

**ASSESSMENT OF MERCURY METHYLATION AND  
DEMETHYLATION WITH FOCUS ON CHEMICAL SPECIATION  
AND BIOLOGICAL PROCESSES**

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**ASSESSMENT OF MERCURY METHYLATION AND  
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## SUMMARY

Mercury occurs naturally in the environment and is released by human activities. Mercury exists in gaseous, liquid, and solid phases, and all phases are of importance when fate affects mercury in terrestrial, fresh and marine water, and atmospheric environments. Mercury can be transformed to a highly toxic form of methylmercury. Humans are exposed to the toxicity of methylmercury through consumption of fish. Methylmercury is bioaccumulated up the food chain by transfer of residues of methylmercury in smaller organisms that are food for larger organisms in the chain. This sequence of process results in higher concentrations in organisms at higher levels in the food chain with humans at the top of the food chain.

Understanding speciation of mercury and biological processes of methylmercury transformation plays an important part in toxicity and exposure of mercury to living organisms. Speciation also influences transport of mercury within and between environmental media while biological processes of methylmercury transformation influence methylmercury production and its transport to the biological communities. This study will provide a comprehensive evaluation of chemical speciation and biological processes that govern mercury distribution and transformation among three environmental media: atmosphere, water, and sediments. The study also covers demethylation process that can convert methylmercury to inorganic mercury species. Demethylation and methylation processes therefore may occur in parallel further complicating the assessment of mercury fate in the environment.

In the end, the study will provide integrated fundamental pathways of mercury species transformation through chemical and biological pathways and will contribute to an understanding of fate and transport of mercury species in environmental media. It will also provide a foundation for a state- and region-wide examination of mercury monitoring and control strategies.

# CHAPTER 1

## INTRODUCTION

Mercury is a naturally occurring element found in rocks, soil, water, and volcanic dust, and is ubiquitous in the environment. Mercury is among the group of elements known as transition metals. As with all metals, transition elements are both ductile and malleable, and conduct electricity and heat. Valence electrons, or electrons used to combine with other elements, are present in more than one shell. This is the reason why mercury exhibits three oxidation states: elemental (metallic,  $\text{Hg}^0$ ), monovalent (mercurous,  $\text{Hg}_2^{2+}$ ), and divalent (mercuric,  $\text{Hg}^{2+}$ ). Elemental mercury is the most common form of mercury found in nature (King, 1999) and the physical and chemical properties of elemental mercury are presented in Table 1.

**Table 1** - Physical and chemical properties of elemental mercury ( $\text{Hg}^0$ )<sup>a</sup>

Property	Value/description
Molecular weight	200.59 g/mol
Atomic number	80
Appearance	Silver-white, heavy, mobile, liquid metal
Odor	Odorless
Henry's law constant	0.11 M/atm (at 25°C) <sup>b</sup>
Density	13.55 g/mL (at 20°C)
Specific gravity	13.6
Boiling point	356.72°C (675°F)
Melting point	-38.87°C (-38°F)
Vapor pressure	0.246 Pa at 25°C <sup>c</sup>
Log $K_{ow}$ <sup>d</sup>	5.95

<sup>a</sup> Agency for Toxic Substances and Disease Registry (2005)

<sup>b</sup> Mason and Sheu (2002)

<sup>c</sup> Mason and Sheu (2002), Sanemasa (1975)

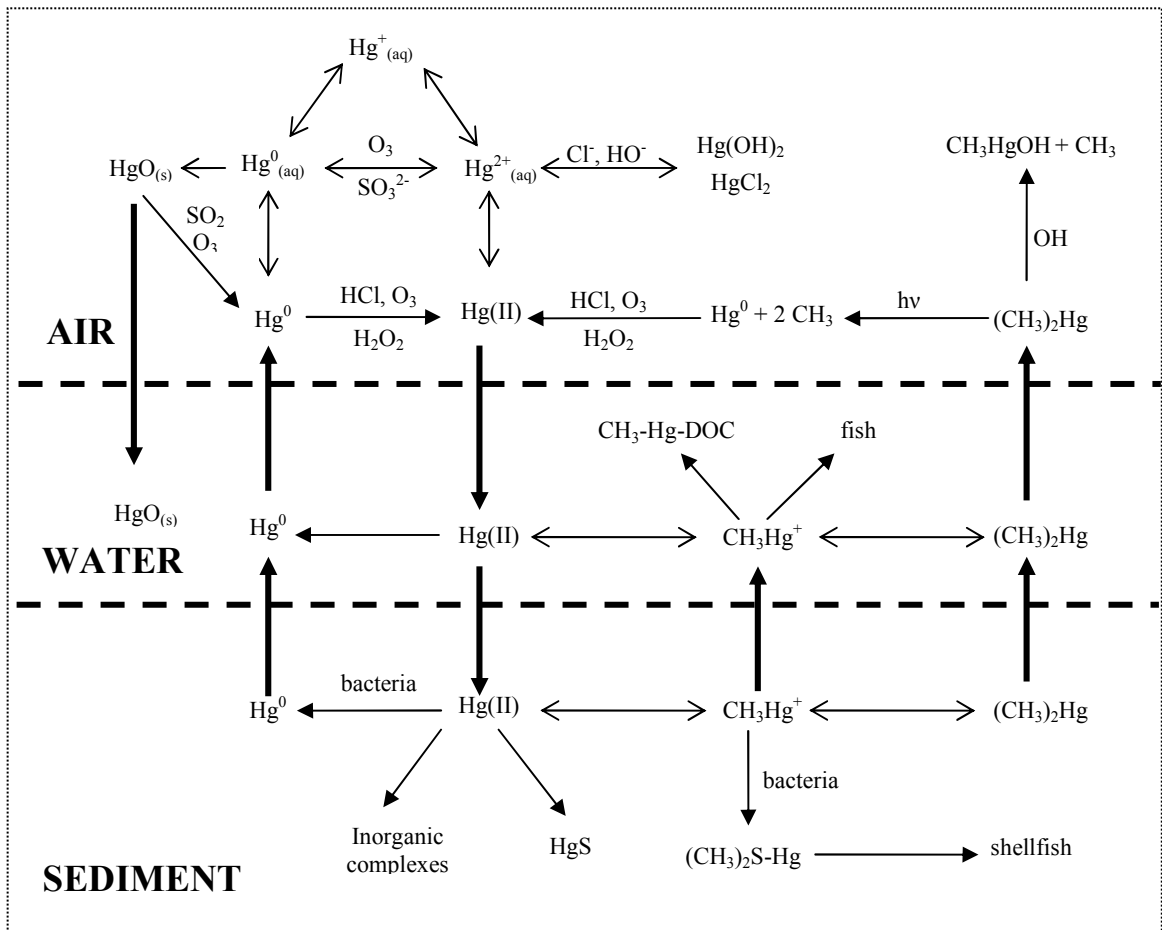
<sup>d</sup>  $K_{ow}$  = Octanol-water partition coefficient

The physical and chemical properties in Table 1 make mercury unique and applicable in many uses in industry. Elemental mercury is liquid at room temperature. As listed in Table 1, it has a silver-white, hence the term ‘quicksilver’, and mirror-like appearance (Figure 1). This silvery color makes it clearly visible in the capillary tube of thermometer. It is important to note that many hospitals are phasing out the use of mercury thermometer and a variety of mercury-free thermometer is widely available and recommended for general public. From the figure, one can also see that liquid mercury will form droplets when spilled. It will then emit vapors into the air. As mercury vapor is odorless, it adds on to its characteristics as a hazardous contaminant, toxicity of which will be discussed in the later chapter. Its low Henry’s Law constant brings about its low water solubility, which makes the removal of gaseous elemental mercury by rainwater less efficient compared to other atmospheric species of mercury, such as reactive and particulate mercury (Mason and Sheu, 2002). Table 1 also shows that elemental mercury is extremely dense and heavy compared to water (density of water = 0.998 g/mL at 20°C (White, 2008)), therefore it is used in pressure detection devices such as barometers. The usage of mercury in thermometer is because of its wide liquid state temperature with very high boiling point and extremely low melting point.



**Figure 1.** Silver-white, mirror-like appearance of liquid mercury (Picture courtesy of American Chemical Society)

In addition to elemental mercury, other forms of mercury (i.e., inorganic and organic mercury compounds) also occur in the environments. Not only does mercury occur in solid and dissolved states, it also occurs in liquid and gaseous phases (Figure 2). Consequently, its biogeochemical cycle involves three phases in nature, encompassing terrestrial, oceanic, and atmospheric processes (Sigel and Sigel, 1997).



**Figure 2.** Biogeochemical mercury cycle as a three-phase nature (adapted from Stein *et al.* (1996))

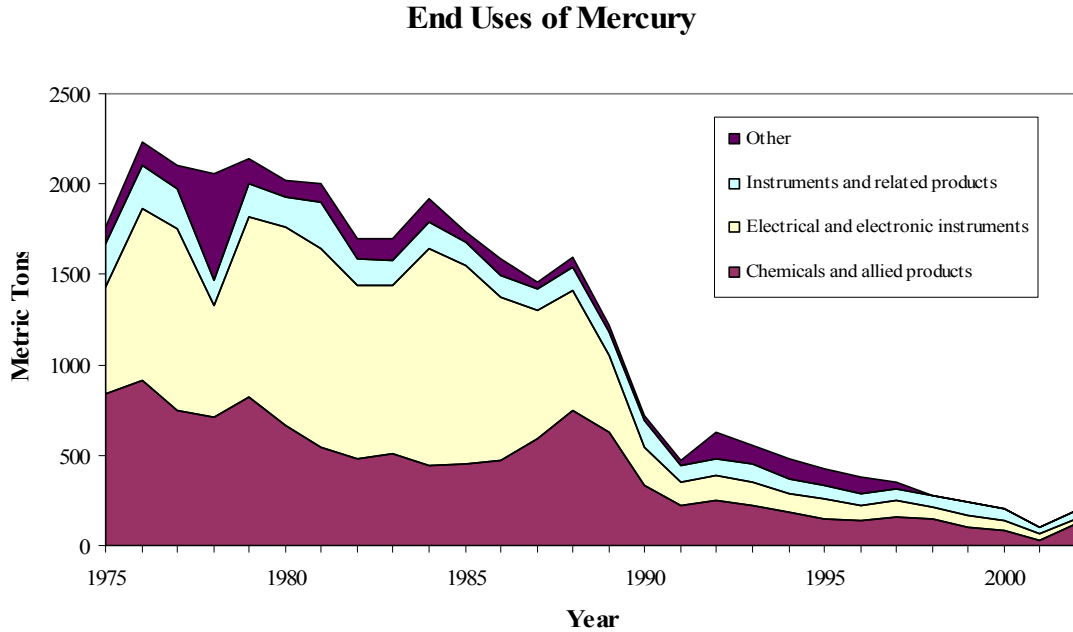
The physical and chemical properties of selected inorganic and organic mercury compounds that are involved in the important reactions or processes in Figure 2 are presented in Table 2.

**Table 2** - Physical and chemical properties of selected mercury compounds. Data are from ATSDR (2005)

Property	Mercuric Chloride (HgCl <sub>2</sub> )	Mercuric Sulfide (HgS)	Dimethylmercury ((CH <sub>3</sub> ) <sub>2</sub> Hg)
Molecular weight	271.52 g/mol	232.68 g/mol	230.66 g/mol
Melting point	277°C	Transition temp (red to black) 386°C	No data
Boiling point	302°C	No data	92°C
Density	5.4 g/mL at 25°C	7.55-7.70 g/mL (black mercuric sulfide), 8.06 - 8.12 g/mL (red mercuric sulfide)	3.1874 g/mL at 20°C

Mercury is mined as cinnabar ore, which contains mercuric sulfide (HgS). The metallic form of elemental mercury is refined from mercuric sulfide ore by heating the ore to temperature above 593°C. This vaporizes mercury in the ore (mercury vaporizes at 356.72°C - see Table 1), and vapors are then captured and cooled to form liquid metallic mercury. Liquid metallic mercury can be used in production of chlorine gas and caustic soda (NaOH or lye) and for extracting gold from ore, or other articles containing gold. It is also used in thermometers, barometers, batteries, and electrical switches but these uses are being restricted or minimized because of mercury toxicity. As shown in Figure 3, the use of mercury in industrial sectors or products shows a decline in the 90's and thereafter, especially in electrical and electronic instruments category which include the use of

mercury in electrical lighting, wiring devices and switches, batteries, and electrical apparatus.



