Unraveling the importance of solid and adsorbed phase mercury speciation for methylmercury formation, evasion and bioaccumulation

Sofi Jonsson
Till Mathias – min älskade och saknade
# Table of Contents

List of publications ii
Abstract iii
Abbreviations v
Sammanfattning (Summary in Swedish) vi

1. **Introduction** 1

2. **Experimental systems and approaches** 8
   2.1 Hg speciation and fractionation analysis in environmental samples 8
   1.1 Application of Hg stable isotope experimental approaches 10
   1.1.1 Determination of gaseous Hg$^0$, Hg$^{II}$, MeHg and DMHg 12
   1.1.2 Methylation and demethylation rates and rate constants 14
   1.1.3 Determination of methylation rate constants using solid or adsorbed chemical forms of Hg$^{II}$ (paper II) 18
   1.2 Scale of experiment – from micro- to mesocosms 19

3. **Mercury methylation, evasion and bioaccumulation** 21
   3.1 Methylation of Hg$^{II}$ 22
   3.2 Demethylation of MeHg 26
   3.3 Evasion of Hg 27
   3.4 Bioaccumulation of mercury 28

4. **Implication and final remarks** 35

Acknowledgements 41
References 44
List of publications

Publications included in this thesis:


II. Sofi Jonsson, Ulf Skyllberg, Mats B. Nilsson, Per-Olof Westlund, Andrey Shchukarev, Erik Lundberg, Erik Björn (2012), Mercury Methylation Rates for Geochemically Relevant Hg^{II} Species in Sediments, *Environmental Science and Technology*, 46, 11653-11659

III. Sofi Jonsson, Ulf Skyllberg, Mats B. Nilsson, Agneta Andersson, Erik Lundberg, Erik Björn, Differentiated reactivity of geochemical mercury pools control methylmercury levels in sediment and biota, *Manuscript*


Paper I and II is reprinted with permission from publisher. Copyright © 2010 and 2012, American Chemical Society.
Abstract

Monomethylmercury, MeHg, is formed under anoxic conditions in waters, sediments and soils and then bioaccumulated and biomagnified in aquatic food webs, negatively effecting both human and wildlife health. It is generally accepted that precipitation of mercury, Hg, and adsorption of Hg to e.g. organic matter and mineral surfaces are important processes limiting the reactivity of Hg mobilized in the environment by natural and anthropogenic activities. However, knowledge concerning the role of different solid and adsorbed chemical forms of Hg for MeHg formation, evasion and bioaccumulation is missing. Such information is vital for the understanding of environmental processes controlling MeHg formation and bioaccumulation, as well as for predicting how changes in e.g. loading rates of atmospheric Hg and the outcome of climate change scenarios and anthropogenic land use could alter Hg concentrations in biota.

In this thesis, a novel experimental approach, using isotopically enriched solid and adsorbed phases of inorganic Hg, Hg$^{II}$, as tracers, was developed. Using this approach, we successfully determined rates of MeHg formation from solid and adsorbed Hg species in sediment slurries and in mesocosm systems under conditions closely resembling those in field. We conclude that the solid/adsorbed phase speciation of Hg$^{II}$ is a major controlling factor for MeHg net formation rates. Microcosm experiments revealed that newly formed MeHg was a major contributor to the evasion of MeHg from the water–sediment system, emphasizing the importance of MeHg formation rate, rather than MeHg concentration, in the sediment for this process. From mesocosm systems, we provide experimental evidence, as well as quantitate data, for that terrestrial and atmospheric sources of Hg$^{II}$ and MeHg are more available for methylation and bioaccumulation processes than Hg$^{II}$ and MeHg stored and formed in sediments. This suggests that the contribution from terrestrial and atmospheric sources to the accumulation of Hg in fish may have been underestimated. As a consequence, in regions where climate change is expected to further increase land runoff, terrestrial MeHg sources may have even higher negative effects on biota than previously thought. Data and concepts presented in this thesis lay the basis for unprecedented in-depth modeling of processes in the Hg biogeochemical cycle that will
improve our understanding and the predicting power on how aquatic ecosystems may respond to environmental changes or differences in loading rates for atmospheric Hg.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAF</td>
<td>Biota Accumulation Factor</td>
</tr>
<tr>
<td>BSAF</td>
<td>Biota-Sediment Accumulation Factor</td>
</tr>
<tr>
<td>DMeHg</td>
<td>Dimethylmercury</td>
</tr>
<tr>
<td>DOM</td>
<td>Dissolved Organic Matter</td>
</tr>
<tr>
<td>d.w.</td>
<td>Dry Weight</td>
</tr>
<tr>
<td>EXAFS</td>
<td>Extended X-ray Absorption Fine Structure</td>
</tr>
<tr>
<td>GC</td>
<td>Gas Chromatography</td>
</tr>
<tr>
<td>GEM</td>
<td>Gaseous Elemental Mercury</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>Hg$^{II}$</td>
<td>Inorganic divalent mercury</td>
</tr>
<tr>
<td>ICPMS</td>
<td>Inductively Coupled Plasma Mass Spectrometry</td>
</tr>
<tr>
<td>IDA</td>
<td>Isotope Dilution Analysis</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>$K_D$</td>
<td>Solid-liquid partition coefficient</td>
</tr>
<tr>
<td>$k_d$</td>
<td>Demethylation rate constant</td>
</tr>
<tr>
<td>$k_m$</td>
<td>Methylation rate constant</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of Detection</td>
</tr>
<tr>
<td>MeHg</td>
<td>Monomethylmercury</td>
</tr>
<tr>
<td>METALLICUS</td>
<td>Mercury Experiment to Assess Atmospheric Loading in Canada and the United States</td>
</tr>
<tr>
<td>NOM</td>
<td>Natural Organic Matter</td>
</tr>
<tr>
<td>$r_d$</td>
<td>Rate of demethylation</td>
</tr>
<tr>
<td>RGM</td>
<td>Reactive Gaseous Mercury</td>
</tr>
<tr>
<td>$r_m$</td>
<td>Rate of methylation</td>
</tr>
<tr>
<td>Sed</td>
<td>Sediment</td>
</tr>
<tr>
<td>UNEP</td>
<td>United Nations Environmental Program</td>
</tr>
<tr>
<td>US EPA</td>
<td>United States Environmental Protection Agency</td>
</tr>
<tr>
<td>Wt</td>
<td>Water</td>
</tr>
<tr>
<td>XAS</td>
<td>X-ray Absorption Spectroscopy</td>
</tr>
<tr>
<td>XANES</td>
<td>X-ray Absorption near-edge Structure</td>
</tr>
</tbody>
</table>
Sammanfattning (Summary in Swedish)

Monometylkvicksilver, MeHg, bildas under anoxiska förhållanden i naturliga vatten, sediment och jordar och bioackumuleras och magnifieras därefter i den akvatiska näringsskedjan med negativa effekter på djur och människor som följd. Det är generellt vedertaget att utfällning av Hg och adsorption av Hg till exempelvis organiskt material och mineralytor begränsar tillgängligheten för biogeochemiska reaktioner av Hg som mobiliserats i miljön via naturliga och antropogena processer. Kunskap om betydelsen av speciationen av Hg i fasta och adsorberade faser för bildning, avgång och bioackumulering av MeHg är dock bristfällig. Denna information är kritisk för att förstå vilka processer som kontrollerar bildning och bioackumulering av MeHg samt för att kunna prediktera hur olika ekosystem kan förväntas svara på exempelvis ändrad deposition av atmosfäriskt Hg eller hur klimatförändringar kan påverka koncentrationerna av Hg i fisk.

I denna avhandling har en experimentell metod utvecklades, där isotopanrikade fasta och adsorberade kemiska former av oorganiska tvåvärt Hg, Hg$^{II}$ används som s.k. "tracers". Denna metod användes för att bestämma MeHg bildningshastigheter i homogeniserade sediment prover samt i mesokosmsystem där förhållandena efterliknar de som förväntas i naturliga ekosystem. Från dessa drar vi slutsatsen att speciationen av Hg$^{II}$ i fast/adsorberad fas är en viktig kontrollerande faktor som begränsar nettobildningen av MeHg. Mikrokosmexperiment visade att i första hand nyligt bildad MeHg avgick till gasfas vilket understryker betydelsen av MeHg bildningshastighet, snarare än koncentration, i sedimentet för denna process. Från mesokosmexperimenten visar vi, med kvantitativa data, att terrestra och atmosfäriska källor av Hg$^{II}$ och MeHg är mer tillgängliga för bildning och bioackumulering av MeHg än Hg$^{II}$ och MeHg lagrat eller bildat i sedimenten. Orsaken till detta är framförallt skillnad i speciationen av Hg i fasta/adsorberade faser. Detta innebär att bidraget från MeHg från terrestra och atmosfäriska källor till koncentrationen av Hg i fisk kan ha underskattats, samt att de negativa effekterna på MeHg exponering i områden där exempelvis klimatförändringar förväntas leda till ökad terrest avrinning kan bli mer allvarliga än vad som tidigare predikterats. Data som
presenteras i denna avhandling möjliggör modellering av Hg’s biogeokemiska cykel på en ny detaljnivå samt möjliggör säkrare prediktioner av hur olika ekosystem kan förväntas svara mot miljöförändringar eller ändrad deposition av atmosfäriskt Hg.
1. Introduction

Anthropogenic activities, such as combustion of fossil fuels and gold mining, have significantly increased the pool of mercury (Hg) mobilized in the environment.\textsuperscript{1-2} This has resulted in an increased chronic exposure of Hg for marine and terrestrial organisms.\textsuperscript{3-4} Figure 1 illustrates the principal transportation and biogeochemical processes in focus for a large community of politicians, policy makers and scientists.\textsuperscript{5} Mercury is emitted, primary to the atmosphere,\textsuperscript{6-8} by both natural and anthropogenic sources. A fraction of this Hg is methylated in sediment,\textsuperscript{9} soils\textsuperscript{10} and waters\textsuperscript{11} by sulfate\textsuperscript{12-13} and/or iron\textsuperscript{14-15} reducing bacteria to monomethylmercury (MeHg) and then bioaccumulated and biomagnified in aquatic food webs.\textsuperscript{16-18}

Consumption of fish is the main exposure route of Hg for humans in most regions.\textsuperscript{19} Acute poisoning of Hg causes severe damage to the nervous system and in worst case death,\textsuperscript{19} such events are however nowadays rare.\textsuperscript{20} For people with a varied diet (including fish but limited intake of certain fish species), the exposure is typically at levels considered being without any associated health risks. Still, 7\% of women in child-bearing age in the USA

![Figure 1. Illustration of the principal biogeochemical processes of Hg, from anthropogenic emission to accumulation in fish.](image-url)
are estimated by the United States Environmental Protection Agency (US EPA) to have a diet with Hg concentrations exceeding this level.19 MeHg is able to cross the blood-to-brain and placenta barriers.21 Fetuses are thus particularly vulnerable and low exposure levels of Hg have been suggested to result in decreased birth weight and negatively affect fine motor, memory and language skills.22 However, at present, unanimous evidence of negative effects from low Hg dose exposure exists only for neurocognitive effects at exposure levels typical in population groups with an unusually high intake of fish, marine mammals or highly Hg contaminated fish from areas with local Hg pollution sources.

