a$ data, with the balance of the particulate material assumed to be detritus. The theoretical basis for modeling metal accumulation in living plankton can be summarized in a simple equation (Slide 12), which we call Sunda’s Law after Bill Sunda at the Beaufort Lab of the National Marine Fisheries Service. Sunda’s Law states that the concentration of a metal in biota is equal to the ratio of the net uptake rate of a metal to the growth rate of the biota. That is, the concentration in an organism depends on the rate of metal uptake and the rate of dilution by growth. The rate of biotic uptake of a metal, as we know from laboratory studies with controlled metal speciation, depends on the speciation of the metal (Hudson, 1998). Although which metal species controls uptake rates depends on the mechanism, it is generally observed that strong organic complexes of metals are less bioavailable. The denominator in the equation, the growth rate, often is controlled by factors other than the metal concentrations, such as light or major nutrients. But in when metal toxicity becomes significant or an essential metal reaches low, limiting concentrations, the metals can actually affect the growth rates. For the systems we are interested in, mercury does not occur at high enough levels to affect the growth rate of any of the aquatic organisms in the lakes and it is never an essential nutrient. So, for the purposes of this study we have assumed that the growth rate is constant in all the Wisconsin lakes that we are modeling.

In fitting the data (Slide 13), the model for adsorption on detritus was similar to the abiotic model, but for the phytoplankton several different models were tested, each assuming different dominant mechanisms of $\text{meHg}$ uptake. First, the orthodox assumption of passive absorption of methylmercury chloride and hydroxide was tested. We also tested the hypothesis that there might be facilitated transport of methylmercury, i.e., that there might be transport proteins whose function is to transport another ion into phytoplankton and that perhaps methylmercury is sneaking through these channels. For example, it is well established that copper or cadmium can be taken up by the channel for manganese in phytoplankton (Sunda and Huntsman, 1998). For $\text{meHg}$, such a mechanism, if it exists has not been identified and its dependence on $\text{meHg}$ speciation is not known. For this reason, we tried a variety of possible dependencies on speciation for the uptake into the phytoplankton fraction With some models, we were able to fit the data significantly better than with the abiotic particle model ($r^2 = 0.89$).
Now, of course this approach cannot prove that the hypothesized mechanism actually exists or controls metal uptake. Furthermore, caution demands that we ask whether the improved fit is just the result of introducing new fitting parameters. But, an additional indication that particulate methylmercury actually does partition into live cells differently than into detrital particles comes from an examination of the ratios of Hg$^{II}$ and meHg to the mass of organic matter in the dissolved and particulate fractions (Slide 14). Other workers have demonstrated that in water, almost all the mercury, both meHg and Hg$^{II}$, is complexed by organic matter. So, these ratios compare the relative binding of mercury to organic matter in water and in seston. If the dissolved matter and the particles were similar chemically as in the abiotic model above, they should have the same ratios of Hg$^{II}$ and of meHg to organic matter. The data show that for Hg$^{II}$, seston has approximately a factor of two higher apparent mercury content per unit of organic matter. For methylmercury, the ratio is about three. So, the comparisons suggest that even Hg$^{II}$, which is known to be very surface reactive, may not be strictly adsorbed on surfaces. It also appears that the higher ratio for meHg, indicates that live cells are accumulating meHg more effectively than Hg$^{II}$. This suggestion is consistent with the laboratory study of Mason et al. (1996), which found that about 85% of Hg$^{II}$ was found on cell membranes and cell surfaces and 15% in the cytosol, whereas meHg was 62% intracellular and 38% on surfaces.

Another suggestion that these findings are meaningful comes from a comparison of the observed bioconcentration factors in fish with different factors for the seston (Slide 15). First, we compare meHg bioconcentration factors for age one yellow perch to seston. We would hope to see a stronger correlation ($r^2 = 0.38$), if the uptake into seston is driving accumulation of meHg in the food web. The correlation with our modeled accumulation in the phytoplankton fraction of the seston with the fish Hg data is somewhat stronger ($r^2 = 0.62$), which we argue that this corroborates our modeling approach.