**Figure 3.** The use of mercury in industrial sectors or products. Graphic is produced from the data in Brooks (2006).

Data in Figure 3 reflect changes in mercury consumption with the following establishments of regulations, following the 1971 milestone when mercury was designated as a hazardous pollutant:

- 1972 - Federal Water Pollution Control Act gave EPA authority to enforce and regulate the discharge of mercury into waterways. In September 1973, mercury was designated as a toxic pollutant and in October, the dumping of mercury or mercury compounds in the ocean became prohibited.

- 1974 - Safe Drinking Water Act authorized EPA to set standards for hazardous substances in drinking water and mercury ( $\text{Hg}^{2+}$ ) was set at 0.002 mg/L.
- 1978 - Resource Conservation and Recovery Act (RCRA) established regulations for disposal of mercury-bearing waste.
- 1980 - Comprehensive, Environmental Response, Compensation, and Liability Act established Superfund to clean toxic waste sites.
- 1992 - EPA banned land disposal of high mercury content wastes generated from chlor-alkali facilities.
- 1993 - EPA canceled registrations of last two mercury-containing fungicides, Calo-chlor and Calo-gran at manufacturer's request.
- 1994 - Congress suspended mercury sales from National Defense Stockpile due to EPA questions associated with environmental problems.
- 1996 - The Mercury-Containing and Rechargeable Battery Management Act prohibited sales of regulated batteries without recyclability or disposal labels and phased out most batteries containing intentionally added mercury.

### **1.1. Toxicity of Mercury**

The toxicity of mercury strongly depends on its speciation. Methylmercury ( $\text{CH}_3\text{Hg}^+$ ) and dimethylmercury ( $(\text{CH}_3)_2\text{Hg}$ ) pose the highest threats of toxicity because these organic species can be adsorbed into living tissues where they accumulate with no

way to be expelled. At room temperature, metallic mercury will evaporate and form mercury vapors (ATSDR, 1999; 2005). The central nervous system is very sensitive to mercury although the numerous forms of mercury have different effects because they do not all move through the body in the same way. The effects of mercury on the nervous system are primarily the consequence of reaction of mercury with sulfur atoms of brain proteins, enzymes, and other macromolecules, which results in perturbation of their normal or usual function (Jitaru and Adams, 2004). Metallic mercury vapor affects many areas of the brain and their associated functions, resulting in a variety of symptoms such as personality changes (e.g., irritability, shyness, nervousness), tremors, changes in vision, deafness, muscle incoordination, loss of sensation, and difficulties with memory (ATSDR, 1999; 2005). In humans, mercury accumulates in the kidneys resulting in higher exposures to these tissues, and thus more damage should large amounts of mercury enter the body (Jitaru and Adams, 2004). According to ATSDR (2005), short-term exposure (hours) to high levels of metallic mercury vapor in air can damage the lining of the mouth and irritate lungs and airways, causing tightness of breath, a burning sensation in the lungs, and coughing. Although literatures on exposure levels resulting in the above symptoms are limited, workers accidentally exposed to mercury vapors at an estimated concentration of up to  $44.3 \text{ mg/m}^3$  for 4-8 hours exhibited chest pains, cough, difficult breathing, and impairment of pulmonary function (ATSDR, 2005).

Toxicity may also occur due to exposure to inorganic mercury compounds when mercury combines with elements such as chlorine, sulfur, and oxygen. These mercury salts, in addition to effects on the kidneys, can damage the stomach and intestines, producing symptoms of nausea, diarrhea, or severe ulcers if swallowed in large amounts

(ATSDR, 1999; 2005). Should high-dose acute intoxication of mercury salts occur, the main symptoms consist of abdominal pain, lack of appetite, difficulty in moving, headache, vomiting, metallic taste, excessive salivation and thirst, bloody diarrhea, prostration, kidney damage, and ultimately death can occur within a few days or weeks of high doses (Jitaru and Adams, 2004). The levels of intoxication of mercury salts resulting in the above symptoms are not reported in the literatures. Although comparison cannot be made to intoxication levels of organic mercury compounds, report confirmed the death of two women exposed to diethylmercury vapor with estimated exposure level of 1 - 1.1 mg/m<sup>3</sup> for 4 - 5 months (ATSDR, 2005)

Mercury poisoning is also linked to the madcap milliner in Lewis Carroll's classic children's book, Alice in Wonderland. The true origin of the saying related to a disease peculiar to the hat making industry in the 1800s. A mercury solution was commonly used during the process of turning fur into felt, which caused the hatters to breathe in the fumes of this highly toxic metal, a situation intensified by the poor ventilation in most of the workshops. This led in turn to an accumulation of mercury in the workers' bodies, resulting in symptoms such as trembling (known as "hatters' shakes"), loss of coordination, slurred speech, loosening of teeth, memory loss, depression, irritability and anxiety (Connealy, 2006)

### **1.1.1. Mercury exposure for general population**

According to ATSDR (2005) everyone is exposed to very low levels of mercury in air, water, and food. Between 10 and 20 ng/m<sup>3</sup> of air has been measured in urban outdoor air (ATSDR, 2005). The regulatory limit for acceptable ceiling concentration of elemental mercury in air set by Occupational Safety and Health Administration is 0.1

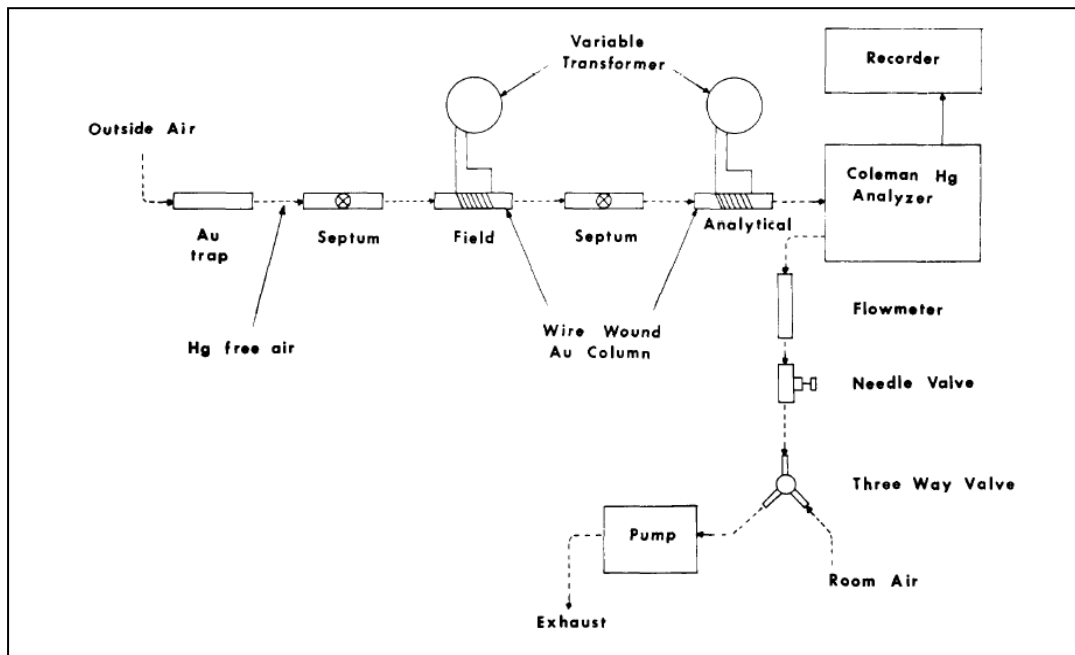
mg/m<sup>3</sup>, therefore the concentration measured for urban outdoor air is still safe to breathe. Mercury levels in surface water are generally less than 5 ng/L, which is approximately a thousand times lower than safe drinking water standards (2 µg/L is the maximum contaminant level set by USEPA). No concern over health risks of mercury in drinking water should be observed.

One potential source of mercury vapor for individuals is from dental amalgam (ATSDR, 1999; 2005; Clarkson and Magos, 2006). Dental amalgam fillings contain about 50% metallic mercury in an amalgam with silver or copper, and small amounts of other metals such as zinc. The total amount of mercury per amalgam can range from 327-928 mg depending on the size of the amalgam. The benefits of using mercury as dental amalgam fillings are ease of preparation, fits the oral cavity tightly as it expands slightly after introduction into the tooth, and has a long life, measured in tens of years (Clarkson and Magos, 2006). The total amount of mercury released from dental amalgam depends upon the total number of fillings and surface area of each filling, chewing and eating habits of person, and other chemical conditions in the mouth. The removal of previously-placed amalgam fillings is not recommended because it does not necessarily pose a health risk (ATSDR, 1999; 2005). Note however that dentists are now required to trap all dental amalgams and filling within their dental clinics and not allow discharge to sewerage system. In addition, since 1984, the ADA has recommended use of precapsulated amalgam alloy instead of liquid mercury, which provides a safe practice for mixing amalgam.

## 1.2. Analytical methods for mercury quantification

Quantifying mercury is a difficult task because mercury concentration is normally on the order of nano ( $10^{-9}$ ) or pico ( $10^{-12}$ ) g/L in solution. In addition, the volatile nature of mercury, which has a significant vapor pressure (i.e., 0.246 Pa) even at room temperature, requires methods that can deliver an accurate result through techniques that are safe for individuals involved as well as environment.

Two-stage gold (Au) amalgamation and gas-phase detection is a method applied in atmospheric analyses, capable of measuring mercury in the vapor phase at concentrations in the lower ng/L level (Fitzgerald and Gill, 1979). A schematic diagram of this method is illustrated in Figure 4.



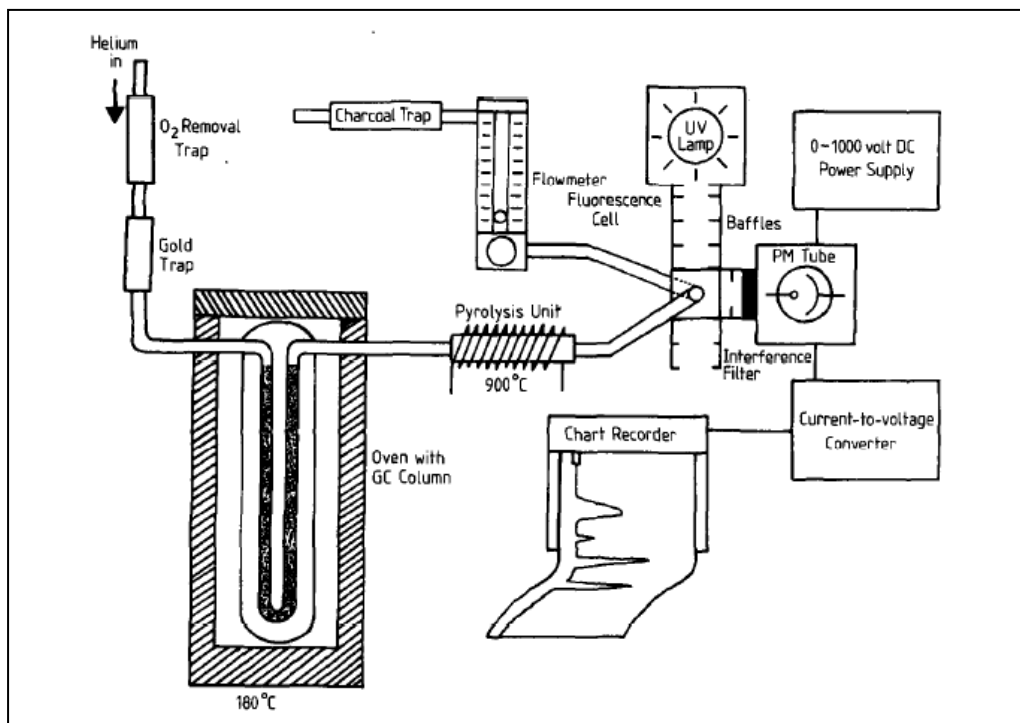
**Figure 4.** Schematic diagram of two-stage gold amalgamation gas flow system as adapted from (Fitzgerald and Gill, 1979).