In Sweden alone, tenths of thousands of lakes have Hg concentrations in piscivorous fish exceeding the levels considered safe for human consumption.23-24 In addition to the concerns for human and wildlife health, Hg in fish results in financial losses (primary for commercial and sport fisheries) and forces to changes in way of life for populations traditionally depending on local fish sources.8,25-26 The bioaccumulated Hg primary originates from atmospherically deposited Hg and it has been suggested, and in some studies experimentally supported, that newly deposited Hg at a higher extent contribute to fish Hg than past depositions do.24-25,27-28 The atmospheric pool of Hg has increased 3-5 times since preindustrial time.29 It has however been difficult to establish an unambiguous relationship between recent changes in the atmospheric load and fish concentration of Hg.25,30-31 In Sweden and in the USA a general decrease in fish Hg was observed from the beginning of the 1980s to the end of the 1990s which was linked to decreased atmospheric deposition rates of Hg.4,32-34 Recent studies have however revealed a shift in this trend and increasing concentrations of Hg in fish has been reported during the last decade.31,33-34 In Sweden, Åkerblom31 et al. reported increased concentration of Hg in pike for 36 % of lakes (n = 25), between 1994 and 2006. The increase was positively related to total organic carbon in the lake water. It has been suggested that variations in hydrological conditions to (at least on a shorter time scale of decades to centuries) possibly can counteract the positive effects expected from further reduced atmospheric Hg load.33-34
Global emission inventories, although containing large uncertainties, reveals that the anthropogenic emissions of Hg to the atmosphere have decreased during the last decades due to implementation of emission controls and restricted use of Hg in high income countries (mainly Europe and North America). However, emissions are increasing in low income countries with a growing economy, industrialization and an increasing demand of energy and heat production. Pacyna et al. have predicted (for main atmospheric emission sources of Hg in an illustrative model) that the anthropogenic emissions could i) increase by 25% from year 2000 to 2020 if current control practices remain unchanged and with the expected global economic growth, ii) be reduced by 40% if currently implemented and planned emission controls for Europe and North America are implemented worldwide; and iii) be reduced by 55% if the technologically best available emission controls would be implemented worldwide. United Nations Environmental Program (UNEP) is currently leading international negotiations for a “global legally binding instrument on Hg” (negotiations are planned to result in an agreement open for signature in the beginning of 2013).

The severeness of Hg as a global pollutant is to a large extent controlled by different environmental processes resulting in MeHg formation from fresh Hg deposits (from natural and anthropogenic emissions sources) and from Hg stored in the environmental compartments, and the subsequent bioaccumulation of MeHg in aquatic food webs. The largest fraction of Hg emitted is accumulated and stored in deep ocean waters, or in sediment and soils as solid and adsorbed phases of inorganic divalent Hg. Because fresh Hg deposits and Hg stored in the environmental compartments at the same time are contributing to the formation of MeHg formation and its bioaccumulation, the link between current anthropogenic Hg emissions and MeHg in fish is neither direct nor simple. It is currently uncertain to what extent and within what timeframe a reduction of anthropogenic emissions of Hg will result in reduced concentrations of Hg in fish. There is a consensus among scientist, as well as environmental organizations, that an improved understanding of Hg biogeochemistry is required to develop effective strategies for decreasing the anthropogenic contribution of Hg in fish. Unraveling the biogeochemical processes for MeHg formation, transportation and bioaccumulation is a key objective in this aspect. The biogeochemistry of Hg is obviously far more complex than
illustrated in Figure 1, involving multiple chemical forms of Hg (Table 1) that undergo photo-, biological-, and chemical reactions and that are readily cycled among the atmosphere, soils, wetlands, waters and sediments. A chemical specie is defined by IUPAC as “specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure” and speciation is defined as “distribution of an element amongst defined chemical species in a system”. Throughout this thesis, the sum of all possible species of inorganic divalent mercury and monomethylmercury is referred to as Hg$^{II}$ and MeHg, respectively. I will refer to geochemical pools of Hg$^{II}$ and MeHg as differing with respect to their i) spatial distribution (e.g. buried in sediment or localized at the sediment surface), and/or with respect to their ii) chemical speciation. In the latter case, chemical species includes well-defined solid phases like α-HgS, β-HgS and Hg$^{II}$ and MeHg adsorbed to more complex components such as natural organic matter (NOM) and iron sulphide minerals (Mackinawite, FeS(s)).

Geochemical pools of Hg have been assumed to differ with respect to their availability for methylation, bioaccumulation and transportation. At what extent the different geochemical pools control Hg geo- and biogeochemistry is however to a large extent unknown. Current insights have been derived from experiment using quite simple model system but establishment of the importance of the processes in complex system at conditions realistic for natural environments are missing. This can partly be addressed to a previous lack of suitable experimental approaches. Understanding the importance of different geochemical Hg$^{II}$ and MeHg pools is further complicated by the fact that analytical methods for determination of individual Hg species in most cases have a limit of detection (LOD) far above the concentrations found in environmental matrices. We thus rely on chemical speciation modelling, sequential extraction procedures isolating operationally defined phases or measurement techniques requiring addition of Hg in the lab. These uncertainties and lack of knowledge contributes to MeHg and Hg$^{II}$ often being viewed as uniform pools in sediment and soils and the chemical speciation of solid-phase Hg is often neglected. Obviously, new analytical methods and experimental approaches are needed.
| Sample Type | Concentration (unit) | Hg Species | % of Hg 
$^{19,39,54-55}$ |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>1-170 ng m$^{-3}$</td>
<td>$\text{Hg}^0$ (g)</td>
<td>&gt;95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\text{Hg}^{II}$ (OH/Cl/Br)$_n$(aq), NOM, -(O/N/S)$\equiv$</td>
<td>&lt;2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\text{MeHg}$ (OH/Cl/Br)(aq), SH(g), NOM, -(O/N/S)$\equiv$</td>
<td>&lt;3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\text{DMeHg}$ (g, aq)</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>0.2-15 ng L$^{-1}$</td>
<td>$\text{Hg}^0$ (g, aq)</td>
<td>10-54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\text{Hg}^{II}$ -(OH/Cl/Br)$_n$(aq), -DOM(aq), NOM, -(O/N/S)$\equiv$</td>
<td>30-90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\text{MeHg}$ -(OH/Cl/Br)(aq),-DOM(aq), -(O/N/S)$\equiv$,</td>
<td>&lt;1-30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\text{DMeHg}$ (g, aq)</td>
<td>&lt;1.7</td>
</tr>
<tr>
<td>Sediment</td>
<td>2-2200 ng g$^{-1}$ d.w.</td>
<td>$\text{Hg}^0$ (g, aq, l)</td>
<td></td>
</tr>
<tr>
<td>/Soil</td>
<td></td>
<td>$\text{Hg}^{II}$ -(OH/Cl/Br)$_n$(aq), (SH)$_2$(aq), $S_2H^+(aq)$, $S_2^-(aq)$, DOM(aq), $\alpha$ and $\beta$-HgS(s), NOM, -(O/N/S)$\equiv$</td>
<td>&gt;96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\text{MeHg}$ NOM, DOM(aq), SH(g), S' (aq), -(O/N/S)$\equiv$</td>
<td>0.06-4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\text{DMeHg}$ (g, aq)</td>
<td></td>
</tr>
<tr>
<td>Fish</td>
<td>10-1300 ng g$^{-1}$ d.w.</td>
<td>$\text{Hg}^{II}$ -S-biomolecules</td>
<td>&lt;5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\text{MeHg}$ -S-biomolecules</td>
<td>&gt;95</td>
</tr>
</tbody>
</table>

Table 1. Summary of concentrations and speciation of Hg in air, water, sediment/Soils and fish (≡ denotes mineral surfaces).
in order to improve our understanding of the role played by different geochemical pools of Hg$^{II}$ and MeHg in the biogeochemical cycle of mercury.

This thesis involves work with a recently developed analytical method for speciation analysis of gaseous MeHg, dimethylmercury (DMeHg), Hg$^{II}$ and elemental Hg (Hg$^0$) (paper I), and a new experimental approach for determination of Hg methylation of solid/adsorbed phase isotope tracers (paper II-IV). Both of these methods provide great opportunities to expand our understanding on Hg biogeochemistry and will be described and discussed in the following chapter. Using these methods, we have studied formation, transportation and bioaccumulation of Hg chemical forms originating from different geochemical pools as indicated with colored arrows (assigned paper I-IV) in Figure 2.
In summary, we (i) have showed a substantial emission of newly methylated Hg from a contaminated water-sediment system (paper I), (ii) have quantified the rates of methylation and bioaccumulation originating from different geochemical pools of Hg$^{II}$ (paper II-IV) in sediment slurries as well as in larger-scale sediment-water systems with intact sediment cores, (iii) showed that newly deposited Hg$^{II}$ and MeHg is significantly more available for MeHg formation and bioaccumulation than Hg$^{II}$ and MeHg accumulated or formed in the sediment, and (iv) showed that, in the estuarine system, formation of MeHg from sediment pools of Hg increases with pelagic primary production whereas enhanced loading of terrestrial NOM results in higher formation of MeHg, as well as higher bioaccumulation, from newly deposited Hg (paper IV).
2. Experimental systems and approaches

This chapter describes the possibilities and limitations of current methods when it comes to the determination of Hg speciation in environmental matrices. Thereafter I describe and discuss the new methods and approaches used in papers I-IV, as well as the experimental scale (from microcosm (papers I-II) to mesocosm (papers III-IV)).

2.1 Hg speciation and fractionation analysis in environmental samples

Analytical methods available for quantifying concentrations of total Hg and concentrations of specific chemical forms of Hg present at trace levels (i.e. <100 parts per million atoms (ppma) or <100 µg g⁻¹) in sediment, soil, water and air (Table 1) have provided the basis from which our current understanding of environmental Hg processes has evolved. These methods typically include procedures for i) extraction, purification and preconcentration of the analyte from the matrix, ii) separation of chemical species and iii) element specific detection. The most commonly used method is the determination of total Hg in solid, aqueous or gaseous samples. Methods for determination of individual Hg species (e.g. the dissolved Hg²⁺ complexes Hg-(OH)₂, -Cl₂, and specific (-SR)₂) generally requires softer extraction procedures and/or separation techniques e.g. high performance liquid chromatography (HPLC), which results in relatively high LOD (typical LOD for HPLC-ICPMS of 0.0001-1 µg L⁻¹). In most cases, such as for the determination of specific Hg²⁺-thiols, LOD for suitable methodologies are far above the concentrations expected in e.g. natural water or sediment pore waters. As an alternative, fractionation, defined by IUPAC as “process of classification of an analyte or a group of analytes from a certain sample according to physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties”, are commonly used. The analytical methods and modelling approaches used for Hg fractionation and speciation in Papers I-IV are listed in Table 2.
Table 2. Analytical methods and model approaches used for total Hg, Hg fractionation and Hg speciation in Papers I-IV.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Analyte</th>
<th>Method</th>
<th>Paper</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>Hg\textsuperscript{0}, MeHg, DMeHg</td>
<td>On-line ethylation of MeHg (NaBEt\textsubscript{4}), purge and trap GC-ICPMS analysis</td>
<td>I</td>
<td>59-60</td>
</tr>
<tr>
<td>Water</td>
<td>total Hg</td>
<td>Sample digestion (BrCl), Hg reduction (SnCl\textsubscript{2}), purge and trap ICPMS analysis</td>
<td>I, II</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>total Hg</td>
<td>Sample digestion (BrCl), on-line Hg reduction (SnCl\textsubscript{2}), ICPMS analysis</td>
<td>III, IV</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Hg\textsuperscript{II} speciation</td>
<td>Chemical equilibrium modeling</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>MeHg</td>
<td>Ethylation of MeHg (NaBEt\textsubscript{4}), purge and trap GC-ICPMS analysis</td>
<td>I-IV</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>MeHg speciation</td>
<td>Chemical equilibrium modeling</td>
<td>I</td>
<td>47,63</td>
<td></td>
</tr>
<tr>
<td>Sediment</td>
<td>total Hg</td>
<td>Microwave assisted acid digestion (HNO\textsubscript{3}, HCl) and ICPMS analysis</td>
<td>I-IV</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Hg\textsuperscript{II} speciation</td>
<td>Chemical equilibrium modeling</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hg\textsuperscript{II} speciation</td>
<td>Hg L\textsubscript{III-edge} EXAFS</td>
<td>II</td>
<td>65-66</td>
</tr>
<tr>
<td>MeHg</td>
<td>Double extraction of MeHg (CuSO\textsubscript{4}/KBr/H\textsubscript{2}SO\textsubscript{4} followed by CH\textsubscript{2}Cl\textsubscript{2}) into MQ water, ethylation of MeHg (NaBEt\textsubscript{4}), purge and trap GC-ICPMS analysis</td>
<td>I-IV</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Biota</td>
<td>total Hg</td>
<td>Sample digestion (80°C, HNO\textsubscript{3} and H\textsubscript{2}SO\textsubscript{4}) ICPMS analysis</td>
<td>III-IV</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>MeHg</td>
<td>Alkaline digestion (tetraethylammonium hydroxide), ethylation of MeHg (NaBEt\textsubscript{4}), purge and trap GC-ICPMS analysis</td>
<td>III-IV</td>
<td>69</td>
</tr>
</tbody>
</table>

