



WATER RESOURCES RESEARCH GRANT PROPOSAL

Title: Factors controlling methylmercury degradation in Pine Barrens lakes.

Proposal submitted to the New Jersey Water Resources Research Institute

By

Tamar Barkay
Department of Biochemistry and Microbiology
Cook College, Rutgers University

Funds Requested: \$24,148

Priority issue addressed (I[c]): Integrity of aquatic and water associated ecosystems. Linkage between indicators and causal factors of ecosystem degradation.

Mercury concentrations in fish of the Pine Barren lakes are elevated as a consequence of the atmospheric deposition of mercury (Hg) and in-lake methylation processes (NJDEP, 1994; Ruppel et al., 1994). A range of tissue concentrations of Hg in fish collected from different lakes suggests that the production of methylmercury (MM)¹ is affected by factors that are unique to each lake. Recent work (Pak and Bartha, 1998)

The term MM relates to monomethylmercury throughout this document. Dimethylmercury while maybe formed (Baldi et al., 1997) has very rarely been detected in natural waters and is rapidly lost to the atmosphere.

Suggested similar production rates of MM in the sediments of three Pine Barren lakes suggesting that transport to, and/or MM degradation in, the water column, may be responsible to between-lakes variability. Availability of MM to the aquatic food chain might be controlled by degradation (and possibly production) of MM in the water column following the flux of MM from sediments. The proposed research addresses the degradation of MM in water samples and how it is affected by the physico-chemical and biological parameters in Pine Barren lakes.

Specific Objectives: To test the hypotheses: (i) that in Pine Barren lakes water the rate of MM degradation is inversely related to the MM/total Hg ratio, and (ii) that this inverse relationship is due to the abundance and expression of *mer* genes in the microbial communities of the lakes.

Background: Public health concerns with Hg contamination is mostly focused on the neuromuscular toxin, MM. This is the most toxic form of Hg and it is the form accumulated and biomagnified through trophic interactions of the food chain. Consequently, consumption of MM tainted fish is a major route for man exposure to Hg (Clarkson, 1997). Thus, processes that affect the production (i.e., synthesis and

degradation) of MM in aquatic environments are a key issue in determining Hg exposure and toxicity. These processes are a part of the geochemical cycling of Hg in the environment (illustrated in Fig. 1) and understanding the dynamics of this cycle would facilitate strategies and means for reducing MM exposure (Barkay, in press).

Fig. 1: The geochemical cycle of Hg in the environment. Full and broken arrows depict Hg transformations and transport processes, respectively. Arrow sizes reflect the magnitude of the corresponding reactions.

Elevated Hg concentrations in fish from oligotrophic acidic lakes is a wide spread phenomenon caused by the atmospheric deposition of Hg and the subsequent methylation by anaerobic microorganisms in lake sediments (Winfrey and Rudd, 1990). This process is the likely source for elevated Hg concentrations in fish from the Pine Barren lakes in southern NJ (NJDEP, 1994; Ruppel et al., 1994) and other lakes with a no known point source of Hg. Interestingly, in lake water as well as in groundwater from the Kirkwood-Cohansee aquifer that underlies the lakes, the concentration of MM does not directly relate to the concentration of total Hg. Furthermore, an inverse relationship was observed between MM/total Hg ratios and total Hg concentrations (Murphy et al., 1994). This “paradox” is not unique to the Pine Barren ecosystem. In general, fish in highly contaminated water (with $\mu\text{g L}^{-1}$ total Hg, Turner et al., 1985) contain as much MM as fish from some pristine oligotrophic lakes (with pg L^{-1} total Hg). The explanation for this “paradox” must be found in the processes that control MM production in aquatic systems, i.e., either methylation is enhanced, or MM degradation is inhibited at low Hg concentrations.

Recent results from a study on the potential for methylation and demethylation in anaerobic sediments suggested similar rates of MM production in three Pine Barren lakes Pak and Bartha, 1998). The two opposing processes converged to produce 20 – 40 ng ml^{-1} of MM after 2 weeks of incubation of sediment slurries that were spiked with either Hg(II) or MM. Thus, the flux of MM from sediment to the water column and the degradation of MM in the water column (where exposure of the aquatic food chain takes place; Watras et al., 1998) might control MM accumulation in Pine Barren lakes fauna./p>