The third line of supporting evidence comes from the agreement of our initial observations with subsequent direct measurements of the stability constant for DOM complexation of methylmercury (Slide 16). In our analysis, this equilibrium constant was one of our fitting parameters. Hintelmann et al. (1996) subsequently published measurements of the affinity for meHg of dissolved organic matter from different lakes. Their results bracket our predictions. So, we also felt quite encouraged that we were on the right track and that we have been able to discriminate between different models of the mechanisms that control meHg bioaccumulation in freshwater phytoplankton.

At this time (Slide 17), we are continuing to refine our methods in order to clarify what is happening in the phytoplankton fraction. We’d like to make a more direct measurement of the meHg in live cells, so we’re exploring methods removing detritally-bound meHg from particulate material retained on filters. We’re also planning to manipulate live samples in order to raise the ratio of phytoplankton to detritus. Finally, in the modeling area, we will be improving our mechanistic model formulations and improving our use of statistical methods.
Finally, we acknowledge the generous support provided by the sponsors of the various phases of this study?the Electric Power Research Institute, Wisconsin DNR, and USEPA Office of Water. We also wish to acknowledge the work of Frontier Geosciences in performing the mercury analyses.

REFERENCES


QUESTION AND ANSWER SESSION

MR. TELLIARD: Questions for Bob? Thank you, Bob.
Methylmercury Uptake by Freshwater Phytoplankton:
Analytical and Modeling Methods

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Hypothesized Mechanism of Methylmercury Bioaccumulation

\[ \text{CH}_3\text{Hg} \rightarrow \text{Y}_{\text{DOM}} \]

\[ \text{CH}_3\text{Hg}_{\text{INORGANIC}} + \text{Y}_{\text{DOM}} \]

\[ \text{X-CH}_3\text{Hg} \]

Phytoplankton

\[ \text{R-S-CH}_3\text{Hg} \]

Zooplankton

\[ \text{Ca}^{2+}? \]

Fish
What Environmental Factors Govern Methylmercury Bioaccumulation?

A. $b[0] = 713.85$
   $b[1] = -92.87$
   $r^2 = 0.72$

B. $b[0] = 61.72$
   $b[1] = 9.20$
   $r^2 = 0.33$
Methods

Field sampling:

EPA 1669

Analytical:

Total Hg  EPA 1631

Methyl Hg  Bloom (1989)
            Liang et al. (1994)
            Horvat et al. (1993)
Method Summary (QA/QC)

<table>
<thead>
<tr>
<th></th>
<th>$\text{Hg}_T$</th>
<th>$\text{meHg}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Filter Blank (pg)</td>
<td>32±8.9</td>
<td>-0.1±1</td>
</tr>
<tr>
<td>2. MDL (pg)</td>
<td>32.2</td>
<td>3</td>
</tr>
<tr>
<td>3. Yield CRM (%) (DOLT-2)</td>
<td>100.6±7.0</td>
<td>101+22</td>
</tr>
<tr>
<td>4. RPD (%)</td>
<td>18.7</td>
<td>43.8</td>
</tr>
</tbody>
</table>
## Method Comparison

**Dual-filtration vs $\text{Hg}_T - \text{Hg}_D$**

<table>
<thead>
<tr>
<th>Method</th>
<th>RPD (%)</th>
<th>meHg$_P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dual-Filtration</td>
<td>19</td>
<td>44</td>
</tr>
<tr>
<td>$\text{Hg}_T - \text{Hg}_D$</td>
<td>50</td>
<td>370</td>
</tr>
</tbody>
</table>