Mercury in air is commonly fractionated into gaseous elemental Hg (GEM, i.e. total gaseous Hg passing 0.45 µm filter, presumably Hg\textsuperscript{0}), reactive gaseous Hg (RGM; i.e. gaseous Hg collected in mist chambers or denuders, presumably Hg\textsuperscript{II}(aq)) and particulate Hg (Hg\textsuperscript{II}(s), i.e. Hg collected using filter based techniques or denuder methods).\textsuperscript{54,70-71} Elemental Hg constitutes approximately 95 % of the atmospheric pool of Hg but RGM and particulate Hg\textsuperscript{II}(s) are the primary forms of Hg deposited to land and water and hence in many cases desirable to quantify.\textsuperscript{20}
In water (including pore waters of sediment and soil), total Hg is often fractionated by a separate determination of MeHg, Hg\(^0\) and Hg\(^{\text{II}}\) (as total Hg - MeHg).\(^{61-62}\) The apparently dissolved fraction of MeHg and total Hg are determined after removal of particles, most commonly larger than 0.45 µm, by filtration.\(^{61}\) However, Hg\(^{\text{II}}\) and MeHg both have a strong affinity for binding sites on solid particles and the fraction defined as “dissolved” can be assumed to contain a significant part (12-93%)\(^{72-74}\) of Hg\(^{\text{II}}\) and MeHg adsorbed to particles with a size less than 0.45 µm. Analytical methods that enable quantification of specific MeHg and Hg\(^{\text{II}}\) species at natural concentration levels in waters are at present not available. Chemical equilibrium modeling approaches (thermodynamic calculations) thus play an important role since the speciation of dissolved MeHg and Hg\(^{\text{II}}\) control the mobility and bioavailability of Hg (paper I).\(^{47-49}\) As discussed by Skyllberg\(^49\), these models still involve large uncertainties and we are far from fully understanding Hg speciation in natural matrices. Further research is needed to determine accurate thermodynamic stability constants and understanding the adsorption properties of Hg\(^{\text{II}}\) and MeHg on e.g. metal sulfide surfaces.

Total Hg in the solid phase (sediment and soils) is typically fractionated by a separate determination of MeHg and Hg\(^{\text{II}}\).\(^{20,67,75}\) In samples contaminated by mining or chloro–alkali industry, the solid phase speciation of Hg\(^{\text{II}}\) (e.g. cinnbar, metacinnbar, HgSO\(_4\), HgO and HgCl\(_2\)) can be determined using synchrotron radiation based X-ray Absorption Spectroscopy (XAS) techniques, e.g. X-ray Absorption Near-Edge Structure (XANES) and Extended X-ray Absorption Fine Structure (EXAFS) Spectroscopy.\(^{76-78}\) For less contaminated sediments or soils, the concentrations of Hg are however too low and additions of Hg are needed for XAS analysis.\(^{66,79}\) Such addition have however been proven useful in establishing the main adsorbed and solid phases of Hg\(^{\text{II}}\), as well as adsorbed phases of MeHg in sediments and soils.\(^{49,53,66}\)

### 1.1 Application of Hg stable isotope experimental approaches

For elements such as carbon and nitrogen, the occurrence of two stable isotopes (\(^{12}\)C, \(^{13}\)C and \(^{14}\)N, \(^{15}\)N, respectively) is widely used i) for quantification purposes by isotope dilution analysis, IDA, ii) to study reactions and processes in natural samples by addition of an isotope enriched
reactant or iii) to trace environmental processes in the field from natural isotope fractionation. Mercury has as many as seven naturally occurring stable isotopes (average natural abundance in %); $^{196}$Hg (0.14 %), $^{198}$Hg (10.0 %), $^{199}$Hg (16.8 %), $^{200}$Hg (23.1 %), $^{201}$Hg (13.2 %), $^{202}$Hg (29.8 %) and $^{204}$Hg (6.9 %). Multiple isotope enriched Hg tracers can thus be added to a single system and be quantified using mass specific detection and signal deconvolution of the detected Hg isotopic composition. This allow us to simultaneously study several individual environmental processes in complex experimental systems containing a “background” of ambient Hg. Commercially available isotopically enriched Hg (often as Hg$^0$, HgO or HgCl$_2$) are typically enriched in the given isotope from 30 to $>$99 %. In most studies, isotopically enriched aqueous Hg$^{II}$ or MeHg complexes are used, however isotopically enriched DMeHg and Hg$^0$ has also been synthesized and used for quantification purposes. In this thesis also the isotopically enriched solid phases cinnabar ($\alpha$-HgS(s)) and metacinnabar ($\beta$-HgS(s)) and, Hg$^{II}$ reacted with Makinawite ($\equiv$FeS-Hg$^{II}$), and Hg$^{II}$ and MeHg adsorbed to natural organic matter (Hg$^{II}$-NOM, MeHg-NOM) have been synthesized and used (paper II-IV).

In IDA, a known amount of an isotopically enriched Hg standard is added as internal standard to the experimental sample before sample workup is initiated. In contrast to external calibration methods, losses or incomplete extraction of the analyte during the analytical procedures can be accounted for (under the assumptions that added internal standard is properly equilibrated with the sample matrix and exhibits the same (or very similar) chemical and physical properties as the analyte). Larsson and co-workers recently developed a method, applied in paper I, for determination of Hg species/fractions in gaseous samples. By using an isotope dilution approach, Larsson and co-workers successfully addressed several critical issues (most importantly incomplete recoveries and species transformation) associated with other methods.

In paper II-IV, up to 5 different isotopically enriched tracers were used to study the reactivity and/or transportation of Hg (the $6^{th}$ and $7^{th}$ isotope are reserved for detection of ambient Hg and for internal standard). The use of isotopically enriched Hg as tracers is most commonly used to study
methylation and demethylation processes in sediment, soils and water.\textsuperscript{50} Typically, Hg\textsuperscript{II}(aq) and MeHg(aq) tracers are added to experimental samples and incubated for up to 48 h. Thereafter the rate constants for Hg\textsuperscript{II} methylation and MeHg demethylation can be calculated. The main concern with this approach is if tracers (added as labile aqueous Hg\textsuperscript{II} or MeHg complexes) will react differently than ambient Hg and MeHg, which under relevant environmental conditions can be assumed to bind to thiol groups in NOM, at FeS surfaces or precipitate as \(\beta\)-HgS(s). To address this issue we developed an approach, discussed below, where we synthesized and added isotopically enriched tracers as solid or adsorbed forms (paper II).

Isotope fractionation of C, N and H have been studied since the 1950’s and is today widely used to study natural processes of these elements.\textsuperscript{85} Given the much lower natural isotope fractionation of the Hg atoms, and a previous lack of analytical techniques with sufficient accuracy of isotope ratio measurements, isotope fractionation of Hg have only during the last decade been recognized as a powerful tool to study biogeochemical processes of Hg.\textsuperscript{86} At present a number of environmental processes have been shown to fractionate Hg isotopes. Different anthropogenic sources of Hg are also known to differ in isotopic signature, but to what extent isotope fractionation could be used to e.g. track sources of Hg in the environment remains uncertain.\textsuperscript{86-87} The approach will however most likely contribute to future advances in our understanding of the environmental processes of Hg and gain a wide interest among Hg scientist. Although such techniques were not used in this thesis they are worth highlighting as emerging experimental tools based on stable Hg isotope measurement techniques.

\subsection*{1.1.1 Determination of gaseous Hg\textsuperscript{0}, Hg\textsuperscript{II}, MeHg and DMHg}

The low concentrations of Hg\textsuperscript{II}, MeHg and DMeHg, in air makes preconcentration of the analytes (typical ambient concentrations around 2-100 pg m\textsuperscript{-3} and instrumental absolute LOD about 2 pg) the main challenge in the quantification of these chemical Hg forms.\textsuperscript{84} Available approaches, such as liquid absorbers, cryogenic trapping and solid adsorbents suffer from incomplete recoveries and potentially high blank levels and/or species transformation. The use of external calibration is thus neither reliable nor practical. By administrating isotopically enriched Hg\textsuperscript{II}(g), MeHg(g),
DMeHg\(_{(g)}\) and Hg\(^0\)(g) as internal standards to the gaseous sample, followed by ethylation of ionic Hg species (Hg\(^{II}\)(g) and MeHg\(_{(g)}\)) and preconcentration on a solid sorbent (Carbotrap or Tenax), Larsson et al., could demonstrate reliable quantification of Hg\(^0\), MeHg, DMeHg and Hg\(^{II}\).\(^{59,60,84}\) The gaseous standards are generated from temperature controlled cells with permeation tubes and a constant transport gas flow. The permeation tubes contain the isotopically enriched standard as a solution or salt, and for Hg\(^0\) as a liquid. The permeation rates will depend on temperature, the concentration of standard, and material and dimension of the permeation tubes. Larsson et al. could quantify species transformation during the sampling and fairly extensive species transformations was observed.\(^{60}\) For transformation during sampling of ambient air as much as 24 \% of the DMeHg\(_{(g)}\) standard added was detected as MeHg\(_{(g)}\) and 40 \% as Hg\(^0\). During collection of only the internal standard in inert atmosphere (N\(_2\)), less than 5 \% of the DMeHg\(_{(g)}\) standard was transformed to other chemical forms of Hg. This emphasizes the large variances that can be expected in Hg transformation reactions depending on the composition of the gaseous sample.

Larsson recognized traceability as a challenge for the proposed methodology, since no closely defined standard methods or certified reference materials exist to assure traceability of the analytical results gained.\(^{84}\) Further, the amount of internal standard added in IDA should be matched to the expected ambient analyte concentration in the sample to minimize the uncertainty of the quantification.\(^{88-89}\) Adjusting the isotope standard additions close to the sampling occasion might however be challenging for gaseous samples due to the time (days-weeks) needed to obtain stable permeation rates of the gaseous standards (caused by e.g. disturbances in adsorption-desorption equilibria of gaseous tracers on tube walls). These challenges are however minor in comparison to the challenges exhibited by methods not based on IDA. The method is thus a great improvement for the determination of chemical forms of gaseous Hg and have been successfully applied in microcosm experiments (Paper I, Björn et al.\(^{50}\), Larsson et al.\(^{60}\)) and for Hg speciation analysis in natural gases (Larsson et al.\(^{90}\)). Further, Baya and Hintelmann\(^{91}\) were, for the first time, able to quantify MeHg and DMeHg in the Arctic lower troposphere using this methodology (average detected concentrations of 5.5 ± 2.0 and 2.3 ± 3.6
pg/m³ of MeHg and 2.8 ± 3.6 and 4.1 ± 2.3 pg/m³ for DMeHg in Hudson bay and the high Arctic, respectively. They could thus support the possibility that MeHg bioaccumulating in the Arctic system may partly originate from MeHg and DMeHg volatilized from oceans.

1.1.2 Methylation and demethylation rates and rate constants

Methylmercury accumulated in fish originates from Hg²⁺ methylated by sulfate and/or iron reducing bacteria under anoxic conditions in soils, waters and sediments.⁹-¹⁵ The concentration of MeHg in fish is thus under control of the activity of Hg²⁺ methylating bacteria, the concentration and availability of Hg²⁺ for these bacteria (i.e. speciation of dissolved, adsorbed and solid Hg²⁺), as well as of the rate of MeHg demethylation.⁴² Because all these factors vary among ecosystems, so will the expected net methylation rates of Hg²⁺. The rates for Hg²⁺ methylation and MeHg demethylation in natural environments are traditionally described by pseudo first-order kinetic models (Equation 1) where the rates of methylation and demethylation, \( r_m \) and \( r_d \), are calculated as the products of concentrations of Hg²⁺ and MeHg, and the methylation and demethylation rate constants, \( k_m \) and \( k_d (d^{-1}) \), respectively.⁴⁵

Typically the rate constants, \( k_m \) and \( k_d \), are experimentally determined using isotopically enriched aqueous \(^{18}\)Hg²⁺ and Me\(^{18}\)Hg tracers. Figure 3 illustrates the expected MeHg/Hg²⁺ ratio for \(^{18}\)Hg²⁺ and Me\(^{18}\)Hg as a function of time, following first-order kinetics (Equation 1). The rate constants are typically determined within the timeframe when \( r_m \gg r_d \) for \(^{18}\)Hg²⁺ and \( r_m \ll r_d \) for Me\(^{18}\)Hg (typically 1-48 h). By assuming negligible demethylation and methylation of formed Me\(^{18}\)Hg and \(^{18}\)Hg²⁺, respectively, during this time period, \( k_m \) and \( k_d \) can be calculated by Equation 2 and Equation 3, respectively. For the calculation of \( k_m \), the pool of Hg²⁺ is assumed to be several orders of magnitude larger than the pool of MeHg formed and can be approximated as constant during the time of incubation.
Figure 3. Expected MeHg/Hg$^{\text{II}}$ ratio as a function of time as a consequence of first-order methylation and demethylation of the $^{A}$Hg$^{\text{II}}$ and Me$^{B}$Hg tracers, enriched in isotope A and B, respectively. A situation is described where steady state is reached with $r_m = r_d$.