This method analyzes mercury using a two-stage gold (Au) amalgamation gas train with detection of the eluting elemental mercury by flameless atomic absorption. Gaseous mercury binds to gold because gaseous mercury readily forms a covalent bond with the gold, which can then be broken in a controlled manner with heat. Gold also has great resistance to corrosion, which will also avoid sample contamination. In Figure 4 above, mercury collected on a gold-coated glass bead tube in the field is transferred to a standardized analytical gold-coated glass bead column by controlled heating of gas, using mercury-free air as the carrier gas.

Quantification of mercury in solution by atomic absorption spectrophotometry (AAS) (Lindqvist *et al.*, 1991) was pioneered by Hatch and Ott (1968). This method is accurate for mercury quantification of concentration as low as 1 µg/L in solution and is still used today with slight modification. The method pioneered by Hatch and Otch (1968) utilizes a vapor sample that is transformed into liquid solution by an oxidizing acid digestion. Mercury is then reduced to elemental states and aerated from solution in a closed system. The mercury vapor passed through a quartz absorption cell of an atomic absorption spectrophotometer where its concentration is measured.

More recent research for the quantification of mercury in solution (Bloom and Fitzgerald, 1988; ATSDR, 1999; Bloom *et al.*, 2003; Bloom *et al.*, 2004; ATSDR, 2005; Neculita *et al.*, 2005) has modified the AAS technique by using cold vapor atomic absorption for mercury quantification. In this method, an acidified solution containing mercury is reacted with stannous chloride, a reducing agent, in a vessel external to the

atomic absorption instrument. Ground-state mercury atoms are produced which are subsequently transported by an inert-gas flow to an absorption cell installed in the atomic absorption instrument. When this technique is combined with gas chromatography, mercury species (e.g., inorganic mercury, dimethylmercury, diethylmercury, and methylmercury chloride) present in the air can be separated. Figure 5 includes a schematic diagram of this cold vapor atomic fluorescence spectrophotometer, as adapted from Bloom and Fitzgerald (1988).



**Figure 5.** Schematic diagram of cold vapor atomic fluorescence spectrophotometer (adapted from (Bloom and Fitzgerald, 1988)).

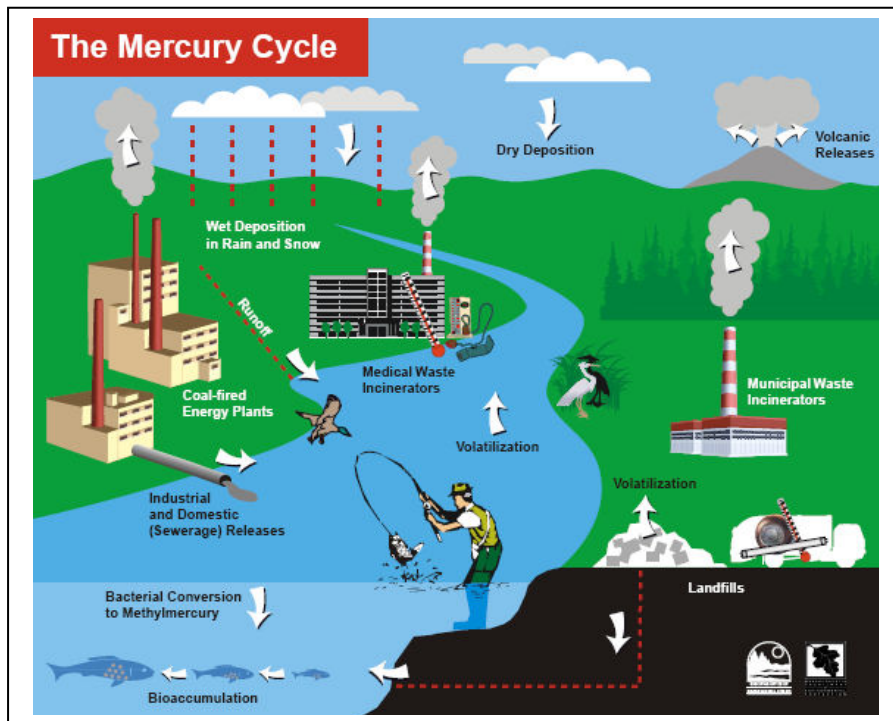
The detector is a UV-visible photomultiplier, shielded from stray light with a 254-nm interference filter. The photomultiplier current is amplified and converted to a millivolt signal with an electrometer, and then passed through an electronic filter to smooth high-

frequency noise. The output is quantified with a dual-pen chart recorder. The excitation source is a short-wave UV mercury vapor lamp. The cryogenic gas chromatograph (GC) is where speciation of mercury species is achieved. The GC column is in the form of a Pyrex U-tube sealed into a glass sheath which enables this air-jacket to temper the rate of heat transfer during mercury elution, and allow clear separation of all species in a single heating step (Bloom and Fitzgerald, 1988). This method is very sensitive for mercury detection or quantification in water (lower  $\mu\text{g/L}$ ) and has been proven to be reliable. Water samples generally do not require digestion, but mercury in the samples is usually reduced to the elemental state prior to analysis (ATSDR, 1999; 2005)

### **1.3. Sources of mercury in the environment**

#### **1.3.1. Natural occurrences**

Mercury is released to the environment by natural processes and from anthropogenic sources, as illustrated in Figure 6. Natural inputs of mercury to the atmosphere come from degassing and wind entrainment of dust particles, volcanic eruptions, forest fires, biogenic emissions of volatile and particulate compounds, and degassing from natural surfaces (Mitra, 1986; Rasmussen, 1994; EPA, 1997; Nriagu and Becker, 2003; Jitaru and Adams, 2004). Early estimate of global natural mercury emissions range from 2,500 to 30,000 ton/yr (Innanen, 1998). No comprehensive report on global natural mercury emissions can be found in literatures.



**Figure 6.** Sources and pathways of mercury in the environment (with permission from The Northeast Waste Management Officials' Association (2007))

Global inventories of volcanic mercury emissions can be found in a recent study by Nriagu (2003). The study is derived from a time-averaged inventory of subaerial (released directly to the atmosphere) emissions from volcanoes that were active between 1980 and 2000 based on the Hg/SO<sub>2</sub> ratios of exhalations. The difficulty to direct methods for measuring the flux of mercury from volcanoes is because volcanoes are often located in inaccessible regions, tend to be unpredictable and are often hazardous. Of the volatiles likely to be released from an active volcano, sulfur dioxide (SO<sub>2</sub>) is the only compound whose flux can be directly measured because of its optical properties, high concentration in volcanic plume (relative to the atmospheric background), and

improvements in remote sensing technology (Nriagu and Becker, 2003). Due to the availability of data of SO<sub>2</sub> emissions, flux of many volatile compounds and trace metals can be estimated by normalizing with reference to SO<sub>2</sub> flux. The result of this study is an estimate of approximately 1140 tons of mercury was released during volcanic eruptions into the global atmosphere and 752 tons of mercury from degassing plumes. The worldwide flux of mercury from volcanic eruptions is equal to 57 ton/yr while the flux from degassing activities is equal to 37.6 ton/yr (Mitra, 1986; EPA, 1997; Nriagu and Becker, 2003; Jitaru and Adams, 2004). This flux of 94.6 ton/yr does not include volcanoes where the SO<sub>2</sub> fluxes have not been measured for political, logistic or economic reasons. After the addition of unmeasured SO<sub>2</sub> flux, estimated by power law equation mentioned in the study, a total of 112 ton/yr of global emissions is reported. According to the authors, this figure is almost identical to the degassing rate of mercury (110 ton/yr) from the mid-ocean ridge volcanoes in 1997 study by Rubin, as cited by Nriagu (2003).

The release of mercury from soils and rocks is also a significant source of mercury to the atmosphere. The highest emission occurs from mercury-enriched soils and rocks. Total global direct emissions from mercury-enriched soils and rocks are estimated to contribute 1500 tons/yr (Rytuba, 2005). Atmospheric mercury is also accumulated in plants and it can subsequently be transferred to terrestrial and aquatic ecosystem by way of litter fall. The total global flux of mercury from litter fall has been estimated to be in the range of 2,400 - 6,000 tons/yr (Rytuba, 2005).

There are a lot of uncertainties in the amount of mercury released to the atmosphere from natural sources. One factor being the difficulty of measuring re-

emissions of mercury because natural emissions from soil, water, vegetation cannot be distinguished from re-emissions. In addition, the technology to measure these emissions and deliver a comprehensive result is not yet available.

### **1.3.2. Anthropogenic sources**

Mercury has many applications in industry due to its unique properties such as high surface tension, uniform volume expansion over a large temperature range (boiling point of 356.72°C and melting point of -38.87°C - refer to Table 1), low electrical resistivity and high thermal conductivity, inability to wet and cling to glass, and ability to form alloy with other metals (Stein *et al.*, 1996; ATSDR, 1999; Jitaru and Adams, 2004; ATSDR, 2005). One of the main consumers was and still is the chlor-alkali industry, where mercury cells are used for production of chlorine and sodium hydroxide by electrolysis of a brine solution (Hylander and Meili, 2003). In 1996, approximately 1344 tons of mercury, 40% of all mercury produced that year, was consumed by the world's chlor-alkali industry (Sznoppek and Goonan, 2000; Hylander and Meili, 2003). The chlor-alkali industry in the USA still accounts for 35% of the domestic mercury consumption, approximately 136 ton per year in 1996 and close to 50% in 2000 (Sznoppek and Goonan, 2000; Hylander and Meili, 2003; Brooks, 2005). According to EPA website (<http://www.epa.gov/epaoswer/hazwaste/mercury/cleanup.htm>), many mercury-cell chlor-alkali plants in the United States have closed or converted to mercury-free processes, however 10 facilities still use the mercury-cell process. One of the 10 facilities has temporarily suspended its mercury-cell operations. The best available alternative is membrane technology which eliminates emissions of mercury and lower energy consumptions.

ATSDR (2005) published an atmospheric mercury emission inventory for the United States (Table 3). Regional Lagrangian Model of Air Pollution (RELMAP), a model built by the EPA produced the inventory result. The purpose of the model was to simulate emission, transport, chemical transformation, and wet and dry deposition of elemental mercury gas, divalent mercury gas, and particulate mercury from various point and area source types. The model was part of EPA's mercury study report to Congress in 1997. The model simulated the regional-scale transport of anthropogenic mercury emissions over a one-year period. The predicted anthropogenic mercury emissions were added to a uniform elemental mercury background concentration of  $1.6 \text{ ng/m}^3$  which represented natural and recycled anthropogenic sources of mercury worldwide. As with any models, RELMAP has a number of uncertainties such as comprehensive emissions data for a number of anthropogenic and natural sources were not available, atmospheric chemistry data were incomplete, and there was inadequate information on the atmospheric processes that affect wet and dry deposition in mercury. The resulting prediction is shown in Table 3, although atmospheric mercury emission has since changed dramatically due to several regulations on mercury issued thereafter. Nevertheless, Table 3 indicates that in 1990's various combustion processes, which include electric utility power production as one of its sources, accounted for 83% of all anthropogenic emissions in the United States. Elemental mercury vapor ( $\text{Hg}^0$ ) and mercuric ( $\text{Hg}^{2+}$ ) comprise 82% of the emissions, with 18% of the emissions associated with particulate mercury ( $\text{Hg}_p$ ). Table 3 also shows that medical waste incineration and municipal waste incineration contributed 48% of total emissions. However, mercury additions to both municipal and medical wastes have been reduced, mainly by

eliminating use of mercury-containing batteries by mandate, and by use of a new class of electronic medical instrumentation to replace those that formerly required mercury, for example, medical thermometers and blood pressure gauges. Unfortunately, no revision on the model that reflects current trends of mercury emission based on evolved understanding in mercury speciation, which plays an important role in model prediction, can be found in the literature.