Photodegradation and microbial processes have been implicated in MM degradation. Methylmercury may be degraded by a combination of photodegradation and microbial processes. In wetlands and lakes the former was shown to be the major route for demethylation (Sellers et al., 1996) and the importance of this process in Pine Barren lakes will be examined. Microbes may degrade MM by either oxidative or reductive pathways. Oxidative demethylation was suggested by the production of $^{14}\text{CO}_2$ when sediment slurries were incubated with ^{14}C -MM (Oremland et al., 1991; Hines et al., in press). The metabolic pathways responsible for oxidative demethylation are not known. Curiously, oxidative demethylation appears to be a more significant process in anaerobic environments as more $^{14}\text{CO}_2$ is produced from ^{14}C -MM during anaerobic than aerobic

the confluence of the Idrijca with the Soca river and the outfall of the later into the Gulf of Trieste (Gosar et al., 1997). We have measured the level of expression of *merA* and *merB* genes in RNA that had been extracted from the biomass of the microbial community and correlated it with the percent MM in total Hg in the river samples from which the biomass was collected (Table 1).

Table 1: Pearson correlations testing the null hypothesis that the expression levels of *mer* genes in microbial communities from Hg contaminated river water are uncorrelated to the proportion of MM in total Hg.

Parameter	Pearson coefficients	fg <i>merA</i> mRNA/ng total RNA	fg <i>merB</i> mRNA/ng total RNA	MM/total Hg (%)
fg <i>merA</i> mRNA/ng total RNA	r P Observations			
fg <i>merB</i> mRNA/ng total RNA	r P Observations	0.51 < 0.05 17		
MM/total Hg (%)	r P Observations	- 0.48 < 0.3 8	- 0.43 < 0.3 8	

First, the positive correlation between the expression of *merA* and *merB* confirms that *mer* operons carried by river microbes are co-transcribed as is known from the molecular analysis of this operon in pure bacterial cultures (see above). Second, these preliminary results suggested a negative correlations between the expression of microbial *mer* genes and the percent of MM in total Hg in water samples collected along the Hg gradient in the Idrijca River. The low P values (< 0.3) likely reflect the low number of observations (8) that is available at this time. This finding means that the proportion of MM declines when *mer* expression is increased and tentatively supports our hypothesis. Obviously, additional sampling in Slovenia and in other locations is needed for a more rigorous testing of our hypothesis.

Research methods, experimental design and expected results:

(i). Experimental design and expected results: Our experimental design is depicted in Fig. 2. Water samples will be collected in the fall, winter, spring and summer of 2000-

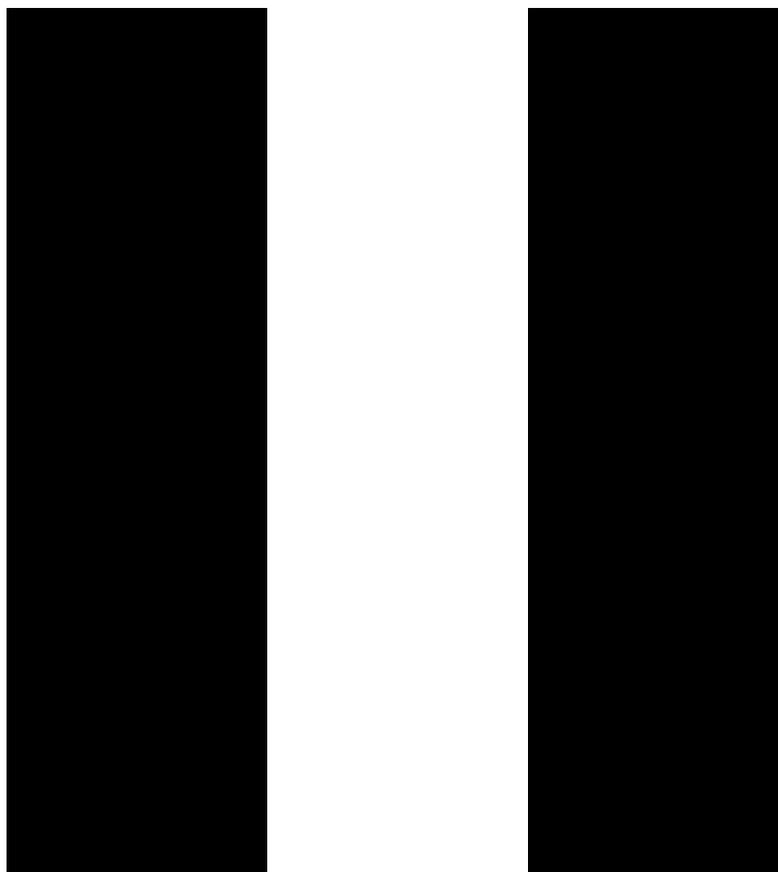


Fig. 2: Broad experimental design for the proposed project.