Equation 1

\[ [\text{Hg}^{\text{II}}] \leftarrow \frac{r_m}{r_d} = \frac{k_m \times [\text{Hg}^{\text{II}}]}{k_d \times [\text{MeHg}]} \rightarrow [\text{MeHg}] \]

Equation 2

\[ k_m = \frac{[\text{MeHg}]_t}{[\text{Hg}^{\text{II}}]_{t=0} \cdot t} \]

Equation 3

\[ k_d = -1 \times (\ln([\text{MeHg}]_{t=0} - [\text{MeHg}]_t) - \ln([\text{MeHg}]_{t=0})) \]
In paper II, $k_m$ and $k_d$, were instead calculated by fitting a nonlinear reversible reaction model, Equation 5 (integrated from Equation 4), to determined MeHg concentrations (formed from a Hg$^{II}$ tracer) as a function of time (Figure 1 in Paper II). This approach does not require the assumptions made in Equation 2 and Equation 3 and allows estimating rate constants also for changes in ambient Hg. Experimental conditions may induce a net methylation of ambient Hg as a consequence of changes in the environmental conditions (increased temperature and mixing of sediment slurries). In paper II, we were able to compare $k_m$ determined for ambient Hg and $k_m$ determined for traditional aqueous $^\text{A}Hg^{II}$ tracers and our newly synthesized solid/adsorbed tracers. Due to the typically small net methylation of ambient Hg, the $k_m$ values were associated with large uncertainties and the approach is thus primary useful for method development and evaluation purposes. In our experiment, the $k_m$ values for ambient Hg were 20-30% lower when calculated from Equation 2, as compared when calculated from Equation 5. This suggests that by neglecting demethylation a reasonably small (considering the experimental complexity and typical variability associated with determination of $k_m$), however statistically significant, error is induced. The magnitude of this error is expected to vary considerably between samples depending on the magnitude of the methylation and demethylation rates. The nonlinear reversible reaction

**Equation 4**

$$
\frac{d}{dt} \left[ \frac{[Hg^{II}](t)}{[MeHg](t)} \right] = \begin{bmatrix} -k_m & k_d \\ k_m & -k_d \end{bmatrix} \begin{bmatrix} [Hg^{II}](t) \\ [MeHg](t) \end{bmatrix}
$$

**Equation 5**

$$
[MeHg](t) = \left( \frac{k_d}{k_m + k_d} e^{-(k_m+k_d)t} + \frac{k_m}{k_m + k_d} \right) [MeHg]_{t=0}
$$

$$
+ \left( \frac{k_m}{k_m + k_d} \left( 1 - e^{-(k_m+k_d)t} \right) \right) [Hg^{II}]_{t=0}
$$
model requires a larger minimum number of data points compared to the traditional approach, based on Equation 2 and Equation 3, and the latter approach may thus be more practical in large scale surveys and monitoring studies.

The $^{\text{A}}\text{Hg}^{\text{II}}$ and $\text{Me}^{\text{B}}\text{Hg}$ tracers are typically added as labile aqueous species at concentrations corresponding to 10-100 % of ambient MeHg and Hg. The current consensus concerning the availability of $\text{Hg}^{\text{II}}$ for uptake in bacteria responsible for methylation is that i) dissolved complexes of $\text{Hg}^{\text{II}}$ constitute the available pool (neutral Hg-S and specific Hg-thiol complexes have been suggested as particular available) and ii) precipitation and adsorption of $\text{Hg}^{\text{II}}$ to the solid and adsorbed phases limit the pool available for methylation. It is generally assumed that added aqueous tracers have a higher availability than ambient $\text{Hg}^{\text{II}}$. This have also been experimentally supported (Paper II). The terms potential, specific or conditional rate/rate constant have thus been used to acknowledge the presumably overestimated $k_m$. For the determination of biogeochemically more relevant values on $k_m$ and $k_d$, we have proposed solid and adsorbed phase isotopically enriched Hg tracers to be used (Paper II, see 2.2.3)

As discussed below (see 3.2); there are reasons to consider, in contrary to the general view, the existence of a sediment MeHg pool that is not readily available for demethylation. Assuming the presence of $\text{Hg}^{\text{II}}$ and MeHg pools that are not readily available for methylation and demethylation reactions, Equation 1 should include one second term for $\text{Hg}^{\text{II}}$ and one for MeHg (Equation 6) in addition to the once describing pools readily available for methylation, $\text{Hg}^{\text{II}}_A$ and $\text{MeHg}_A$.

Equation 6

\[
\begin{align*}
[Hg^{\text{II}}] & \quad \downarrow \quad k_m \times [Hg^{\text{II}}] & \quad [MeHg] \quad \downarrow \quad k_d \times [MeHg] \\
[Hg^{\text{II}}_A] & \quad \downarrow \quad r_m = \quad & \quad [MeHg_A] \\
\end{align*}
\]
1.1.3 Determination of methylation rate constants using solid or adsorbed chemical forms of Hg\textsuperscript{II} (paper II)

In paper II, we synthesized the following solid and adsorbed Hg\textsuperscript{II} isotope tracers; \( \alpha^{-199}\text{HgS(s)} \), \( \beta^{-201}\text{HgS(s)} \), Hg\textsuperscript{II} reacted with Mackinawite (=FeS-\( ^{200}\text{Hg}^\text{II} \) or \( =\text{FeS}^{-202}\text{Hg}^\text{II} \)), and Hg\textsuperscript{II} bound to natural organic matter (NOM-\( ^{196}\text{Hg}^\text{II} \)). These tracers were incubated in brackish water estuarine sediment slurries together with the traditionally used aqueous Hg\textsuperscript{II} nitrate tracer, \( ^{198}\text{Hg(NO}_3\text{)}_2(aq) \). Determined average \( k_m \) values are shown in Equation 7 and followed the order; \( \beta^{-201}\text{HgS(s)} < \alpha^{-199}\text{HgS(s)} < =\text{FeS}^{-202}\text{Hg}^\text{II} < \text{NOM}^{-196}\text{Hg}^\text{II} < ^{198}\text{Hg(NO}_3\text{)}_2(aq) \). The constants were calculated using the nonlinear curve fitting of Equation 5 and thus a \( k_m \) value could also be assigned for ambient Hg \((0.011 \pm 0.005 \text{ d}^{-1})\), calculated from a small increase in the MeHg/Hg\textsuperscript{II} ratio (an experimental artifact due to disturbance of sediment layering and increased temperature). As a comparison, a \( k_m \) value of \( 0.010 \pm 0.0011 \text{ d}^{-1} \) for ambient Hg was calculated from rate constants determined from solid/adsorbed tracers and with the distribution of ambient Hg\textsuperscript{II} between solid (HgS) and organically adsorbed phases (Hg-NOM) as determined using Hg L\textsubscript{III}-edge EXAFS. The good correspondence between the measured and calculated values of \( k_m \) for ambient Hg is a strong support for the new methodology used. It was concluded that specific solid and adsorbed Hg\textsuperscript{II} tracers are much better representatives of ambient Hg than aqueous phase tracers like \( ^{198}\text{Hg(NO}_3\text{)}_2(aq) \), which yielded a \( k_m \) 12 times higher than the value estimated for ambient Hg. It was furthermore shown that net methylation of solid/adsorbed tracers, in addition to thermodynamic properties of solid/adsorbed Hg\textsuperscript{II} phases, was controlled by the kinetics of dissolution/desorption (Figure 4 in paper II).

\textbf{Equation 7}

\[
\begin{align*}
\begin{bmatrix}
\alpha^{-199}\text{HgS(s)} \\
\beta^{-201}\text{HgS(s)} \\
\equiv \text{FeS}^{-200}\text{Hg}^\text{II} \\
\text{NOM}^{-196}\text{Hg}^\text{II} \\
198\text{Hg(NO}_3\text{)}_2(aq)
\end{bmatrix} & \quad k_m = 0.0066 \pm 0.0013 \text{ d}^{-1} \\
\equiv \text{FeS}^{-202}\text{Hg}^\text{II} & \quad k_m = 0.0012 \pm 0.0013 \text{d}^{-1} \\
\text{NOM}^{-196}\text{Hg}^\text{II} & \quad k_m = 0.014 \pm 0.0033 \text{ d}^{-1} \\
198\text{Hg(NO}_3\text{)}_2(aq) & \quad k_m = 0.029 \pm 0.0030 \text{d}^{-1}
\end{align*}
\]

\[
\begin{bmatrix}
\text{MeHg} - X_{UA} \\
\text{MeHg} - X_{A}
\end{bmatrix} \quad \downarrow
\]

\[
\begin{bmatrix}
\text{MeHg} - X_{UA} \\
\text{MeHg} - X_{A}
\end{bmatrix} \quad \downarrow
\]

\[
\begin{bmatrix}
\text{MeHg} - X_{UA} \\
\text{MeHg} - X_{A}
\end{bmatrix} \quad \downarrow
\]

\[
\begin{bmatrix}
\text{MeHg} - X_{UA} \\
\text{MeHg} - X_{A}
\end{bmatrix} \quad \downarrow
\]

\[
\begin{bmatrix}
\text{MeHg} - X_{UA} \\
\text{MeHg} - X_{A}
\end{bmatrix} \quad \downarrow
\]

\[
\begin{bmatrix}
\text{MeHg} - X_{UA} \\
\text{MeHg} - X_{A}
\end{bmatrix} \quad \downarrow
\]

\[
\begin{bmatrix}
\text{MeHg} - X_{UA} \\
\text{MeHg} - X_{A}
\end{bmatrix} \quad \downarrow
\]

\[
\begin{bmatrix}
\text{MeHg} - X_{UA} \\
\text{MeHg} - X_{A}
\end{bmatrix} \quad \downarrow
\]

\[
\begin{bmatrix}
\text{MeHg} - X_{UA} \\
\text{MeHg} - X_{A}
\end{bmatrix} \quad \downarrow
\]

\[
\begin{bmatrix}
\text{MeHg} - X_{UA} \\
\text{MeHg} - X_{A}
\end{bmatrix} \quad \downarrow
\]
Applying this solid/adsorbed-phase tracer methodology to other systems requires knowledge of the chemical solid/adsorbed-phase speciation of ambient Hg\textsuperscript{II} in the sample. Even though current approaches to obtain such information (Hg-EXAFS measurements, sequential extraction methods, or chemical equilibrium modeling) have constraints, they can be used to make qualified assumptions about the speciation of Hg. For the sediment used in papers II-IV, Hg L\textsubscript{III}-edge EXAFS measurements (and speciation modeling) suggested a solid-phase speciation of 70 \% as $\beta$-HgS(s) and 30 \% as Hg-(SR)\textsubscript{2} (paper II). In paper III and IV we demonstrate how the proposed approach also can be applied to differentiate the reactivity of different geochemical pools of Hg in systems of larger scale.