**Table 3 - Atmospheric Mercury Emission Inventory for the United States by Anthropogenic Source Type (adapted from ATSDR (2005))**

Source type	Ton/yr	% of total emissions	% mercury species		
			Hg <sup>0</sup>	Hg <sup>2+</sup>	Hg <sub>p</sub>
Medical waste incineration	58.6	26	20	60	20
Municipal waste collection	49.8	22	20	60	20
Electric utility boilers (coal, gas, oil)	48.5	22	50	30	20
Non-utility power and heat generation	28.5	13	50	30	20
Non-ferrous metal smelting	8.7	4	85	10	5
Chlor-alkali factories	6.5	3	70	30	0
Other point sources	16.2	7	80	10	10
Area sources ( i.e. dental amalgams, fluorescent lighting fixtures)	6.9	3	100	0	0
Total	223.7	100	41	41	18

In 2000, USGS published a report of domestic mercury flow based on the study by Sznoppek and Goonan (2000). The report confirmed that in 1996, the single largest

point source of anthropogenic mercury emissions was coal-fueled utility boilers used for electrical generation, representing 76 tons of emissions into the atmosphere (Table 4).

**Table 4** - Domestic flow of mercury in 1996, as published by USGS (data are taken from Sznoppek and Goonan (2000))

Emissions from	Amount (in tons)	% of total emissions
Coal combustion	66.0	50.9
Oil and natural gas combustion	10.0	7.7
Municipal solid waste combustion	27.0	20.8
Medical waste combustion	14.6	11.3
Hazardous waste combustion	6.4	4.9
Cement kiln operation	4.4	3.4
Sewage sludge incinerators	1.0	0.8
Non-ferrous smelters	0.2	0.2
Landfills	0.1	0.08
<b>TOTAL</b>	<b>129.7</b>	<b>≈100</b>

In addition to the above reported amount of 129.7 tons of total emissions, about 13.9 tons entered the environment from spills, breakage, and other leaks as mercury was used. These two combined resulted in a total of 144 tons of mercury entered the U.S. environment from all anthropogenic sources in 1996. This amount is 79.7 tons lower than predicted by the model published in EPA report in 1997 which represents mercury emissions in 1990's.

Global anthropogenic emissions of mercury in the year 2000 is estimated to be 2260 tons (Pacyna and Pacyna, 2005). As much as 1915 tons of mercury is emitted to the atmosphere from electric and heat-generating power plants, industrial boilers, small residential and commercial furnaces, non-ferrous metal smelters, pig iron and steel plants, cement plants, and waste incinerators that year. A summary of global emissions of total mercury from anthropogenic sources in the year 2000 is shown in Table 5.

**Table 5** - Global emissions of total mercury from anthropogenic sources in 2000 (in tons) (adapted from Pacyna and Pacyna (2005))

Continent	Africa	Asia	Australia	Europe	South America	North America	Total
Various combustions	215	912	112	114	32	107	1492
Cement production	5	82	n/a	30	6	n/a	123
Non-ferrous metal production	8	87	4	15	25	25	164
Pig iron & steel production	1	12	n/a	13	1	n/a	27
Caustic soda production	n/a	31	1	40	5	2	79
Mercury production	n/a	n/a	n/a	n/a	23	n/a	23
Gold production	178	47	8	n/a	n/a	2	235
Waste disposal	n/a	33	n/a	12	n/a	64	109
Other	n/a	n/a	n/a	15	n/a	2	17
Total	407	1204	125	239	92	202	2269

Various combustions of 1492 tons come from electric- and heat-generating power plants, industrial boilers as well as residential and commercial furnaces. A total of 202 tons of mercury emissions is reported in the year of 2000. The data are compiled from national estimates reports from 17 countries worldwide, including the United States, and where national estimates are not available, estimates are performed using various reports published by the United Nations and other worldwide agencies such as the World Bureau of Metal Statistics. The estimated figures is within 30% accuracy overall (Pacyna and Pacyna, 2005). While one may assume that the 2000 emissions is lower than 1996 emissions due to more stringent regulations imposed on mercury emissions and disposal from various industries thereafter, this figure is up 56% from 1996 report. The increase may be contributed by population growth (The U.S. Census Bureau reported a 13.2% growth from 1990 to 2000) which subsequently translates to increased energy demand. Should stricter regulations on mercury emissions not be in place since 1990, the increase of emissions may represent a much higher value due to this population growth.

## CHAPTER 2

### DISTRIBUTION OF MERCURY

Once mercury enters the ecosystem, it can cycle indefinitely among three environmental media: soils, water and air. Atmospheric deposition has been identified as the dominant source of mercury to water bodies that are not within the vicinity of deposition sources as well as to most remote locations, such as the Arctic and Greenland (Mason *et al.*, 1994; Mason and Sheu, 2002). Regulation in USA has targeted anthropogenic mercury emission sources for reduction because of their substantial contribution to mercury emissions.

#### 2.1. Mercury regulations

Over the past two decades, EPA and U.S. government have released several rules pertaining to the emission of mercury. In 1990, Congress amended the Clean Air Act, requiring EPA to complete a study on hazardous emissions from power plants, and mercury emissions from utilities, municipal waste incinerators and other sources. In 1997, EPA released its Mercury Study Report to Congress. One year later, EPA released a study on the health effects of power plant emissions. This study labeled mercury as the most hazardous emission. In 2000, EPA determined that power plants are responsible for large amounts of dangerous air pollutants. As a result, mercury needs to be regulated, resulting in the development of maximum achievable control technology (MACT) standards. In 2001, EPA informed the industry that MACT standard would require

national reductions in mercury emissions by 89%, 90%, or 98% by December 2007. In 2004, EPA reversed the 2000 determination to establish Cap-and-Trade system.

The Cap-and-Trade system on mercury is based on EPA's Acid Rain program, which is aimed at reduction of sulfur dioxide (SO<sub>2</sub>) emissions. In general, the Cap-and-Trade systems creates a financial incentive for emissions regulators by assigning a cost to polluting (Mathers and Manion, 2005). Companies are free to buy and sell permits in order to continue operating in the most profitable manner available to them. So, those that are able to reduce emissions at a low cost can sell their extra permits to companies facing high costs. This system gives companies flexibility in the manner in which they may achieve their emissions targets (Mathers and Manion, 2005).

The Clean Air Mercury Rule (CAMR) was issued on March 15, 2005. This document builds on EPA's Clean Air Interstate Rule (CAIR) and sets standards for new and existing coal-fired utilities. Included in these standards are emissions limitations, control technologies, and a cap-and-trade program. The document stipulates that not all coal-fired utility units are subjected to the CAMR. Utility units capable of firing more than 73 MW heat input of fossil fuel are subject to the rules. Any industrial cogeneration facility that sells more than 25 MW of electrical output and more than 1/3 of their potential output capacity to any utility power distribution system are also subject to CAMR.

CAMR contains a total national mercury budget that is apportioned to the states. For 2010 to 2017, the nationwide cap for mercury emissions is 38 tons per year. For 2018 and beyond, the nationwide cap for mercury emissions is 15 tons per year. Mercury

emissions from both existing and new mercury budget units must remain within the national caps. Under CAMR, Georgia's annual electric generating unit mercury budget is 1.227 tons/yr for phase 1 and 0.484 tons/yr for phase 2. Mercury budget units are the largest remaining source category of mercury emissions in the country. CAMR relies heavily on mercury emissions reductions expected from the addition of nitrous oxide (NO<sub>x</sub>) and sulfur dioxide (SO<sub>2</sub>) pollution control devices at mercury budget units in the United States under EPA's CAIR. When fully implemented, EPA expects CAMR to reduce national mercury budget unit emissions of mercury from 48 tons/yr (in 1999) to 15 tons, a reduction of nearly 70%.

The CAMR New Source Performance Standards (NSPS) establish mercury emissions for new mercury budget units constructed on or after January 30, 2004. New mercury budget units must meet the following standards of performance based on gross energy output:

NSPS Mercury Limits for New Mercury Budget Units	
Unit Type	Average pounds per megawatt hour for every 12-month period (lbs/MWh)
Bituminous coal units	20 x 10 <sup>-6</sup>
Subbituminous coal (wet units)	66 x 10 <sup>-6</sup>
Subbituminous coal (dry units)	97 x 10 <sup>-6</sup>
Lignite coal units	175 x 10 <sup>-6</sup>
Coal refuse units	16 x 10 <sup>-6</sup>

Integrated gasification combined cycle units	20 x 10 <sup>-6</sup>
<p>Source: US EPA, <i>Revision of December 2000 Clean Air Act Section 112(n) Finding Regarding Electric Utility Steam Generating Units; and Standards of Performance for New and Existing Electric Utility Steam Generating Units: Reconsideration</i>, 71 Fed. Reg. 33388, 33395 (June 9, 2006)</p>	

CAMR also establishes “standard of performance” for existing mercury budget units. Rather than adopt emissions standards for existing units, EPA interpreted the term “standards of performance” to include a market-based cap-and-trade program, and therefore established the Mercury Budget Trading Program.

Similar to CAIR, EPA adopted a model cap-and-trade rule that States may adopt to demonstrate compliance with the requirements of CAMR, however States can choose to achieve the required reductions without joining the national cap-and-trade program. The mercury apportioned to each state is binding on the State, even if it does not participate in the EPA-administered national Mercury Budget Trading Program. For States that choose not to join the national Mercury Budget Trading Program, the State must impose control requirements that will limit statewide emissions from new and existing mercury budget units to the amount of the State budget. Moreover, States are authorized to require emissions reductions beyond those required by the State budget, and nothing in the final CAMR preclude States from requiring stricter controls. Even if a State does not participate in the national Mercury Budget Trading Program, mercury budget units are required to comply with the monitoring, recordkeeping and reporting requirements, including submitting electronic data reports of mercury emissions to EPA each calendar quarter beginning January 1, 2009. Monitoring options include 1) continuously collecting mercury emissions data from each affected unit using a

Continuous Emissions Monitoring System; 2) an appropriate long-term method (i.e. sorbent trap) that can collect an uninterrupted, continuous sample of the mercury in the flue gases emitted from the unit; 3) stack testing for low emitters; or 4) an EPA-approved facility-specific alternative monitoring system, for which any facility may petition. CAMR also requires the owner or operator of a mercury budget unit to maintain records of all information needed to demonstrate compliance with the applicable mercury emissions limit, including the results of performance tests, data from the continuous monitoring systems, fuel analyses, calculations used to assess compliance. States have eighteen (18) months from the data of the final CAMR to submit a State Plan demonstrating how they will meet the assigned CAMR Phase I and 2 mercury budget. Each State Plan must fully adopt state rules to achieve mercury reductions by 2010 and 2018.

In Georgia, the responsibility to draw a State plan is undertaken by the Environmental Protection Division (EPD) of the Georgia Department of Natural Resources. The Georgia EPD is a state agency charged with protecting air, land, and water resources through the authority of state and federal environmental statutes. These laws regulate public and private facilities in the areas of air quality, water quality, hazardous waste, water supply, solid waste, surface mining, underground storage tanks, and others. EPD issues and enforces all state permits in these areas and has full delegation for federal environmental permits except Section 404 (wetland) permits. In 2006, they proposed two options for reducing mercury emissions from the state's coal-fired power plants. Under option 1, Georgia would opt out of the EPA-administered CAMR trading market and would not allow interstate trading of mercury allowances. The

mercury emissions limit during Phase I would be equivalent to either 80% (approximately 0.51 tons/yr) or 85% (approximately 0.38 tons/yr) average capture efficiency. Actual emissions from affected units were 1.89 tons in 2004. During Phase 2, mercury emissions limit would be equivalent to 90% (approximately 0.26 tons/yr) average capture efficiency. Under option 2, Georgia would adopt the Phase I (1.227 tons/yr) and Phase 2 (0.484 tons/yr) emission budgets and deadlines specified in the regulations as well as join the EPA-administered cap-and-trade program for CAMR. No final decision has been made at the time this thesis is written.