May 1998
Abiotic Particle Model of Seston-Water Partitioning

Complexation equilibria:

\[ \theta_{\text{H}^+} : \text{CH}_3\text{Hg}^+ + \text{Cl}^- \leftrightarrow \text{CH}_3\text{HgCl}^0 \]

\[ \theta_{\text{OH}^-} : \text{CH}_3\text{Hg}^+ + \text{OH}^- \leftrightarrow \text{CH}_3\text{HgOH}^0 \]

\[ \theta_{\text{H}^+\text{DOM}} : \text{H}^+ + \text{Y}_{\text{DOM}} \leftrightarrow \text{HY}_{\text{DOM}} \]

\[ \theta_{\text{CH}_3\text{Hg}^+\text{Y}_{\text{DOM}}} : \text{CH}_3\text{Hg}^+ + \text{Y}_{\text{DOM}} \leftrightarrow \text{CH}_3\text{HgY}_{\text{DOM}} \]

\[ \theta_{\text{H}^+\text{Y}_{\text{POM}}} : \text{H}^+ + \text{Y}_{\text{POM}} \leftrightarrow \text{HY}_{\text{POM}} \]

\[ \theta_{\text{CH}_3\text{Hg}^+\text{Y}_{\text{POM}}} : \text{CH}_3\text{Hg}^+ + \text{Y}_{\text{POM}} \leftrightarrow \text{CH}_3\text{HgY}_{\text{POM}} \]

\[ K^*_\text{PARTICLE} = \frac{[\text{CH}_3\text{Hg}^+][\text{Y}_{\text{DOM}}]}{[\text{CH}_3\text{Hg}^+\text{Y}_{\text{DOM}}] + [\text{HY}_{\text{DOM}}]} n_{\text{Y}_{\text{POM}}} \theta_{\text{HY}_{\text{POM}}} \]

Assumptions:

\[ \theta_{\text{H}^+\text{DOM}} = \theta_{\text{HY}_{\text{DOM}}} \]

\[ n_{\text{Y}_{\text{DOM}}}, \theta_{\text{HY}_{\text{DOM}}}, n_{\text{Y}_{\text{POM}}}, \theta_{\text{HY}_{\text{POM}}} = \text{constant} \]
Metal Uptake follows Sunda’s Law

\[ C_{biota} = \frac{V_{net}}{\mu} \]

- \( C_{biota} \) = metal concentration in biota,
- \( V_{net} \) = net uptake rate,
- \( \mu \) = growth rate (biodilution term).
Abiotic Particle + Phytoplankton Model of Seston-Water Partitioning

Mechanisms of uptake by phytoplankton:

1. Facilitated transport at pronated site:
   
   \[ H^+ + L_{\text{cells}} \rightleftharpoons HL_{\text{cells}} \]

2. Thermodynamic control:
   
   \[ CH_3Hg^+ + L_{\text{cells}} \rightarrow CH_3Hg_{\text{cells}} \]

3. Kinetic control:
   
   \[ CH_3Hg_{\text{inorganic}} + L_{\text{cells}} \rightarrow CH_3Hg_{\text{cells}} \]

4. Passive absorption through membrane:
   
   \[ CH_3HgCl_0 \rightarrow CH_3Hg_{\text{cells}} \]

\[ K'_{\text{PLANKTON}} = \frac{\text{Uptake rate constant}}{\text{Growth rate}} \]

Graphical representation:

- Average error = 0.22
- \( r^2 \) = 0.89
- Slope = 0.81

DOC (mg L\(^{-1}\)) on the x-axis and \( \log K_{\text{seston}} (L \cdot kg^{-1}) \) on the y-axis.
Mercury Bioconcentration in Seston

<table>
<thead>
<tr>
<th>Form of Hg</th>
<th>Hg Content (G Hg/g-dw)</th>
<th>Hg in Phytoplankton(^a) (% in cytosol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOM</td>
<td>Seston</td>
<td></td>
</tr>
<tr>
<td>Hg(^II)</td>
<td>85</td>
<td>170</td>
</tr>
<tr>
<td>MeHg</td>
<td>12</td>
<td>34</td>
</tr>
</tbody>
</table>

\(^a\) Mason et. Al. (1996).
Relationships between observed meHg bioconcentration factors in age-1 yellow perch and A) measured seston-water partition coefficient and B) model BCF for phytoplankton fraction of seston. After Hudson Et al. (1994).
Future Research Directions

I. Distinguishing detrital and cellular meHg in field samples.
   A. Filter washing techniques
   B. Manipulation of live samples

II Modeling seston-water partitioning
   A. Mechanistic modeling
   B. Statistical analysis
      (Model discrimination analysis)
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