1.2 Scale of experiment – from micro- to mesocosms

A goal in science is to recognize and determine the causality in order to be able to explain, predict and understand ongoing or coming natural phenomena. This requires experiments at multiple scales and with variable and increasing complexity.\textsuperscript{95-97} Microcosm (micro = small; cosm = world) provides less complex and more controlled conditions, giving an opportunity to study individual processes than cannot be singled out in larger scale experiments. According to scaling theory, some patterns and processes will however only be evident over a certain threshold of scale and complexity.\textsuperscript{95} The number of experiments conducted at mesocosm (meso=intermediate) or field scale have thus increased during the last 30 years.\textsuperscript{97} The mesocosm experiments summarized by Petersen et al.\textsuperscript{97} had a median volume of 1.7 m\textsuperscript{3} and a median duration of 49 days. As concluded by the authors, this is a small and brief scale in relation to the physical and time scale of ecosystems. They further identified the presence of walls as a problem since the biological, material and energy exchange with the outside world is restricted and the walls also provide surfaces for growth of artificial edge habitats. The third and last main limitation of mesocosm experiment originates from experimental artifacts arising from the decisions made by the experimental designer (i.e. number of replicates, size, duration, control of additives etc). It should be noted that field and whole ecosystem experiments also suffer from scale dependence since all ecosystems vary in physical scale, manipulations are done by experiment designer and the time scale of experiments /monitoring is limited.
We studied methylation of solid and adsorbed phases of Hg\textsuperscript{II} in microcosm (up to 0.005 L of sediment slurries; paper II) as well as in mesocosms (2000 L of water and intact sediment cores; papers III-IV). Even though absolute rates of net methylation between tracers differed, the order and the relative differences of availability for methylation of the tracers was the same in micro- and mesocosms (Table 3, 3.1). Similar conclusions concerning the availability of solid/adsorbed Hg\textsuperscript{II} phases can thus be drawn from both scales. This illustrates that traditional microcosm incubation for determination of $k_m$ and $k_d$ are useful experimental tools that do reflect environmental processes occurring also in larger scale. This is not obvious since sediments are dynamic and complex systems with a drastic change in chemical properties with depth. Thus, mixing of sediment into slurries likely alters the chemical and biological properties of the material. Important differences between the microcosm sediment slurries and the mesocosm experiment were: i) the use of intact sediment cores in the mesocosm study (keeping the natural redox gradient and presumably bacterial community structure), ii) establishment of a pelagic–sediment coupling in the mesocosm systems and maintenance of a continuous supply of autochthonous organic carbon to the benthic zone, and iii) that atmospheric and terrestrial loadings of Hg\textsuperscript{II} and MeHg could be simulated in a realistic way in the mesocosm. The combination of molecular scale detail (i.e. solid phase speciation of Hg\textsuperscript{II} and MeHg) with the more complex meso-scale experimental system help to significantly improve our biogeochemical understanding of Hg in natural, complex systems.
3. Mercury methylation, evasion and bioaccumulation

This thesis contains a summary of three experimental studies, as illustrated in Figure 4, covering the processes of mercury methylation, demethylation and bioaccumulation.

**Figure 4.** Illustration summarizing the three experimental studies in this thesis
3.1 Methylation of Hg$^{II}$

Mercury methylation in sediments is mediated by specific strains of sulfate and iron reducing bacteria.\textsuperscript{12-15} It is generally accepted that methylation of Hg occurs inside the cell,\textsuperscript{98-99} even if the transportation mechanisms of Hg$^{II}$ through the cell membrane is not clear. Different dissolved complexes of Hg$^{II}$ have been suggested to be available for uptake by methylation bacteria including neutral Hg-sulfide complexes and Hg complexes with specific low molecular mass thiols.\textsuperscript{44,48,100-101} Nano-particulate (colloidal) HgS(s) has recently been proposed to be directly available for bacterial uptake, as judged from studies using pure bacterial cultures.\textsuperscript{46,102} Proposed mechanisms for uptake of such particles are however lacking. In accordance with bacterial culture studies, we detected methylation in sediment slurries of Hg$^{II}$ likely originating from nanoparticles (<0.02 µm) of α-HgS(s) (paper II). Our study showed that methylation of solid/adsorbed Hg$^{II}$ phases is controlled by thermodynamics and as well as kinetics of Hg$^{II}$ dissolution/desorption. The higher availability for methylation of nano-particulate HgS(s) than crystalline micro-particulate HgS(s) observed in previous bacterial culture experiments\textsuperscript{46,102}, might thus also be explained by higher desorption rates from smaller particles instead of a direct bacterial uptake of HgS(s) nanoparticles. Until further evidence for direct bacterial uptake of HgS(s) nano-particles can be demonstrated, it is reasonable to retain the consensus stating uptake of only dissolved Hg$^{II}$ species.

The phase distribution of Hg$^{II}$ in sediments is largely shifted to the solid and adsorbed phases (typical solid-liquid partition coefficient, K_d, of Hg in sediments is 10$^{3.5-6}$ (L kg$^{-1}$)).\textsuperscript{43, 68} The main chemical forms of Hg$^{II}$ in these phases include Hg$^{II}$ adsorbed to thiol groups in natural organic matter,\textsuperscript{65,103} or at the surfaces of FeS(s) minerals\textsuperscript{104} and incorporated in the crystalline Hg phases cinnbar (α-HgS) and metacinnabar (β-HgS).\textsuperscript{105} Remarkably few studies have discussed, or studied in detail, the importance of solid phase speciation, even though it has been generally accepted that precipitation and adsorption of Hg$^{II}$ are important processes limiting Hg methylation.\textsuperscript{42-45} Dissolution of HgS(s) and desorption of Hg$^{II}$ from surfaces are to a large extent controlled by the composition of the pore water chemistry.\textsuperscript{103,106-107} Formation rates of MeHg, derived from complex natural system at realistic conditions, are thus required to evaluate different solid and adsorbed Hg$^{II}$
species contribution to MeHg formation. In papers II-IV we provide such quantitative data, which previously have not been available (Table 3; net methylation represented as the MeHg/\text{Hg}^{II} molar ratio, further discussed in paper III). We conclude, that the solid/adsorbed phase speciation of \text{Hg}^{II} is a major controlling factor for rates of MeHg net formation. This was shown in traditional sediment slurries (paper II) as well as in mesocosm systems with intact sediments (i.e. maintained natural redox gradient, presumably maintained bacterial community structure) and a continuous supply of autochthonous organic carbon to the benthic zone from pelagic food webs with different structure and productivity.

The higher availability of \text{Hg}^{II}\text{-NOM} to methylating bacteria, as compared to \text{HgS(s)} phases (papers II-IV, Table 3) can be explained by the higher thermodynamic stability of \text{HgS(s)}. As shown by EXAFS measurements, \text{Hg}^{II} bind to NOM by two-coordinated complexation to thiol-groups, and when these are saturated, \text{Hg}^{II} binds to weaker O/N sites. The estimated concentration of thiol-groups (RHS) in the NOM material used in papers II-IV (~25 µmol RHS g\textsuperscript{-1} NOM)\textsuperscript{79} and the concentrations of \text{Hg}^{II} in the \text{Hg}^{II}\text{-NOM} tracer (~3 µmol g\textsuperscript{-1} NOM) suggest a supply of thiol-groups large enough for the formation of a \text{Hg}^{II}\text{-(SR)}\textsubscript{2} complex. In the mesocosm systems (papers III and IV), we observed higher net methylation rates in the sediment of \text{Hg}^{II}\text{ wt} (\text{Hg}^{II}\text{(aq)} added to the water of the mesocosm) than of the

<table>
<thead>
<tr>
<th>Paper II</th>
<th>Papers III-IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>^{198}\text{Hg}^{II}\text{(aq)}</td>
<td>0.96 (0.004)</td>
</tr>
<tr>
<td>^{198}\text{Hg}^{II}\text{-NOM}</td>
<td>0.050 (0.003)</td>
</tr>
<tr>
<td>\text{HgS(s)}</td>
<td>0.0054 (0.0007)</td>
</tr>
<tr>
<td>\text{HgS(s)}</td>
<td>0.0078 (0.0008)</td>
</tr>
<tr>
<td>\equiv\text{FeS}\text{Hg}^{II}</td>
<td>0.044 (0.004)</td>
</tr>
<tr>
<td>\equiv\text{FeS}\text{Hg}^{II}</td>
<td>0.044 (0.004)</td>
</tr>
<tr>
<td>\equiv\text{FeS}\text{Hg}^{II}</td>
<td>0.044 (0.004)</td>
</tr>
<tr>
<td>\equiv\text{FeS}\text{Hg}^{II}</td>
<td>0.044 (0.004)</td>
</tr>
<tr>
<td>\equiv\text{FeS}\text{Hg}^{II}</td>
<td>0.044 (0.004)</td>
</tr>
<tr>
<td>\equiv\text{FeS}\text{Hg}^{II}</td>
<td>0.044 (0.004)</td>
</tr>
<tr>
<td>\equiv\text{FeS}\text{Hg}^{II}</td>
<td>0.044 (0.004)</td>
</tr>
</tbody>
</table>

\textbf{Table 3.} Average net methylation, as \text{MeHg/\text{Hg}^{II}} ratio (± Standard Error,SE), of \text{Hg}^{II} determined in paper II (n=5) and paper III-IV (range for 3 mesocosm treatments, n=3-9)
$^{201}\text{Hg}^{\text{II}}$-$\text{NOM}_{\text{sed}}$ tracer (injected 5 mm below the sediment surface). As discussed in paper III, we suggest that $^{204}\text{Hg}^{\text{II}}_{\text{wt}}$ is deposited to the surface of the sediment as primary bound to NOM particles present in the water column and that differences in binding strength to the NOM is a contributing cause for the higher net methylation of $^{204}\text{Hg}^{\text{II}}_{\text{wt}}$ compared to $^{201}\text{Hg}^{\text{II}}$-$\text{NOM}_{\text{sed}}$. The variation in reactivity between different types of Hg$^{\text{II}}$-NOM complexes is however most likely smaller (because Hg will always bind to an excess of RSH groups with similar binding strength) than the difference in reactivity between Hg$^{\text{II}}$-NOM and $\beta$-HgS(s). For Hg$^{\text{II}}$ reacted with Mackinawite (FeS(s)), the availability for methylation of a newly prepared tracer was similar as of $^{196}\text{Hg}^{\text{II}}$-NOM (paper II). The availability of the \equiv\text{FeS}-$^{202}\text{Hg}^{\text{II}}$ tracer when it had been aged for 24 days before the incubation was however lower and similar to the availability of $\beta$-HgS(s), suggesting a gradual dissolution of the Mackinawite by Hg$^{\text{II}}$ and precipitation of $\beta$-HgS(s) (as previously shown in adsorption$^{104}$ and Hg EXAFS studies$^{79}$). Our results support the idea that precipitation of $\beta$-HgS(s) in sediment is an important “sink” for Hg$^{\text{II}}$ that limits the bioavailability of Hg$^{\text{II}}$ for methylating bacteria.$^{92-94}$ Pure $\beta$-HgS(s) is metastable at temperatures below 315°C but only slowly transforms to $\alpha$-HgS(s).$^{108}$ The inclusion of impurities also affects the stability of $\beta$-HgS(s) in natural systems. It is plausible that $\beta$-HgS(s) precipitated in sediment pore water, with a complex chemical composition, is less stable (shows a greater solubility) than commercially available $\beta$-HgS(s) and $\beta$-HgS(s) precipitated in more pure model systems (MQ water). Further, $\beta$-HgS(s) is (as typically for sulfur minerals) a non-stoichiometric solid and differences in the stoichiometry (i.e. Hg/S ratio) may be a second cause of differing availability between $\beta$-HgS(s) phases. Indeed, differentiated solubility of $\beta$-HgS(s) phases has been demonstrated in laboratory experiment.$^{109}$

In addition to the solid/adsorbed Hg$^{\text{II}}$ speciation, MeHg formation is controlled by additional environmental processes limiting or enhancing the bioavailability of Hg$^{\text{II}}$ or the activity of methylating bacteria. In field studies, variations in MeHg formation and concentrations among sites have been correlated to the concentrations of dissolved sulfide, NOM and to pH.$^{42}$ The effects of these chemical factors are complex since they may affect MeHg formation in multiple ways. For example, NOM immobilizes Hg$^{\text{II}}$ in sediments by adsorption$^{42,66,106}$ but may also increase the solubility of $\beta$-
HgS(s), inhibit β-HgS(s) precipitation, and enhance bacterial activity, resulting in a potentially increased methylation rate. Further, specific low molecular mass HgII-thiols have been suggested to be available for methylating bacteria. The possible HgII species accumulated and methylated by bacteria in natural environments are still under debate mainly due to the uncertainties in the chemical speciation of HgII in pore water. For sulfide, correlation to MeHg concentrations has been suggested to be an effect of decreased availability of HgII due to a higher concentration of charged dissolved HgII-sulfide complexes at higher concentrations of sulfide. Finally, the observed correlation between MeHg concentration in sediments and pH has been suggested to be caused by an increased association with solid phases of HgII at lower pH values, thus limiting the availability of HgII.