In Georgia, there are currently a total of 10 power plants. Savannah Electric operates two facilities, Plant McIntosh and Plant Kraft that have four coal-fired units. Georgia Power has an additional 29 coal-powered units in eight Georgia plants. According to Landers (2006), the companies and their parent, Southern Company, support EPA's national cap-and-trade approach.

The concern on mercury emission is because once mercury is emitted to the atmosphere, it cannot be broken down or degraded into harmless substances. It may change between different states and species in its cycle. Once mercury has been liberated from either ores or from fossil fuel and mineral deposits hidden in the earth's crust and released into the biosphere, it can be highly mobile, cycling between the earth's surface and the atmosphere. Elevated levels of mercury in aquatic environments remote from industrial sources have been broadly attributed to long-range atmospheric transport and deposition of anthropogenic mercury (Fitzgerald *et al.*, 1998).

## 2.2. Global Mercury Cycle

Mercury can enter the atmosphere as a gas or bound to other airborne particles (EPA, 1997). Once emitted, mercury may be deposited by wet and dry processes to environmental surfaces such as soils and water bodies. The main form of atmospheric mercury is elemental vapor ( $\text{Hg}^0$ ), a relatively stable compound, making up at least 95% of the total mercury in atmosphere. The remaining fraction consists of trace levels of gaseous divalent mercury ( $\text{Hg}^{2+}$ ) such as mercuric dichloride ( $\text{HgCl}_2$ ) and mercury associated with particulate matter ( $\text{Hg}_p$ ). Unlike divalent forms of mercury which are removed from the atmosphere relatively quickly, the high vapor pressure (0.246 Pa at 25°C) and low water solubility (0.11 M/atm at 20°C) (Sanemasa, 1975; Mason and Sheu, 2002) of elemental mercury ( $\text{Hg}^0$ ) extend its residence time in the atmosphere, thus facilitating the long-range transport of elemental mercury away from points of release (Carpi, 1997). As shown in Figures 5 and 6, mercury pollution is global, affecting remote areas of the planet because its residence time is between 6 months and 2 years (Mason and Sheu, 2002) which provides sufficient time for elemental mercury ( $\text{Hg}^0$ ) to be distributed over the entire planet before returning to the land, lakes, seas, and ice (Morel *et al.*, 1998).

Wet and dry particle deposition and evasion of dissolved gaseous mercury from the ocean and the land have been identified as key controls over mercury cycling and fate on earth's surface (Mason and Sheu, 2002). In addition, elemental mercury ( $\text{Hg}^0$ ) has been considered to be the main form of mercury in evasion from both ocean and terrestrial environment. Three types of mercury are identified in the atmosphere based on their physical and chemical properties (Lindberg and Stratton, 1998; Munthe *et al.*, 2001;

Stratton *et al.*, 2001; Mason and Sheu, 2002): gaseous elemental mercury ( $\text{Hg}^0$ ), reactive gaseous mercury (RGM), and particulate mercury, which consists of mercury bound or adsorbed on atmospheric particulate matter (Munthe *et al.*, 2001). RGM is water-soluble mercury with sufficiently high vapor pressure to exist in gas phase, and is assumed to be gaseous mercuric dichloride ( $\text{HgCl}_2$ ) or other mercuric halides (Morel *et al.*, 1998; Shia *et al.*, 1999; Munthe *et al.*, 2001; Mason and Sheu, 2002). These compounds have a high surface reactivity and water solubility (i.e., 0.069g/mL for  $\text{HgCl}_2$  (ATSDR, 2005)) and they are rapidly removed from the atmosphere by deposition processes (Mason and Sheu, 2002). Gaseous elemental mercury ( $\text{Hg}^0$ ) has low water solubility, which makes its removal by rainwater less efficient compared to RGM and also to the scavenging of particulate mercury.

The global mercury cycles for current and preindustrial periods are presented in Figures 7 and 8. Estimation of fluxes shown in the figures is based on available data from a number of studies and previous estimates of mercury evasion. Table 6 lists the emission fluxes to the atmosphere and deposition estimates in Figure 7.

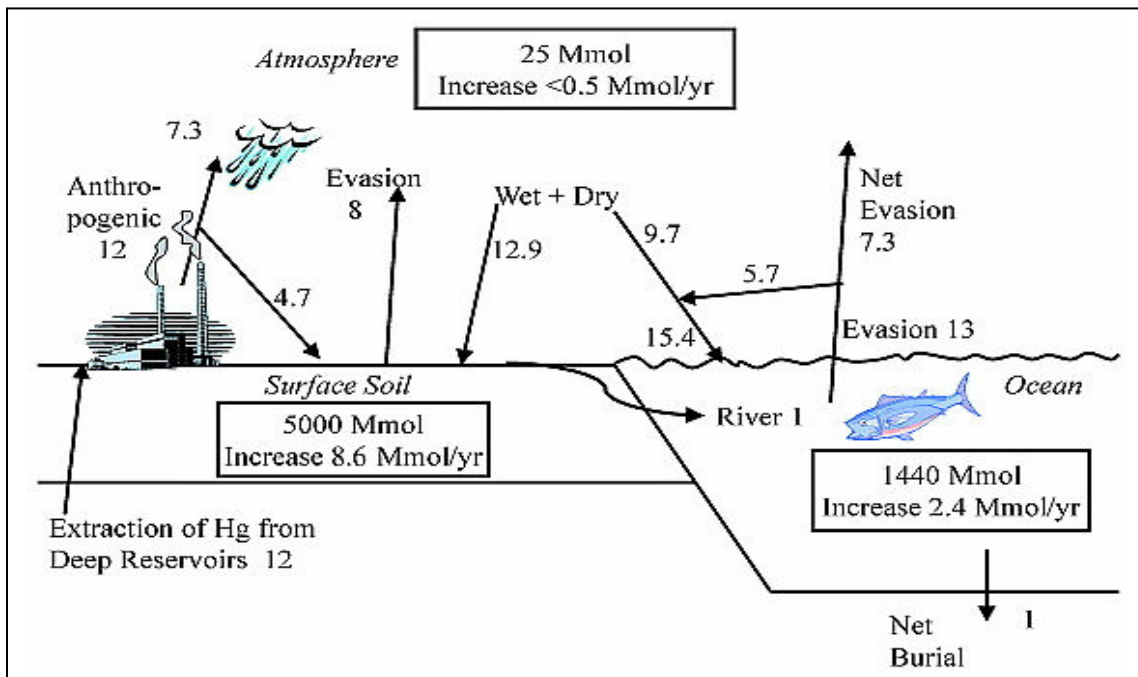
**Table 6** - Emission fluxes to the atmosphere and deposition estimates.  
All values are in Mmol/yr

Sources to the atmosphere	Flux (Mmol/yr)	Atmospheric sinks	Flux (Mmol/yr)
Direct anthropogenic	12	Wet deposition to ocean	9.6
Land natural	4.05	Dry deposition to the ocean	5.8
Land reemission	3.95	Wet deposition to land	10.0

Ocean natural	6.5	Local dry deposition to land	4.7
Ocean reemission	6.5	Remote dry deposition to land	2.9
Total	33		33

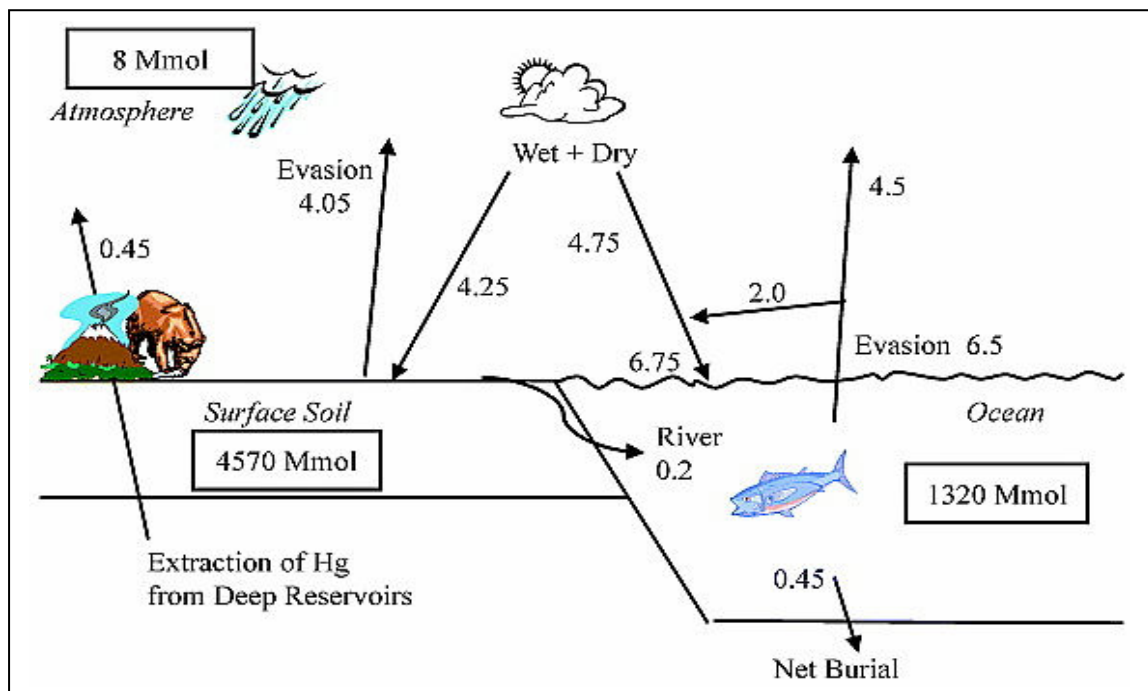
As with all estimates, the evasion flux estimates listed in Table 6 is based on data set with high variability, there are errors associated with this estimate (Mason and Sheu, 2002). Nevertheless, the estimates represent how air-sea exchanges of mercury is a critical part of the global mercury cycle. Direct anthropogenic of 12 Mmol/yr is estimates of point source inputs to the atmosphere that also includes crude oil processing. Crude oil processing purifies the raw natural gas extracted from underground gas fields and brought up to the surface by gas wells. The processed natural gas, used as fuel by residential, commercial and industrial consumers, is almost pure methane but also consists of small amounts of mercury primarily in elementary form. A total of 9.7 Mmol/yr represents dry and wet deposition flux to the ocean. The wet deposition flux to the ocean (9.6 Mmol/yr) is taken from a previous study, and with the addition of the dry particulate flux estimate of 0.1 Mmol/yr, given the very low concentration of particulate mercury generally encounter over the open ocean. Wet and dry deposition to land (12.9 Mmol/yr) combines a flux of 10.0 Mmol/yr of wet deposition and another 2.9 Mmol/yr of remote (not within the vicinity of point sources) dry deposition. Ocean evasion of 13 Mmol/yr represents equal fluxes of natural evasion and reemission, however the net evasion to the atmosphere is equivalent to 7.3 Mmol/yr given the rapid oxidation of elemental mercury

in the marine boundary layer, the layer of moist air above the sea. An increase of 2.4 Mmol/yr to the deep ocean is taken into consideration water mixing between the surface and deep ocean, which brings only a small increase to this reservoir. The net burial to the deep ocean is equivalent to 1 Mmol/yr, which is preserved in sediment. The local deposition of anthropogenic inputs is estimated at 4.7 Mmol/yr compared to deposition of RGM over the ocean of 5.7 Mmol/yr. The overall mass balance for this global mercury cycle includes a rate of increase of 8.6 Mmol/yr in the terrestrial surface layer and with the deep ocean accumulating 2.4 Mmol/year of increase. Finally, assuming that the mercury deposition has increased relatively linearly over the last 100 years and that the distribution in deposition has not changed, the ocean currently has a total deposition of 1440 Mmol and 5000 Mmol in the terrestrial surface layer.