2001 from lakes known to have fish with various Hg tissue concentrations (NJDEP, 1994). If possible, 4 independent analyses will be obtained from each lake for each parameter. We will determine the concentrations of various Hg species, the potential of methylation and demethylation, and the abundance of *mer* genes and their transcripts. In addition, we will measure a physical-chemical parameters that affect Hg availability and microbial activities directly and indirectly influencing MM production. Data collected will be statistically analyzed to allow acceptance or rejection of our hypotheses. Hypothesis (i) will be accepted if MM degradation rates inversely correlate with MM/total Hg ratios. Hypothesis (ii) will be accepted if *mer* gene and transcript abundance is directly related to MM degradation rates and inversely related to MM/total Hg ratios. The results of this study will add to our on-going investigation on the processes that cause inverse relationships between total Hg and MM concentrations in natural waters. Because Hg is methylated in anoxic sediments, most research on MM production has focused on the benthic component of aquatic system. However, the aquatic food chain is exposed to MM in the water column and its degradation in this environmental compartment might be an important process determining exposure levels. To assure that our study is not an isolated effort we will integrate it with on-going research on the mobility and bioavailability of Hg in aquatic systems (The Superfund hazardous Substances Basic Research Program) that is currently initiated by the Dept. of Pharmacology and Toxicology at Rutgers. To the best of our knowledge the proposed

project together with on-going projects in the PI's laboratory, which use similar approaches to those described here to study Hg cycling in riverine ecosystems, are the first attempt to quantitatively relate the expression of a gene that affects a biogeochemical cycle with the dynamics of that cycle in the environment. This approach could lead to the placing of molecular events in the context of an ecosystem response to contaminants and to an understanding that would refine our ability to manage environmental contamination.

(ii). **Methods:** Many of the methods needed to carry out the proposed research have been routinely used in the PI's laboratory and previously tested protocols will be used here. These include methylation and demethylation assays using $^{203}\text{Hg}(\text{II})$ and ^{14}C -MM substrates, respectively (Vaithyanathan et al., 1996; Hines et al., in press), and determining the abundance of *mer* genes and their transcripts (Barkay, 1987, Barkay et al., 1989, Nazaret et al., 1994; Barkay et al., 1999).

Hg analysis: Clean protocols will be used during the collection of water samples (Gill and Fitzgerald, 1985). Total Hg and MM will be quantitated by ICP-MS at EOSHI's analytical facilities through a collaboration with Dr. Brian Buckley (see attached letter). If sensitivities obtained by this method (presently at the 10 pg L^{-1} range) are found to be insufficient, water samples will be sent to Frontier Geosciences in Seattle for analysis.

Physical-chemical factors: pH, O_2 and salinity will be measured in the field using portable equipment. DOC and particulate matter content will be measured as previously described (Barkay et al., 1997).

Statistical analysis: Regression analysis (Pearson correlations) will be used to test the significance of the dependency between concentrations of MM and total Hg, and the ratio between the two, and MM degradation rates, *mer* gene and transcript abundance. Multiple regression analysis will be performed to determine how each of the measured variables affects MM degradation in presence of all other variables.

Literature cited:

Baldi, F. 1997. Microbial transformation of mercury species and their importance in the biogeochemical cycle of mercury. In: A. Sigel and H. Sigel (Editor), Metal Ions in Biological Syst, 34, Marcel Dekker, pp. 213-257.

Barkay, T. 1987. Adaptation of aquatic microbial communities to Hg^{2+} stress. Appl. Environ. Microbiol. 53:2725-2732.

Barkay, T. The mercury cycle. 2000. Encyclopedia of Microbiology. 2nd edition. Academic Press, Inc., San Diego. in press.

Barkay, T., M. Hines, J-C. Bonzongo, K. Scott, P. Kringelum, W.B. Lyons, J.J. Warwick, And J. Faganelli. Molecular Analysis of a Mercury Contaminated Aquatic Ecosystem. 99th Annu. Meet. Am. Soc. Microbiol. Chicago, May 30 - June 3, 1999.