Climate change scenarios for the Baltic Sea region predict an increased terrestrial input of allochthonous NOM and nutrients to lakes and estuaries. This may fuel the pelagic bacterial pathway, reduce phytoplankton primary production and cause a shift from a phytoplankton based to a bacteria based pelagic food web with decreased sedimentation of autochthonous NOM as a consequence. We hypothesized that this could decrease the activity of methylating bacteria and thus also MeHg formation. In the reversed direction, we hypothesized that increased sedimentation of autochthonous NOM, caused by an increased biomass from increased nutrients load to lakes and estuaries as a consequence of eutrophication, could increase the production of MeHg. It is obvious from the number of factors influencing MeHg formation and as discussed later, its bioaccumulation, that properly conducted meso- and large scale experiments are needed if the effect of e.g. climate change or eutrophication is to be predicted. In paper IV, methylation of geochemical Hg pools in mesocosm model ecosystems with different pelagic food web structure and productivity are discussed. In the experiment, three different treatment schemes were applied: high and low addition of nutrients (referred to as NPhigh and NPlow, respectively) and addition of a humic soil extract (simulating an increased terrestrial load of humic material, referred to as HSE). For NPhigh mesocosms, fluctuations in the net formation of MeHg from \( ^{201}\text{Hg}^{II}\)-NOM_{sed} tracer correlated to that of primary production in the pelagic zone (measured as primary production rate or chlorophyll α), whereas only small fluctuation
in net Hg methylation and primary production was observed in HSE mesocosms. This shows that net methylation of Hg$^{II}$ may respond quickly to changes in pelagic productivity (days in these mesocosm systems). The fluctuation in Hg methylation observed for $\beta$-$^{200}$HgS$_{sed}$ was less pronounced than for $^{201}$Hg$^{II}$-NOM$_{sed}$. This suggests that formation of MeHg from $\beta$-$^{200}$HgS$_{sed}$ tracer was limited by a slow rate of dissolution of Hg$^{II}$ from the solid phase i.e. the dissolution process was the rate limiting step. Interestingly, net methylation of $^{204}$Hg$_{wt}$ (simulating recent atmospheric and terrestrial Hg$^{II}$ loads) increased with time in HSE mesocosm and did not show the same pattern as the $^{201}$Hg$^{II}$-NOM$_{sed}$ tracer. The higher (however not statistically different) average net methylation of $^{204}$Hg$_{wt}$ tracer in HSE was opposite to the hypothesis proposed prior to the experiment, expecting a lower net methylation of also recent Hg$^{II}$ loads due to a lower primary production. A number of different possible explanations are discussed in paper IV. The results in paper IV again emphasize the importance of the chemical speciation of solid/adsorbed forms of Hg$^{II}$. It is clear that depending on the chemical speciation of solid/adsorbed Hg$^{II}$, ecosystems can be expected to differ with respect to the outcome and magnitude of the response to environmental changes.

### 3.2 Demethylation of MeHg

Processes of MeHg demethylation in surface waters include both biotic and photo induced abiotic mechanisms.$^{39,118-119}$ In sediments, demethylation has been ascribed mainly to biological processes.$^{39,120-121}$ Significant abiotic demethylation has also been suggested, but a mechanistic understanding is lacking.$^{122-123}$ In the mesocosm systems (paper III), we report steady-state MeHg/Hg$^{II}$ ratios in the sediment obtained from net demethylation of the Me$^{198}$Hg-NOM$_{sed}$ tracer, and net methylation of Hg$^{II}$ solid/adsorbed phase tracers as well as of ambient Hg (during the 52 days on experiment). Based on these quotients it was estimated that up to 30% of the Me$^{198}$Hg-NOM$_{sed}$ tracer was not readily available for demethylation. This observation is consistent with previous findings from Marvin-Dipasquale et al.$^{124}$ An initial quick demethylation of added tracers (also observed in papers III-IV and by Marvin-Dipasquale et al.), has been used in other studies to determine the typical MeHg turnover, $(k_m+k_d)^{-1}$ of 1-3 days.$^{125}$ These turnover rates are however misguiding. They are determined from the initial decrease in MeHg
when the rate of demethylation is highly exceeding the rate of methylation ($r_m << r_d$, Figure 3), as controlled by the high availability of MeHg tracer added as an aqueous labile complex, and do not take into account the pool of MeHg not readily available for demethylation. Instead, turnover rates determined from the Me$^{198}$Hg-NOM$_{sed}$ tracer (paper III, SI) were on the order of 90 days. It is still uncertain if the mechanism for stabilization of MeHg (making it less available for demethylation), simply is formation of complexes with thiol-groups in NOM, or if additional processes are involved. As illustrated in paper III, these processes are potentially very important for the amount of MeHg accumulated in biota and further research to clarify mechanisms for MeHg stabilization in sediments is warranted.

### 3.3 Evasion of Hg

In paper I, evasion of gaseous chemical forms of Hg, i.e. Hg$^0$, MeHg and DMHg, was determined from sediment-water microcosm systems containing sediments contaminated with Hg from chloro alkali industry and pulp from paper industry. Emission of MeHg($g$) (chemical speciation modeling suggested evasion of MeHg as a neutral CH$_3$HgSH$^0$ complex) was characterized by a transient trend (Figure 2 in paper 1) with highest emission rates reached after 12 days (110 ± 41 and 140 ± 51 fmol h$^{-1}$ for ambient MeHg and MeHg formed from added $^{201}$Hg$^{117}$(aq) tracer, respectively). This transient trend is similar to trends for MeHg concentrations in other sediment incubation experiments (e.g. Figure 1 in paper II). Also, the sediment concentration of ambient MeHg increased 3 times during the course of the experiment whereas the gaseous emission of ambient MeHg was significantly higher in the beginning of the experiment. Our data thus suggest that gaseous emission of MeHg in sediment is controlled by recently methylated MeHg rather than by the total pool of MeHg in sediment. Indeed, estimated sediment–water flux of MeHg from costal marine systems reveals variations among sites and also seasonal variations have been observed. At sites in the continental shelf of southern New England, the seasonal variation in MeHg flux was also correlated to seasonal changes in MeHg gross production, in line with our observation. As previously discussed adsorption of MeHg to e.g. NOM particles may possibly stabilize MeHg and prevent demethylation (see 3.2). Adsorption of MeHg to solid phases may also be the reason for the higher sediment concentration, but lower evasion,
of ambient MeHg compared to Me\textsuperscript{201}Hg from the microcosms observed in paper I. Treatments of the microcosms (dark, light and dark with addition of acetate) did not cause any significant difference in MeHg evasion. For the evasion of Hg\textsuperscript{0}, the treatments suggested a predominance of photo induced oxidation of Hg\textsuperscript{0} rather than reduction of Hg\textsuperscript{II} in this system (light radiation from a tungsten lamp giving radiation in the visual spectral range with an overall lower intensity than solar radiation).

The transport of Hg from sediment to water column occurs via diffusion, advection and/or resuspension of particles.\textsuperscript{131} The importance of \textit{in situ} gross MeHg production, aqueous MeHg speciation and total concentration of MeHg in the sediment for the sediment–water mass transfer of MeHg can thus be expected to vary among ecosystem depending on the dominant transportation mechanism.

### 3.4 Bioaccumulation of mercury

Both Hg\textsuperscript{II} and MeHg are accumulated in micro-sized seston but little is known about the underlying mechanisms. Current literature suggests passive diffusion of lipophilic mercury species such as HgCl\textsubscript{2}, CH\textsubscript{3}HgCl and low molecular mass thiol complexes of MeHg into plankton cells.\textsuperscript{132-135} The similar abilities of HgCl\textsubscript{2} and CH\textsubscript{3}HgCl to pass lipophilic membranes suggest that these chemical forms should have similar accumulation efficiencies.\textsuperscript{134} MeHg and Hg\textsuperscript{II} are however distributed differently within the cells and this result in a higher transfer efficiency of MeHg.\textsuperscript{134,136} The fraction of total Hg occurring as MeHg thus typically increases from 0.3-1.5\% in phytoplankton to 2-22\% in zooplankton (data compiled by Mason et al.\textsuperscript{38}) and >90 \% in piscivorous fish.\textsuperscript{39,137}

In paper III and IV, we quantified Hg\textsuperscript{II} and MeHg accumulated in seston and benthic invertebrates (Chironomids, Amphipods, Polychaetes and Bivalves), after 8 weeks of experiment, from ambient Hg, and from Hg\textsuperscript{II} and MeHg tracers added to the brackish water-sediment mesocosms. The accumulation is presented as the biota–sediment accumulation factor (BSAF), which is the
Table 4. Accumulation of Ambient Hg (Hg occurring as MeHg (%), MeHg-BSAF and Hg\textsuperscript{II}-BSAF) and stable isotope analysis (\(\delta^{13}C\) and \(\delta^{15}N\) (‰)) in seston and benthic invertebrates from mesocosm experiment systems (± 1 SE).

<table>
<thead>
<tr>
<th></th>
<th>MeHg (% of Hg)</th>
<th>BSAF MeHg</th>
<th>BSAF Hg\textsuperscript{II}</th>
<th>(\delta^{13}C) (‰)</th>
<th>(\delta^{15}N) (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seston</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-100 µm</td>
<td>\textbf{8.6 (4.0)} (n=9)</td>
<td>\textbf{4.8 (2.2)} (n=9)</td>
<td>\textbf{0.39 (0.06)} (n=9)</td>
<td>-23 (1.6) (n=8)</td>
<td>\textbf{8.1 (0.4)} (n=8)</td>
</tr>
<tr>
<td>100-300 µm</td>
<td>\textbf{16 (5.3)} (n=8)</td>
<td>\textbf{6.7 (4.7)} (n=9)</td>
<td>\textbf{0.15 (0.04)} (n=8)</td>
<td>-25 (1.5) (n=7)</td>
<td>\textbf{8.8 (0.5)} (n=7)</td>
</tr>
<tr>
<td>300 µm</td>
<td>\textbf{17 (10)} (n=7)</td>
<td>\textbf{7.5 (4.3)} (n=9)</td>
<td>\textbf{0.19 (0.06)} (n=8)</td>
<td>-20 (1.5) (n=5)</td>
<td>\textbf{9.4 (1.1)} (n=5)</td>
</tr>
<tr>
<td>Amphipoda</td>
<td>\textbf{63 (11)} (n=3)</td>
<td>\textbf{22 (6)} (n=8)</td>
<td>\textbf{0.063 (0.050)} (n=4)</td>
<td>-19 (n=1)</td>
<td>\textbf{7.3 (n=1)}</td>
</tr>
<tr>
<td>Polycheate</td>
<td>\textbf{29 (9)} (n=9)</td>
<td>\textbf{36 (14)} (n=9)</td>
<td>\textbf{0.58 (0.08)} (n=9)</td>
<td>-22 (0.3) (n=9)</td>
<td>\textbf{9.4 (0.1)} (n=9)</td>
</tr>
<tr>
<td>Mussel</td>
<td>\textbf{8.6 (0.5)} (n=9)</td>
<td>\textbf{28 (2)} (n=9)</td>
<td>\textbf{2.2 (0.2)} (n=9)</td>
<td>-22 (0.2) (n=9)</td>
<td>\textbf{7.1 (0.1)} (n=9)</td>
</tr>
<tr>
<td>Chironomidae</td>
<td>\textbf{5.6 (1.5)} (n=4)</td>
<td>\textbf{16 (2.2)} (n=7)</td>
<td>\textbf{2.2 (0.3)} (n=5)</td>
<td>-22 (0.3) (n=5)</td>
<td>\textbf{8.5 (0.1)} (n=5)</td>
</tr>
</tbody>
</table>