**Figure 7.** The current global mercury cycle (adapted from Mason and Sheu (2002)). All fluxes are in Mmol/yr

According to Mason and Sheu (2002), the preindustrial mercury cycle (Figure 8) was developed by assuming, based on sediment records of atmospheric deposition, that remote terrestrial deposition in preindustrial times was less than current deposition by a factor of 3 (i.e. 4.5 Mmol/yr). Inputs from volcanoes and other geological sources are estimated at 0.45 Mmol/yr, therefore evasion from other terrestrial sources is 4.05 Mmol/yr. The mass balance implication includes a total inventory of 4570 Mmol in surface soil and 1320 Mmol in deep ocean, which is about 9% lower than the current mercury cycle (Figure 7). The preindustrial mercury cycle has no net increase in any of the reservoirs because this increase is contributed by the burning of fossil fuel and the release of mercury from industrial processes, which do not occur during preindustrial time.



**Figure 8.** The preindustrial global mercury cycle (adapted from Mason and Sheu (2002)). All fluxes are in Mmol/yr

It is important to note that the global mercury cycles (Figures 7 and 8) do not include mercury accumulation in fish and how much this accumulation adds to the deposition of mercury in the reservoirs. It reiterates the fact that mercury biogeochemical cycling is complex, principally because it involves both biological and chemical processes.

### **2.3. Speciation in Environmental Media**

One of the major concerns of mercury and its impact to the environments is its transformation to methylmercury, a highly toxic form of mercury. Environmental factors such as redox conditions, pH, and presence of soluble organics and sulfate contribute to the transformation of mercury species. Speciation plays a major role in determining the bioavailability of mercury species for methylmercury transformation. As different forms of mercury may exhibit differing toxicities and mobilities in the environment, it is important to distinguish individual mercury species as they are emitted, deposited, and transformed in the atmosphere, water, and sediments.

#### **2.3.1. Atmosphere**

The type of mercury most emitted to the atmosphere, both natural and anthropogenic, is the elemental mercury ( $\text{Hg}^0$ ) vapor form, and the remaining balance is made up of particulate-bound divalent mercury ( $\text{Hg}^{2+}$ ) and reactive gaseous mercury (RGM) (Nriagu, 1979; Mason and Sheu, 2002; Krabbenhoft *et al.*, 2005; Parsons and Percival, 2005). According to Mason and Gill (2005) elemental gaseous mercury is

unreactive with a net atmospheric residence time to be between six and twelve months (Hedgecock and Pirrone, 2001). Particulate mercury consists mainly of divalent mercury ( $\text{Hg}^{2+}$ ) species adsorbed to surfaces or incorporated into particles during high level combustion and other processes. Particulate mercury resides predominantly in the fine particulate fraction ( $< 2.5\mu\text{m}$ ). The deposition velocity has been estimated to be  $< 0.5$  cm/s typically. The concentration of particulate mercury is relatively low (i.e.,  $< 10$   $\text{pg}/\text{m}^3$ ), thus the input of mercury via dry deposition of particulate mercury is not a major component of the overall flux (Mason and Gill, 2005). However, particulate mercury is also scavenged by wet deposition. The residence time of particulate mercury has been estimated to be from days to weeks (Bullock, 2000). Reactive gaseous mercury consists of gaseous neutral divalent mercury ( $\text{Hg}^{2+}$ ) complexes such as  $\text{HgCl}_2$ ,  $\text{HgBr}_2$ , and  $\text{HgOBr}$ . Such compounds are highly soluble (i.e.,  $\text{HgCl}_2$  solubility of 6 g/mL) and have a high deposition velocity. The dry deposition velocity for RGM is estimated to be  $< 1$  to 4 cm/s (Bullock, 2000; Mason and Gill, 2005), thus it is rapidly removed from the atmosphere and its residence time is on the order of hours to days, and it will not persist in the atmosphere unless it is being continually produced. RGM concentration also follows a diurnal cycle, increasing after sunrise and decreasing towards evening with the highest concentrations occurring around midday (Lindberg and Stratton, 1998; Gardfeldt, *et al.*, 2001; Sprovieri *et al.*, 2003). Some atmospheric trace gases are characterized by strong day/night differences in their concentrations, generally as a result of photochemistry and/or removal at the surface by nocturnal dry deposition. It was speculated that atmospheric emissions of RGM from many large scale combustion sources probably occur near or above the nocturnal boundary layer (the boundary layer

from sunset and sunrise), allowing for limited downward mixing at night (Lindberg and Stratton, 1998). This observation is a result of a six field sampling campaigns at two eastern U.S. sites, Tennessee and Indiana which was conducted from 1992 to 1995, with over 260 samples of ambient RGM were collected. The study utilized a high-flow refluxing mist chamber developed at NASA Langley Research Center. The high flow refluxing mist chamber has been widely used for various studies of trace gases in the atmosphere, a review which is not covered in this paper. The study also found that RGM was found to reach maximum value of  $\sim 0.05\text{-}0.06\text{ ng/m}^3$  during each day of the experiment but decreased by more than a factor of 3 during the intervening night (Lindberg and Stratton, 1998).

Although gaseous elemental mercury ( $\text{Hg}^0$ ) is relatively volatile and is not easily oxidized to less volatile forms by major atmospheric oxidants, the oxidation of mercury in the atmosphere is important because of the disparate residence times of elemental ( $\text{Hg}^0$ ) and divalent ( $\text{Hg}^{2+}$ ) mercury. Divalent mercury compounds are less volatile than elemental mercury and are deposited close to their point of production within days (Hedgecock and Pirrone, 2001). Of the oxidant species in the atmosphere, ozone is the most important aqueous phase species in conversion of elemental mercury to divalent mercury, although it has been suggested that where ozone concentrations are low, the  $\cdot\text{OH}$  radical can play a role of equal importance (Lin and Pehkonen, 1999; Hedgecock and Pirrone, 2001).

According to Schroeder and Munthe (1998), the transformation of mercury species in the atmosphere is also affected by the air-surface exchange of mercury. Wet deposition of divalent mercury ( $\text{Hg}^{2+}$ ) species and dry deposition (of particulate mercury)

are generally considered to be uni-directional processes involving transfer of mercury from the atmospheric compartment to the earth's surface. However, air-surface exchange of elemental mercury ( $\text{Hg}^0$ ) can occur bi-directionally, allowing reemission or recycling of mercury from a given surface to the atmosphere. For example, divalent mercury ( $\text{Hg}^{2+}$ ) species, one of the oxidized forms of mercury, are deposited on water, soil or vegetative surfaces, tend to remain non-volatile and hence immobile.

Table 7 lists gas-liquid equilibria for various mercury species, as governed by Henry's law. These Henry's law constants show how mercury can equilibrate among different phases. The chemistry of mercury in the atmospheric environment may take place in the gas phase and in the aqueous phase. Elemental mercury ( $\text{Hg}^0$ ) and dimethylmercury ( $(\text{CH}_3)_2\text{Hg}$ ), with comparatively low Henry's Law constants, are moderately soluble in water. The rest of the compounds in Table 7 have very high Henry's Law constants, which indicate that they will not be absorbed into water and will remain in gaseous phase.

**Table 7 - Gas-liquid equilibria of mercury species<sup>a</sup>**

Equilibrium	$K_H$ (M/atm)
$\text{Hg}^0_{(g)} \leftrightarrow \text{Hg}^0_{(aq)}$	0.11
$\text{Hg}(\text{OH})_{2(g)} \leftrightarrow \text{Hg}(\text{OH})_{2(aq)}$	$1.2 \times 10^4$
$\text{HgCl}_{2(g)} \leftrightarrow \text{HgCl}_{2(aq)}^b$	$2.4 \times 10^7$
$\text{CH}_3\text{HgCl}_{(g)} \leftrightarrow \text{CH}_3\text{HgCl}_{(aq)}$	$2.2 \times 10^3$
$\text{Hg}(\text{CH}_3)_{2(g)} \leftrightarrow \text{Hg}(\text{CH}_3)_{2(aq)}$	0.13

<sup>a</sup> Lin *et al.* (1999).

<sup>b</sup> Shon *et al.* (2005)

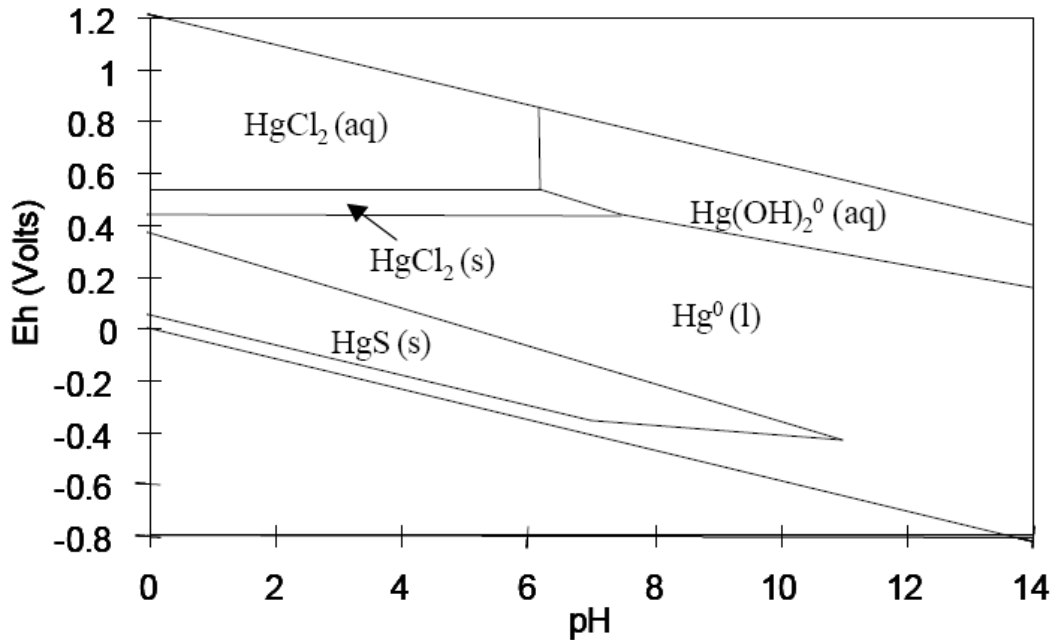
### 2.3.2. Aquatic environment

As in the atmosphere, the speciation of mercury in the aquatic environment will determine its bioavailability for mercury transformation to methylmercury. The speciation of mercury in water is dependent on a number of factors. First, the dissolved solid phase partition coefficient for mercury in water is on the order of  $10^{-4}$  to  $10^{-5}$ , demonstrating the strong affinity of mercury for suspended natural particles in aquatic environment (Babiarz *et al.*, 2001; Krabbenhoft *et al.*, 2005). The pH, pE and composition of the water matrix will also determine the thermodynamically favored species both in the dissolved and particulate phases.

Under acidic conditions ( $\text{pH} < 6$ ), elemental mercury ( $\text{Hg}^0$ ) can be oxidized to divalent mercury ( $\text{Hg}^{2+}$ ) (Stein *et al.*, 1996). The environmental behavior of divalent mercury is determined by its subsequent complexation with inorganic and organic

compounds. Divalent mercury is a necessary precursor for the formation of compounds with increased solubility (i.e.,  $\text{HgCl}_2$  and  $\text{Hg}(\text{OH})_2$ ) and increased bioavailability ( $\text{CH}_3\text{Hg}^+$ ).