- Barkay, T., C. Liebert, and M. Gillman. 1989. Hybridization of DNA probes with wholecommunity genome for detection of genes that encode microbial responses to pollutants: *mer* genes and Hg²⁺ resistance. *Appl. Environ. Microbiol.* 55:1574-1577.
- Barkay, T., M. Gillman, and R.R. Turner. 1997. Effects of dissolved organic carbon andspeciation of Hg(II) on bioavailability of mercury. *Appl. Environ. Microbiol.* 63:4267-4271.
- Barkay, T., R. Turner, A. VandenBrook, and C. Liebert. 1991. The relationships ofHg(II) volatilization from a freshwater pond to abundance of *mer* genes in the gene pool of the indigenous microbial community. *Microb. Ecol.* 21:151-161.
- Clarkson, T.W. 1997. The toxicology of mercruy. *Cri. Rev. Clin. Lab. Sci.* 34:369-403.
- Gill, G.A., and W.F. Fitzgerald. 1985. Mercury sampling of open ocean waters at the picomolar level. *Deep-Sea Res.* 32:287-297.
- Gosar, M., S. Pirc and M. Bidovec. 1997. Mercury in the Idrija river sediments as a reflection of mining and smelting activities of the Idrija mercury mine. *J. Geochem. Explor.* 58:125-131.
- Hines, M.E., M. Horvat, J. Faganeli, J.-C.J. Bonzongo, T. Barkay, E.B. Major, K.J. Scott, E.A, Bailey, J.J. Warwick, and W.B. Lyons. Mercury Biogeochemistry in the Idrija River, Slovenia from above the mine into the Gulf of Trieste. *Environ. Res.* In press.
- Marvin-Dipasquale, M.C., and Oremland, R.S. 1998. Bacterial methylmercurydegradation in Flroida Everglades peat sediment. *Environ. Sci. Technol.* 32:2556-2563.
- Murphy, E.A., J. Dooley, H.L. Windom, and R.G. Smith, Jr. 1994. Mercury species in potable water in Southern New Jersey. *Wat. Air Soil Pollut.* 78:61-72.
- Nazaret, S., W.H. Jeffrey, E. Saouter, R. Von Haven, and T. Barkay. 1994. *merA* gene expression in aquatic environments measured by mRNA production and Hg(II) volatilization. *Appl. Environ. Microbiol.* *Appl. Environ. Microbiol.* 60:4059-4065.
- NJ DEP. 1994. Preliminary assessment of total mercury concentrations in fish from rivers, lakes and reservoirs in New jersey. Report no. 93-15F. NJDEP, Trenton.
- Oremland, R.S., C.W. Culbertson and M.R. Winfrey. 1991. Methylmercury decomposition in sediments and bacterial cultures: Involvement of methanogens and sulfate reducers in oxidative demethylation. *Appl. Environ. Microbiol.* 57: 130-137.
- Pak, K.R. and R. Bartha. 1998. Mercury methylation and demethylation in anoxic lake sediments and by strictly anaerobic bacteria. *Appl Environ Microbiol*, 64: 1013-1017.

Ralston, D.M. and T.V. O'Halloran. 1990. Ultrasensitivity and heavy-metal sensitivity of the allosterically modulated MerR transcription complex. Proc. Natl. Acad. Sci. USA 87: 3846-3850.

Rasmussen, L.D., R.R. Turner and T. Barkay. 1997. Cell-density-dependent sensitivity of a *mer-lux* bioassay. Appl. Environ. Microbiol. 63: 3291-3293.

Ruppel, B., A. Stern, and M. Sheil. 1994. Mercury concentrations in New Jersey fish.

Report of the Toxics in Biota Committee NJDEP, Trenton, NJ.

Sellers, P., C.A. Kelly, J.W.M. Rudd and A.R. MacHutchon. 1996. Photodegradation of methylmercury in lakes. Nature, 380: 694-697.

Silver, S. and L.T. Phung. 1996. Bacterial heavy metal resistance: new surprises. Annu. Rev. Microbiol., 50: 753-789.

Summers, A.O. 1992. Untwist And Shout: A Heavy metal-responsive transcriptional regulator. J. Bacteriol. 174: 3097-3101.

Turner, R.R., G.E. Kemp, M.A. Bogle, J. Switek, and R. McElhaney. 1985. Sources and discharges of mercury in drainage waters at the Oak Ridge Y-12 plant. Oak Ridge Y-12 plant, Y/TS-90.

Vaithyanathan, P., R.G. Kavanaugh, , C.B. Craft, C. J. Richardson, and T. Barkay. 1996. The role of eutrophication in the distribution and potential net methylation of mercury in the peat soils of the Everglade. Env. Sci. Technol. 30:2591-2597.

Walsh, C.T., M.D. Distefano, M.J. Moore, L.M. Shewchuk and G.L. Verdine. 1988. Molecular basis of bacterial resistance to organomercurial and inorganic mercuric salts. FASEB J., 2: 124-130.

Watras, C.J., R.C. Back, S. Halvorsen, R.J.M. Hudson, K.A. Morrison, and S.P. Wentz. Bioaccumulation of mercury in pelagic freshwater food webs. Sci. Total Environ. 219:183-208.

Winfrey, M.R., and J.W.M. Rudd. 1990. Environ. Toxicol. Chem. 9:853-869.