Concentration ratio of Hg\textsuperscript{II} or MeHg between biota and sediment (pmol g\(^{-1}\) d.w./pmol g\(^{-1}\) d.w.). Accumulation in seston is typically given as the biota accumulation factor (concentration ratio between biota and water), but because the concentration in the water was below the detection limits for most tracers, BSAF was used also for seston. The average percent of ambient Hg occurring as MeHg, MeHg-BSAF and Hg\textsuperscript{II}-BSAF for ambient Hg, and \(\delta^{13}C\) and \(\delta^{15}N\) (‰) (used to evaluated trophic and foraging dynamics) in seston and benthic invertebrates are summarized in Table 4. Seston (suspended material including living organism as well as inorganic and organic particles\(^{138}\)) was collected with plankton nets at size fractions of 50-100, 100-300 and >300 µm. The \(\delta^{15}N\) value in seston was comparable to the values in benthic invertebrates. This is similar to findings by Eagles Smith et al\(^{139}\): chironomids, amphipods and seston with a size over 80 µm (assigned by the authors as zooplankton) had similar \(\delta^{15}N\) values whereas the ratio in phytoplankton was lower. This suggests that primarily zooplankton species made up the seston fractions collected (> 50 µm) from the mesocosm. The fraction of total Hg occurring as MeHg in seston collected (Table 4) was also within the range typical for zooplankton (2-22 %).\(^{38}\)

Traditional paradigms envision production of phytoplankton to constitute the base of aquatic food webs\(^{140}\) and the concentrations of MeHg in fish to be a
consequence of dietary exposure of Hg throughout the food web.\textsuperscript{16,141} Bioaccumulation and magnification schemes and models are therefore often based on MeHg entering the food web by accumulation in phytoplankton from surrounding water. MeHg and Hg\textsuperscript{II} accumulating in pelagic plankton will thus originate from either the sediment via advection, diffusion and resuspension of particles or from terrestrial and atmospheric sources.\textsuperscript{131} Even though fish concentration of MeHg typically increases with trophic level,\textsuperscript{17-18} large variations are found among ecosystems and species positioned at the same trophic level.\textsuperscript{18,139,142} Factors regulating the production of MeHg, the entry of MeHg into the food web as well as the trophic transfer of MeHg have been suggested to explain these variations.\textsuperscript{139,142} The relative importance of these factors is however poorly understood.\textsuperscript{131,142-143} Factors suggested includes e.g. concentration of dissolved organic carbon (DOC),\textsuperscript{110,135} pH,\textsuperscript{144} rates of primary production,\textsuperscript{145-146} atmospheric deposition of sulfate\textsuperscript{147} and of Hg\textsuperscript{II} \textsuperscript{27-28} as well as the growth rate\textsuperscript{148} and age\textsuperscript{149} of the biota and the pelagic food web structure.\textsuperscript{18} As discussed in paper IV, we observed higher accumulation of ambient MeHg, and MeHg originating from Me\textsuperscript{199}Hg\textsubscript{wt} and 204Hg\textsuperscript{II}\textsubscript{wt} tracers in seston in HSE compared to NP\textsubscript{low} and NP\textsubscript{high} treated mesocosm. This may to some extent be explained by a higher concentration of DOM circulating in the water column, complexing and keeping the concentrations of Hg and MeHg added as aqueous tracers at a higher level in the water phase of HSE mesocosms. Even though higher rates of sedimentation were observed in HSE mesocosm, the sedimentation of Hg and MeHg obviously was overridden by the effect of higher concentrations of DOC in the water column per se. As discussed in paper IV, this was likely not the only explanation for the higher MeHg concentrations in seston originating from Me\textsuperscript{199}Hg\textsubscript{wt} and 204Hg\textsuperscript{II}\textsubscript{wt} tracer. Additional plausible factors that may have contributed include algal bloom dilution\textsuperscript{150}, a higher degree of accumulation of MeHg-DOM complexes by bacteria and/or larger biomagnification due to an additional trophic level\textsuperscript{151} introduced in HSE mesocosm.

Several studies have stressed the importance of pelagic food web couplings to the benthic zone for trophic transfer of nutrients and energy\textsuperscript{152} as well as MeHg.\textsuperscript{137,139,142-143} Benthic organisms can accumulate MeHg from the dissolved MeHg fraction and via sediment ingestion.\textsuperscript{153} Aquatic organisms feeding on benthic invertebrates, so called benthivores, thus provide a
secondary pathway for the transfer of MeHg from sediment to the pelagic food web (in addition to MeHg entering the food web via accumulation in phytoplankton after transfer of MeHg from sediment to water). In the reversed direction, benthic organisms feeding on deposited planktonic material and necrophages may forage on fish carcasses with a high MeHg concentration. Together, the pelagic-to-benthic and the benthic-to-pelagic foraging results in a trophic feedback cycle of MeHg that not only preserves MeHg within the biota compartment, but may also result in higher MeHg levels in benthivores and piscivorous fish. Figure 5 illustrates the trophic transfer pathways in the aquatic food web.

Amphipods feed both on suspended particulate matter in the water column by filtration, and detritus and sediment by deposit feeding. Amphipods showed higher MeHg-BSAF and Hg\textsuperscript{II}-BSAF for Me\textsuperscript{199}Hg\textsubscript{wt} tracer, and lower for Me\textsuperscript{198}Hg-NOM\textsubscript{sed} added 0.5 cm below the sediment surface, in comparison to the other benthic invertebrates (Figure 6 and Figure 7).

**Figure 5.** Illustration of biaccumualtion and biomagnification of MeHg from sediment and water to the benthic and pelagic foodweb. Solid lines represent abiotc transportation processes and dashed lines bioaccumulation and magnification.
This suggests that the Amphipods to a larger extent feed on material originating from the water column in comparison to the other benthic invertebrates. *Chironomids* and *Bivalves* (mussels), well-known filter feeders, had similar concentrations of MeHg and Hg\(^{\text{II}}\) originated from the tracers and from ambient Hg. They also had a similar fraction of Hg occurring as MeHg (Table 4). *Polychaetes* (ragworms) had higher %C, %N, δ\(^{15}\text{N}\) (‰) as well as a generally higher MeHg-BSAF of sediment tracers. This suggests that Polychaetes, by their digestion of sediment, were exposed for higher concentrations of MeHg than chironomids and bivalves that primary filter feeds. Sediment digestion as a primary exposure route of Hg for Polychaetes have also previously been shown.\(^{153}\)

As shown in Figure 6 and Figure 7, Hg\(^{\text{II}}\) and MeHg from tracers added to the water (Me\(^{199}\)Hg\(_{\text{wt}}\) and Me\(^{204}\)Hg\(_{\text{wt}}\)) accumulated to a higher extent than tracers injected into the sediment (MeHg\(^{196}\)Hg-NOM\(_{\text{sed}}\), MeHg\(^{201}\)Hg\(^{\text{II}}\)-NOM\(_{\text{sed}}\), and β-Me\(^{200}\)HgS\(_{\text{sed}}\)) in both seston and benthic invertebrates. We therefore conclude newly imported Hg\(^{\text{II}}\) and MeHg from terrestrial and atmospheric sources to be more available for accumulation in seston and benthic invertebrates than Hg\(^{\text{II}}\) and MeHg previously accumulated in the sediment or MeHg formed *in situ* from previously accumulated Hg\(^{\text{II}}\). Furthermore, benthic invertebrates accumulated MeHg from the MeHg\(^{196}\)Hg-NOM\(_{\text{sed}}\) tracer and MeHg formed from MeHg\(^{201}\)Hg\(^{\text{II}}\)-NOM\(_{\text{sed}}\) and β-Me\(^{200}\)HgS\(_{\text{sed}}\) tracers to a much higher extent than seston. This suggests that benthic invertebrates may play an important role in transferring MeHg accumulated or *in situ* formed in the sediment to the pelagic food web. Our results in papers III and IV thus emphasize that the traditional bioaccumulation and biomagnification pathways of mercury (MeHg in water → phytoplankton → zooplankton → fish) are too simplified.
Figure 6. MeHg and Hg\textsuperscript{II} accumulation from tracers added 0.5 cm below the sediment surface in seston and benthic invertebrates (shown as MeHg- and Hg\textsuperscript{II}-BSAF ± 1 SD)
Figure 7 MeHg and Hg$^{II}$ accumulation from tracers added to the water column in seston and benthic invertebrates (shown as MeHg- and Hg$^{II}$-BSAF ± 1 SD)
4. Implication and final remarks

In papers I-IV we concluded that:

- the solid and adsorbed speciation of Hg\textsuperscript{II} is a principle factor controlling the rate and net methylation of Hg
- terrestrial and atmospheric sources of Hg\textsuperscript{II} and MeHg are more available for methylation, as well as for bioaccumulation, than sediment pools of Hg\textsuperscript{II} and MeHg
- a fraction of MeHg in sediments is not readily available for demethylation or evasion, but still available for bioaccumulation in benthic invertebrates

Extensive efforts have been undertaken to reduce anthropogenic emissions of Hg since the health effects of MeHg showed in e.g. pollution catastrophes (Minamata, Japan, 1950’s and Iraq, 1970’s).\textsuperscript{35,156} These actions have indeed resulted in decreased atmospheric depositions of Hg and downward trends of mercury concentration in fish in Sweden and the USA, for examples, have also been observed.\textsuperscript{4,32-34,157} While further efforts are planned, negotiated and under implementation there are still large uncertainties how further decreased anthropogenic Hg emissions will affect MeHg concentrations in fish.\textsuperscript{37} Two main concerns are that i) the response time for levels of MeHg in fish may be on the order of decades or up to centuries due to a continuous leakage and export of Hg stored in soils, and that ii) decreased anthropogenic depositions may be counteracted by other environmental changes such as climate changes and eutrophication.\textsuperscript{25,31,33}

In the METALLICUS (Mercury Experiment to Assess Atmospheric Loading in Canada and the United States) project, the response time of reduced atmospheric depositions has been simulated in mesocosm and whole ecosystem scale experiments.\textsuperscript{25,27-28} It was concluded that Hg\textsuperscript{II} deposited directly to the water surface was quickly methylated. The increase of the concentrations of Hg in fish was immediate, suggesting a very fast response time between changes in rates of atmospheric Hg deposition and Hg concentrations in fish. As pointed out in the METALLICUS studies, the fast response time is only true for systems receiving the major Hg input from the
atmosphere as a load directly onto the water body, and not indirectly via runoff from terrestrial ecosystems. For aquatic systems with a relative large watershed area, the response time may be delayed up to centuries due to the legacy of Hg stored and gradually leached and exported from soil. In the METALLICUS whole ecosystem study, isotopically enriched Hg were also added to the watershed (forest and wetland), however these loadings could not, during 3 years after first load addition, be detected in the biota.\textsuperscript{25} Table 5 summarizes literature data on the relative contribution from terrestrial and atmospheric sources of Hg (note: also including Hg\textsuperscript{0}) and MeHg to different aquatic systems. As shown, terrestrial imports of Hg is higher than atmospheric imports in most estuaries and lakes where the loadings of Hg has been modeled.

Transfer of MeHg from sediment to the water systems, determined using flux chambers or pore water MeHg concentration gradients, is typically interpreted as net methylation of Hg accumulated in sediments.\textsuperscript{39, 129-130} Thus, the possible mobilization of MeHg from sediment into the water phase is neglected. We show that Hg\textsuperscript{II} recently deposited to the sediment surface is more readily available for methylation processes than the already accumulated pool of Hg\textsuperscript{II} (paper III). It is also shown that newly formed MeHg was the main source of MeHg evasion from the sediment system (paper I). Based on our results, we suggest that recently (months-years) deposited Hg\textsuperscript{II} and MeHg contribute to a significantly higher degree to sediment-water fluxes than Hg\textsuperscript{II} and MeHg already stored in sediments for a longer time. Separating the contribution from recent and past Hg deposited deposition from field observations is however challenging. It emphasizes the need for quantitative data on the availability of specific geochemical pools of Hg for methylation and bioaccumulation. Such data were successfully derived from our mesocosm scale system, allowing modeling of MeHg formation and bioaccumulation in unprecedented detail. Studies in the METALLICUS project are loading experiments where the atmospheric deposition of Hg is simulated by isotopically enriched, aqueous Hg\textsuperscript{II} tracers. Thus, the study is restricted to a comparison of the reactivity of recent, labile Hg loadings with the reactivity of the entire Hg pool (i.e. ambient Hg) without differentiating between younger and older fractions (corresponding to recent and past loadings) of the sediment pool.
Table 5. Terrestrial/atmospheric ratios for import of total Hg and MeHg to the water body of oceans, estuaries and lakes. The total contribution from terrestrial and atmospheric sources to the total Hg and MeHg import to the water are given in parenthesis.