Under oxidizing conditions and in freshwater systems, any  $\text{Hg}^{2+}$  not bound to organic or sulfide ligands exists as hydroxyl and/or chloride complexes (Stein *et al.*, 1996; Reddy and Aiken, 2001). Mercury is likely to form complexes with  $\text{OH}^-$  and  $\text{Cl}^-$  because of their high abundance and stability, with  $\text{HgCl}_2$  and  $\text{Hg}(\text{OH})_2$  forms being the most abundant over all pH ranges (Gabriel and Williamson, 2004). As can be seen from Figure 9 (reproduced from Davis *et al.* (1997)), the chemical form of mercury in solution is strongly affected by the redox conditions in the system, characterized by its redox potential  $E_h$ , and also depends on pH. The more positive the potential of the system, the higher the valency of mercury that can be expected. The redox potential in natural waters is determined mainly by the concentration of dissolved oxygen, and by the organic matter content. In well-aerated, oxygen-containing waters ( $E_h \sim 0.5\text{V}$ ), mercuric species will be the predominant form of inorganic soluble mercury. Elemental mercury should prevail under mildly oxidizing or reducing conditions, unless enough sulfide is present to stabilize hydrosulfide or sulfide complexes of divalent mercury (Benes and Havlik, 1979). A significant abundance of the sulfidic complexes can be expected in sulfidic marine waters, in interstitial water of bottom sediments or in certain types of wastewaters. The actual form of divalent mercury ( $\text{Hg}^{2+}$ ) in well-aerated surface waters will depend on the pH and the chloride concentration because of the tendency of divalent mercury to form stable chloride complexes.



**Figure 9.** Predominance diagram for mercury species in water at 25°C and 1 atm pressure containing  $10^{-3}\text{M Cl}^-$ . The vertical axis represents oxidation potential (reproduced from Davis *et al.* (1997)).

Figure 9 is a result of a study to assess mercury speciation in soils and sediments in relation to the prediction of mercury bioaccessibility via the ingestion exposure pathway (Davis *et al.*, 1997). Bioaccessibility represents the fraction of ingested mercury solubilized in the gastrointestinal tract, while bioavailability denotes the fraction actually absorbed. Although the study focused on human exposure pathway, Figure 9 can be used to explain the behavior of inorganic mercury species. The oxidized forms are stabilized in oxygenated water, in which the oxidation potential  $\text{Eh} > 0.5 \text{ V}$ . Elemental mercury dissolved in oxygenated surface water is thus thermodynamically unstable with respect to oxidized species (Lindqvist and Rodhe, 1985). At lower pH, mercuric dichloride ( $\text{HgCl}_2$ ) is the dominant solid, and at higher pH hydrated mercuric oxide ( $\text{Hg(OH)}_2$ ) is

found. For example, in an estuarine saltmarsh when sediment pH is 6.5 and Eh value of -0.1 V,  $\text{HgS}_{(s)}$  would tend to dominate the total Hg pool. Divalent mercury ( $\text{Hg}^{2+}$ ) dominates in aerated, neutral, aqueous solutions and is easily complexed by  $\text{Cl}^-$  up to pH 7, resulting in soluble  $\text{HgCl}^+$  and  $\text{HgCl}_2$  even at low  $\text{Cl}^-$  concentrations ( $10^{-5}$  M - see Figure 9).

Table 8 lists the equilibrium constants for divalent mercury complexation with some inorganic ligands in the waters. Other ligands such as  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{CH}_3\text{COO}^-$  and  $\text{HCOO}^-$  may also be present at significant concentrations in cloud water, however the stability constants of  $\text{Hg}^{2+}$  with these ligands are too small to lead to significant complex formation (Lin and Pehkonen, 1999)

**Table 8** - Equilibria for aqueous phase  $\text{Hg}^{2+}$  speciation<sup>a</sup>

Equilibrium	Log ( $K_{\text{eq}}$ )
$\text{Hg}^{2+} + \text{OH}^- \leftrightarrow \text{Hg}(\text{OH})^+$	10.63
$\text{Hg}^{2+} + 2 \text{OH}^- \leftrightarrow \text{Hg}(\text{OH})_2$	22.24
$\text{Hg}^{2+} + \text{SO}_3^{2-} \leftrightarrow \text{HgSO}_3$	12.7
$\text{Hg}^{2+} + 2 \text{SO}_3^{2-} \leftrightarrow \text{Hg}(\text{SO}_3)_2^{2-}$	7.30
$\text{Hg}^{2+} + \text{Cl}^- \leftrightarrow \text{HgCl}^+$	24.1
$\text{Hg}^{2+} + 2 \text{Cl}^- \leftrightarrow \text{HgCl}_2$	14.0
$\text{Hg}^{2+} + 3 \text{Cl}^- \leftrightarrow \text{HgCl}_3^-$	15.0
$\text{Hg}^{2+} + 4 \text{Cl}^- \leftrightarrow \text{HgCl}_4^{2-}$	15.6
$\text{Hg}^{2+} + \text{C}_2\text{O}_4^{2-} \leftrightarrow \text{HgC}_2\text{O}_4$	9.66

<sup>a</sup> Lin *et al.* (1999).

In addition to mercury complexation with inorganic ligands such  $\text{Cl}^-$  and  $\text{OH}^-$ , mercury speciation with organic ligands (i.e. dissolved organic carbon (DOC)) is also taking place in natural waters. Through its binding to DOC, mercury can be mobilized

from the drainage basin and transported to lakes (Morel *et al.*, 1998). Contradicting theories about mercury binding to DOC hence its bioavailability makes this complexation critically important. Gilmour and Henry (1991) reported that, in the water column, increased DOC may increase ligand formation between DOC and dissolved mercury, making it unavailable for microbial methylation. In the contrary, increased DOC at the sediment-water interface may act as a substrate for bacteria, thereby increasing microbial methylation (Stein *et al.*, 1996).

### **2.3.3. Soils and sediments**

Soils and sediments have been considered as the main sinks for mercury. Soils and sediments can also act as a source of mercury to surrounding media (i.e., uptake of mercury-containing sediment by biota). Mercury likely associates with organic matter and iron oxides under oxidizing conditions, and organic matter and sulfides under reducing conditions (Heyes *et al.*, 2004; Krabbenhoft *et al.*, 2005). The strong association of mercury with organic matter is in part because of the overwhelming amount of organic matter in soils and sediments. Humic and fulvic materials make up a large percentage of dissolved organic matter and it is generally reported that humic and fulvic acids are the important complexing agents in soils (Gabriel and Williamson, 2004).

Studies conducted to measure concentrations of total mercury in upland forest soils across the upper Midwest of the U.S. reported a resulting range from about 100-250 ng/g dry weight for the surface humus layers (Hintelmann *et al.*, 2002). Surface enrichment pattern was also reported, with strongly decreasing concentration gradients to about 15 cm below the surface (Krabbenhoft *et al.*, 2005). According to Gagnon, *et al.*

(1997), who studied contaminated sediments in a site formerly occupied by a chlor-alkali plant in Saguenay Fjord, Sweden, the depth distribution of dissolved mercury may depend on the affinity of specific components of the sediments for mercury rather than on total mercury content. Dissolved mercury can be adsorbed by several components of the solid sediment, for example sulfide minerals have been reported to be excellent scavengers for mercury and potential source for secondary contamination.

According to Gabriel and Williamson (2004), the overall adsorption of mercury to mineral and organic particles is correlated to their surface area, organic content, cation exchange capacity, and grain size. In addition,  $\text{Cl}^-$  ion concentration and pH are two factors that affect mercury adsorption in terrestrial soils. An increase in  $\text{Cl}^-$  concentration and a decrease in pH can, together or separately, decrease mercury adsorption. Mercury adsorption generally decreases with decreasing pH, due to  $\text{H}^+$  ions removing and replacing metal ions. A study on the effect of pH on mercury adsorption to organic and various mineral soils confirms that the most efficient sorbent over all pH ranges is the organic soil (Gabriel and Williamson, 2004). Mercury's strong binding to soil is, in part, due to the S-functional groups that are frequently associated with organic molecules. The dominant factor controlling the formation of sulfide-mercury species is redox potential.  $\text{HgS}$  is formed under reducing conditions. Because  $\text{HgS}$  is non-polar, and has a high stability, it commonly does not adsorb to soil or sediment media. Therefore, in reducing environments, formation of  $\text{HgS}$  can be an efficient detoxification process.

In addition to soils and sediments, groundwater inflow and outflow may potentially represent both a mercury source and sink to lakes, transporting mercury associated either with atmospheric deposition or from the dissolution of minerals in the

aquifer (Krabbenhoft and Babiarz, 1992). As mentioned before, mercury may be present at the sediment/water interface in several dissolved forms (ionic, nonionic, complexed, or methylated) or may be associated with solid phases (adsorbed, assimilated, or precipitated). In pore waters (interstitial water near the sediment/water interface, which is usually less than 0.5 m below the interface), dissolved elemental mercury may diffuse back to the lake and possibly volatilize from the surface (Krabbenhoft and Babiarz, 1992). Numerous studies reviewed in this chapter show the complexity of mercury speciation that determines its fate and transport in different environment media. The transformation of mercury species in the environment will ultimately determine its bioavailability to microbial community (i.e. sulfate-reducing bacteria) for methylation of mercury.

## CHAPTER 3

### METHYLATION OF MERCURY

Organic mercury compounds include dimethylmercury ((CH<sub>3</sub>)<sub>2</sub>Hg), phenylmercury (C<sub>6</sub>H<sub>5</sub>Hg<sup>+</sup>), ethylmercury (C<sub>2</sub>H<sub>5</sub>Hg<sup>+</sup>) and methylmercury (CH<sub>3</sub>Hg<sup>+</sup>), with the most common organic mercury compounds in the environment being methylmercury. Methylmercury is formed in aquatic systems, and it cannot be degraded from organisms after uptake, therefore it is biomagnified in aquatic food chains from bacteria, to plankton, through macroinvertebrates, to herbivorous fish and to piscivorous (fish-eating) fish (Watras and Bloom, 1992). At each step in the food chain the concentration of methylmercury in the organism increases (Gilmour and Henry, 1991; Stein *et al.*, 1996; Morel *et al.*, 1998; Hintelmann *et al.*, 2000; Chemaly, 2002; Celo *et al.*, 2006). Fish and other aquatic species are the only significant source of human methylmercury exposure (EPA, 1997).

The concentration of mercury in any given fish depends on species of fish, age and size of the fish and type of water body in which it is found. In general, fish-eating fish such as shark, swordfish, marlin, larger species of tuna, walleye, largemouth bass, and chain pickerel, have higher levels of methylmercury than herbivorous fish such as tilapia, trout, and herring (UNEP, 2003). Within a given species of fish, older and larger fish have higher levels of methylmercury than smaller fish. Humans are exposed to toxicity methylmercury mainly through consumption of fish in our diet, and Minamata disaster in Japan is an example of this. Toxicity of methylmercury targets the central nervous system, with fetal nervous systems being especially sensitive (Stein *et al.*, 1996).

### 3.1. Toxicity of methylmercury

Elemental mercury is absorbed mainly through the lungs and the skin and causes damage to the lungs and the brain while methylmercury is taken up through intestinal absorption (Chemaly, 2002). Following inhalation of elemental mercury vapor, it readily diffuses across the blood-brain barrier. In the brain, it is oxidized to divalent mercury which then damages cells of the nervous system (Rabenstein, 1978; Clarkson and Magos, 2006). In contrast, the Hg-C bond in methylmercury is stable in biological media, and because of its lipid solubility, methylmercury readily crosses the blood-brain barrier and accumulates in the brain, the target organ in methylmercury poisoning (Rabenstein, 1978; Eccles and Annau, 1987; EPA, 1997; UNEP, 2003; Jitaru and Adams, 2004; ATSDR, 2005; Clarkson and Magos, 2006). According to Rabenstein (1978), methylmercury in the brain causes lysis of cells of the central nervous system. Because nervous system cells are replenished only by cell division, cell lysis by methylmercury results in permanent damage on the central nervous system. Thus, nerve cell damage is irreversible and most likely cumulative (Rabenstein, 1978; Eccles and Annau, 1987; EPA, 1997; UNEP, 2003; Jitaru and Adams, 2004; ATSDR, 2005; Clarkson and Magos, 2006). In addition, the biological half time of methylmercury for humans is 70 days, enabling it to concentrate to very high levels (Rabenstein, 1978; D'Itri, 1991). Methylmercury can also cross the placental barrier and this leads to accumulation in the fetus (Chemaly, 2002).