<table>
<thead>
<tr>
<th></th>
<th>Terrestrial/Atmospheric input ratios (100·(Terrestrial+Atmospheric)/(Total inputs))</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total Hg</td>
<td>MeHg</td>
</tr>
<tr>
<td>Ocean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global (pre. industrial)</td>
<td>158</td>
<td>0.030 (100 %)</td>
</tr>
<tr>
<td>Global (current)</td>
<td>158</td>
<td>0.065 (100 %)</td>
</tr>
<tr>
<td>Estuaries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global Estuarine</td>
<td>159</td>
<td>1 (98 %)</td>
</tr>
<tr>
<td>San Francisco Bay</td>
<td>160</td>
<td>1.5 (6.4 %)</td>
</tr>
<tr>
<td>Tokyo Bay</td>
<td>161</td>
<td>1.9 (19 %)</td>
</tr>
<tr>
<td>Long Island Sound</td>
<td>162</td>
<td>7.9 (100 %)</td>
</tr>
<tr>
<td>NY/NJ Harbor Estuary</td>
<td>163</td>
<td>31 (100 %)</td>
</tr>
<tr>
<td>Chesapeake bay</td>
<td>164</td>
<td>1.6 (79 %)</td>
</tr>
<tr>
<td>Lakes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior</td>
<td>165</td>
<td>0.6 (87 %)</td>
</tr>
<tr>
<td>Michigan</td>
<td>165</td>
<td>0.31 (44 %)</td>
</tr>
<tr>
<td>Onondaga</td>
<td>165</td>
<td>310 (91 %)</td>
</tr>
<tr>
<td>Little Rock Lake</td>
<td>165</td>
<td>only atm. (40 %)</td>
</tr>
<tr>
<td>Clear Lake</td>
<td>166</td>
<td>188 (100 %)</td>
</tr>
<tr>
<td>Lake Champlain</td>
<td>167</td>
<td>1.6 (100 %)</td>
</tr>
<tr>
<td>Big Dam West</td>
<td>168</td>
<td>10 (74 %)</td>
</tr>
<tr>
<td>Spring Lake</td>
<td>169</td>
<td>0.09 (93.9 %)</td>
</tr>
</tbody>
</table>

In papers III-IV, we constructed simple mass balance budgets to illustrate different scenarios in an estuarine environment. (Figure 8, Paper III (Figure 5 and Table 1), Paper IV (Figure 5)). The origin of MeHg in sediment and biota was calculated from the determined ratios of net methylation and demethylation, and the Biota Sediment Accumulation Factors (BSAF) derived from specific Hg$^{II}$ and MeHg tracers. These tracers simulated more recently imported Hg$^{II}$ and MeHg from atmospheric and terrestrial sources, as well as older, stored sediment pools of Hg$^{II}$ and MeHg. A challenge when applying these types of models is the selection of scales, including the time and the depth in the sediment at which methylation and demethylation reactions are considered most active. We used calculated cumulative terrestrial and atmospheric loads of Hg$^{II}$ and MeHg, typical for estuarine environments during a 2 months period, and an active sediment layer of 1.5 cm in our model to match the experimental scale from which our quantitative data was derived. Since sediments store the largest reservoirs of
Hg in lakes and estuaries, the mass of sediment considered as “active” can be expected to largely impact the results of Hg mass balance budgets. For estuarine mass balance budgets, “active” sediment layer of 1 and 15 cm has previously been assumed for Chesapeake and San Francisco Bay, respectively\textsuperscript{160,164} and the timescale is typically year\textsuperscript{-1} 160-164.

It is easy to understand that it has been difficult to establish a clear relationship between MeHg in fish and the concentrations of Hg or MeHg in sediment among ecosystems when comparing the mass budget of MeHg in sediment and biota (Figure 8) and by looking at the variations of terrestrial and atmospheric inputs among ecosystem (Table 5). Because of differences in the availability for methylation and bioaccumulation, specific geochemical Hg pools give very different predicted contributions to MeHg in sediment, seston and benthic organisms (Figure 8, Paper III (Figure 4-5)). Since such data have not previously been available, we need to reevaluate the importance of sediment pools versus newly imported Hg from atmospheric and terrestrial sources. Most urgently, there is an obvious risk

![Figure 8. Mass balance budget calculations (denoted model A, in paper III) for pools of MeHg in sediment and biota (seston, Amphipodas and benthic invertebrates) consisting of newly imported MeHg and Hg\textsuperscript{II} from terrestrial and atmospheric sources and older sediment pools of MeHg and Hg\textsuperscript{II} (having a composition of 70 % β-HgS and 30 % Hg-NOM).](image-url)
that contributions of MeHg in fish from terrestrial sources of MeHg and Hg\textsuperscript{II} are underestimated. From the different Hg loading and solid phase speciation scenarios postulated (Paper III) we conclude that at constant loading rates, it can be expected that the sediment pools’ contribution to MeHg in sediment and biota is governed by the solid-phase speciation of Hg\textsuperscript{II} and the formation of MeHg pools not readily available for demethylation. In paper IV, we calculated mass balance budgets for MeHg in sediment and seston under the scenarios simulated in NP\textsubscript{low}, NP\textsubscript{high} and HSE mesocosm. Although associated with large uncertainties, the model suggested that at predicted climate change scenarios for Scandinavia, the concentration of MeHg in plankton could double due to increased inflow of MeHg and Hg\textsuperscript{II} and an increased availability of Hg for MeHg formation and bioaccumulation. For the eutrophication scenario, only small changes in sediment and seston concentrations of MeHg was predicted.

Even though the availability of geochemical pools of Hg\textsuperscript{II} and MeHg for methylation, demethylation and bioaccumulation will differ among sites, our data can, until similar data have been obtained also from other ecosystems, be used to improve our understanding of Hg biogeochemistry and to predict the effect of environmental changes or changes in Hg loading rates. As future outlooks following this thesis work I suggest some key research questions to address important knowledge gaps in Hg biogeochemistry related to environmental changes, atmospheric and terrestrial loading rate changes as well as Hg and MeHg bioaccumulation in fish:

- *Determination of methylation rates of solid/adsorbed phase tracers and solid phase speciation in other types of ecosystems.* Such data could allow an evaluation to what extent differences in the solid phase speciation of Hg among ecosystems could explain differences in sediment as well as biota concentrations of MeHg.
- *Studying mechanisms and processes leading to accumulation of persistent MeHg pools in sediments.* Such knowledge is at the present scant and the importance of such pools in sediments is not well recognized.
- *Application of the mesocosm scale experiment on fresh water system.* We experimentally demonstrated how environmental
changes such as global warming and eutrophication may affect MeHg formation and bioaccumulation of Hg in an estuarine system. Such data is also needed for freshwater ecosystems where the responses might differ in direction and magnitude.

Addressing these three key questions will broaden our understanding of Hg biogeochemistry and help understand ways to mitigate the anthropogenic contribution of MeHg in the environment.
Acknowledgements


Tack Ida, du är underbar! De finns så många stunder som jag inte skulle klarat av utan dig! Tack för all stöttning och att du låtit mig vara en sån stor del av ditt liv.

Ett stort tack till min o Frejas familj som stöttat och hjälp med allt från kärlek o värme till hjälp med barnpassning o alla små ting: mamma, pappa, Eva, svärmor Susanne, svärfar Eskil, bror Jens med familj, Mathias syskon med respektive och övrig familj till Mathias, moster Kerstin.

I would like to thank the staff at the Department of Chemistry and Umeå Marine Sciences Centre for providing me extra support and help so I could come back to work again and finish my thesis. I felt a great support and a genuine care from you. A special thanks to my supervisors and co-authors (especially Erik B, Ulf, and Mats) for listening and for the extra support.

During my time in Umeå, I always felt I was surrounded by people who were true friends, and people that would support me if I ever needed it. When my life then was turned upside down, I got at so much more support than I ever could have imagined. Also from friends I just got to know or that I lost contact with. So I would like to thank all of you who in small and big ways showed your support. Even if I did not have time to have all the coffees, dinners and movie nights you suggested, the gesture meant a lot to me.
I would like to thank the Department of Chemistry and Umeå Marine Sciences Centre for giving me the chance to opportunity to do research.

Tack till min underbara huvudhandledare Erik! Tack för att du gav mig vägledning såväl utrymme att utvecklas och växa. Tack för allt roligt vi haft tillsammans under de här åren. Min tid som doktorand har varit spännande, rolig och helt fantastisk, och mycket av det beror på att du lagt ner din tid och själ i våra forskningsprojekt och i att vägleda mig i forskningen. Tack också för att du varit en av mina närmsta vänner och stöttat mig i både framgångar och motgångar. Tack för skratten, guidningen in i vinernas värld och inte minst alla fartfyllda och legendariska stunder på dansgolvet!

Ulf, tack för att du delar med dig så mycket av din passion för forskning. Det är verkligen inspirerande och jag är väldigt glad att jag fått jobba med dig. Tack också för att du, trots ett fullspäckat schema, tog dig tiden att läsa min avhandling och ge feedback. Erik L, tack för support och all praktiskt hjälp med galna mesocosm-idéer. Någon uppskjutning av en mesocosm till rymden blev det inte (och inte heller sediment-tårta), men de kändes som vi fixade saker som tekniskt var i samma klass. Tack till mina medförfattare: speciellt Mats för ditt engagemang och stöttning och Agneta, utan din kompetens hade mesocosm-projektet inte varit möjligt. Tack Tom, för att du guidade mig under första tiden som doktorand, det blev ett stort tomrum i gruppen sen du slutade och jag saknar din humor och personlighet. Thanks to my super-mesocosm-team including Minh and Helen!!! The mesocosm project and my time as a PhD student would not have been the same without the two of you!!! Tack Lasse för två oförglömliga sommar-projekt i Vietnam och Kambodja. Tack till övriga kollegor i forskningsgruppen: Lars, Sylvain, Andreas, Max, Rose-Marie, Solomon och Wolfgang. Tack Yvonne för tiden som kontorskompisar och för din vänskap.

Tack till alla er andra som bidragit på olika sätt till arbetet som ligger till grund för artiklarna: Tom Larsson, Anna Karlsson, Ida Tjerngren, Johan Nordbäck (Swedish Geotechnical Institute), Dan Boström, Per Hörstedt, Per-Olof Westlund, Andrey Shchukarev, Staffan Åkerblom och framför allt ett stort tack till Henrik Larsson och övrig teknisk personal på UMF.

Till sist, Tack till min underbara och älskade dotter Freja! Du och jag har en underbar liten familj ihop!
References


42. Benoit, J. M.; Gilmour, C. C.; Heyes, A.; Mason, R. P.; Miller, C. L., Geochemical and Biological Controls over Methylmercury Production and Degradation in Aquatic Ecosystems. 2003; p 262-297.


50. Bjorn, E.; Larsson, T.; Lambertsson, L.; Skyllberg, U.; Frech, W., Recent Advances in Mercury Speciation Analysis with Focus on Spectrometric

51. USEPA, Method 3200, Mercury Species Fractionation and Quantification by Microwave Assisted Extraction, Selective Solvent Extraction and/or Solid Phase Extraction. **2005**.


62. Lambertsson, L.; Bjorn, E., Validation of a Simplified Field-Adapted Procedure for Routine Determinations of Methyl Mercury at Trace Levels


64. USEPA, *Method 3052, Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices;* 1996.

65. Skyllberg, U., Competition among Thiols and Inorganic Sulfides and Polysulfides for Hg and MeHg in Wetland Soils and Sediments under Suboxic Conditions: Illumination of Controversies and Implications for MeHg Net Production. *J. Geophys. Res-Biogeo.* **2008**, *113*, G00C03.


75. USEPA Method 3051a; Microwave Assisted Acid Digestion of Sediments, Sludges, Soils and Oils. 2007.


102. Zhang, T.; Kim, B.; Leyard, C.; Reinsch, B. C.; Lowry, G. V.; Deshusses, M. A.; Hsu-Kim, H., Methylation of Mercury by Bacteria Exposed to