The first indication that methylmercury may present a threat to public health came from the epidemics of Minamata Bay, Japan (Bakir *et al.*, 1973; D'Itri, 1991). Minamata

disease was first discovered in Minamata City in 1956. A total of 121 people living in villages around Minamata Bay were poisoned during 1953 to 1960 and 22 infants were poisoned prenatally (Bakir *et al.*, 1973). The neurological syndrome included symptoms such as loss of muscles coordination, numbness in the hands and feet, general muscle weakness, narrowing of the field of vision and damage to hearing and speech.

Investigations revealed that the poisonings were due to the consumption of fish having high concentrations of methylmercury. The contaminations of the waters of Minamata Bay was traced to the release of methylmercury compounds from plastic industries in which inorganic mercury compounds were used as catalysts (Bakir *et al.*, 1973; D'Itri, 1991). According to the Japanese Ministry of the Environment, as of March 2001, 2,265 victims had been officially recognized (1,784 of whom had died) and over 10,000 had received financial compensation.

Methylmercury gained worldwide interest again when an epidemic of methylmercury poisoning in farmers and their families took place in Iraq in 1972. A total of 6,530 cases of poisoning were admitted to hospitals in provinces throughout the country, and there were 459 hospital deaths attributed to methylmercury poisoning (Bakir *et al.*, 1973; D'Itri, 1991). The population became exposed to methylmercury when they ate homemade bread prepared from seed wheat treated with a methylmercurial fungicide.

Methylation of mercury requires the transfer of an alkyl anion group (such as  $\text{CH}_3^-$ ), a strong base highly unstable in water. The methylmercury unit itself is kinetically inert toward decomposition (Stumm and Morgan, 1996) although methylmercury has been shown to be degraded enzymatically by some bacteria or through photochemical

mechanism (Morel *et al.*, 1998), a subject which will be reviewed in subsequent chapter of this thesis. Methylation of mercury occurs biotic and abiotically, and transformation processes are influenced by several environmental factors such as pH, temperature, sulfate deposition, and availability of biodegradable organic carbon.

### **3.2. Transformation to methylmercury**

While it is possible to produce methylmercury abiotically, it is widely believed that biotic methylation of mercury within a watershed is the principal mechanism for methylmercury formation, and that sulfate-reducing bacteria (SRB) are the primary methylators of mercury in the environment (Compeau and Bartha, 1985; King *et al.*, 2000; Krabbenhoft *et al.*, 2005). SRB are found both in a high acid (pH = 4) and a tolerate alkaline (pH = 9.5) environments (Barton 1995). Three pathways how mercury is methylated by SRB have been proposed: (1) the acetyl Coenzyme A pathway in which methyl-tetrahydrofolate is the methyl group donor (Choi *et al.*, 1994); (2) the acetate metabolic pathway using methyltransferase enzymes (King *et al.*, 2000); and (3) the methionine synthase (Siciliano and Lean, 2002). In addition to biologically mediated methylation by SRB, abiotic methylation can also occur photochemically (Lean and Siciliano, 2003; Siciliano *et al.*, 2005).

#### **3.2.1. Environment influences on mercury transformation**

Studies have confirmed that methylation of mercury is influenced by several environmental factors such as concentration of  $\text{Hg}^{2+}$  including its complexes and pH. Low initial  $\text{Hg}^{2+}$  concentrations and  $\text{pH} \geq 7$  result in dimethylmercury ( $(\text{CH}_3)_2\text{Hg}$ ), while

higher initial  $\text{Hg}^{2+}$  concentrations and acidic pH result in monomethylmercury, with optimum pH for monomethylmercury formation in sediments being around 4.5 (Bodek *et al.*, 1988; D'Itri, 1991). Other environmental factors also affect the bioavailability of mercury for methylation.

#### **3.2.1.1. Effects of temperature**

According to Winfrey and Rudd (1990), in freshwater sediments, methylation is inhibited by low temperatures and has a temperature optimum of about 35°C. Mercury methylation also reaches its peak in late summer and is low throughout the remainder of the year. The seasonality of methylation is not due solely to an increase in temperature, however, others factors such as variations in demethylation activity, nutrient loading, and aerobic conditions may contribute to the peak methylation in the summer (Korthals and Winfrey, 1987). Methylation and demethylation may occur in parallel, therefore increases in methylation activity may be in part due to decreased demethylation activity. Nevertheless, temperature plays a role in methylation rate because of its effects on microbial activity in the sediments.

#### **3.2.1.2. Availability of biodegradable organic carbon**

Methylation is enhanced by increased availability of organic carbon because microbial activities that produce and decompose methylmercury are dependent on it. Moreover, methylation is fastest in the surface layer of sediments, where microbial activity and newly sedimented highly degradable organic carbon is concentrated.

### 3.2.1.3. Sulfate deposition

Earlier work on methylation demonstrated that an inhibition of sulfate reduction by molybdate inhibited mercury methylation in both marine and freshwater sediments (Compeau and Bartha, 1985). Similar experiments to the work of Compeau and Bartha (1985) were conducted using anoxic subsurface (< 5 cm depth) sediments from Lake Clara in Wisconsin, however the results demonstrated that over a 21-day period addition of sulfate, increased accumulation of sulfide but slightly reduced rates of mercury methylation was observed (Winfrey and Rudd, 1990). It was suggested that the reason was likely due to precipitation of  $\text{Hg}^{2+}$  by sulfide produced by the enhanced sulfate reduction.

### 3.2.1.4. Effects of pH

Decreased pH does not translate to increased methylation. As pH is lowered, there is a shift from the production of dimethylmercury to the production of monomethylmercury but the total amount of mercury methylated remains approximately the same (Winfrey and Rudd, 1990). Although the authors confirms that this affects the mercury content in fish because monomethylmercury is less volatile than dimethylmercury and thus is retained more efficiently in lakes. Low pH conditions (<5) also favor the production of soluble and bioavailable monomethylmercury over volatile dimethylmercury (Stein *et al.*, 1996). This condition stimulates microbial production while at the same time decreases demethylation process.

As mentioned before, methylation of mercury in the aquatic environment has largely been considered to be the result of biological processes, with sulfate-reducing bacteria as the main contributor to the process, a review which will be covered later in this chapter. However, methylmercury transformation can also be contributed abiotically, through chemical reactions.

### **3.2.2. Abiotic methylation**

Chemical methylation is possible only if suitable methyl donors are present (Celo *et al.*, 2006). The authors suggest that chemical reagents thought to cause abiotic methylmercury formation include small organic molecules such as methyl iodide ( $\text{CH}_3\text{I}$ ) and dimethylsulfide ( $(\text{CH}_3)_2\text{S}$ ), and larger organic components of dissolved organic matter such as fulvic and humic acids. In addition, transmethylation reactions involving organometallic complexes such as methylcobalamin, methyllead or methyltin compounds have also been considered as possible pathways for chemical methylation of mercury in the aquatic environment (Celo *et al.*, 2006). Reactions of methyltin compounds with inorganic mercury in natural waters find methylmercury as the product. Based on typical environmental concentrations of monomethyltin ( $\sim 1200$  ng Sn/L) and  $\text{Hg}^{2+}$  ( $\sim 1$  ng/L), and the methylation rate constant at pH 8 and  $20^\circ\text{C}$ , the authors estimate the half-life of mercury in seawater due to its reaction with monomethyltin to be 4.6 years, and a rate of methylmercury formation under these conditions of ca. 0.5 pg/L.day. Elemental mercury, instead of divalent mercury, is also proven to be methylated slowly by methyl iodide at room temperature in the study. The result shows that in the presence of 200 ng/L methyl iodide, methylmercury production can be as high as 0.2 pg/L.year (Celo *et al.*, 2006).

A study conducted at two lakes in southern Nova Scotia, Canada shows that methylmercury concentration often increase during sunlight hours (Siciliano *et al.*, 2005). The hypothesis is that there are water column processes that generate methylmercury and that these processes are linked to dissolved organic matter (DOM) and solar radiation. The formation of methylmercury is found to be dependent on the size fraction and amount of DOM present in the water. DOM has been hypothesized to play an important role in the fate of methylmercury in the water column due to the following reasons: (1) DOM is a strong sorbant of methylmercury as well as  $Hg^{2+}$  and thereby transports and sequesters mercury; (2) Solar radiation is attenuated by DOM thereby reduces the impact of solar radiation at depth in freshwater lakes; (3) Solar radiation and DOM react to produce oxidants in the water column and DOM can be activated to act as an oxidant; and (4) DOM contains reactive functional groups and associated counterions that may interact with  $Hg^{2+}$  in various ways including methylation. In addition, the authors also find that water from lakes with logged watersheds generates methylmercury when exposed to sunlight, whereas water from lakes with low levels of logging in the undisturbed watersheds does not. Logging of freshwater watersheds increases DOM concentrations in the associated lakewater, and also increases mercury loads in the freshwater biota, which subsequently translates to methylmercury production. However, water chemistry that contributes to this abiotic production of methylmercury is not yet clear.

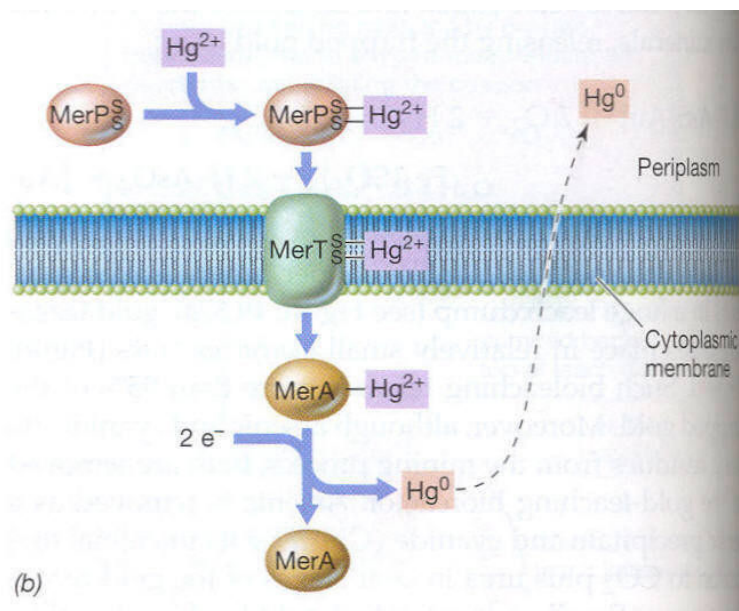
The larger fraction of methylmercury production in the aquatic systems is contributed by microbial community, specifically sulfate-reducing bacteria. According to Celo *et. al* (2006), two observations have confirmed that mercury methylation is biological: (1) Higher concentrations of methylmercury were found in sediments with

higher microbial activity; and (2) Methylation decreased when microbial activity was interrupted.

### 3.2.3. Biotic methylation

As mentioned above, SRB have been identified as the primary methylators in the environment. SRB are widespread in aquatic and terrestrial environments that become anoxic as a result of microbial decomposition processes (Madigan and Martinko, 2005). To be methylated by SRB or to enter the aquatic food chain via phytoplankton or bacteria, mercury must first be transported across the lipid membrane that surrounds unicellular organisms (Morel *et al.*, 1998). Microorganisms have developed a resistance system, an operon (*mer* operon), to toxic mercury compounds, which allows bacteria to detoxify  $\text{Hg}^{2+}$  into volatile metallic mercury by an inducible enzyme, mercuric reductase (Nascimento and Chartone-Souza, 2003). Mercuric reductase is a flavoprotein, which catalyzes the NADPH-dependent reduction of  $\text{Hg}^{2+}$  to  $\text{Hg}^0$ . Since mercury has a high vapor pressure, it volatilizes and the bacterial environment is left mercury free.

According to Nascimento and Chartone-Souza (2003), transport of mercuric ions (i.e.  $\text{HgCl}_2$ ) outside the cell is carried out by a series of transporter proteins, which involves the binding of  $\text{Hg}^{2+}$  by the MerP protein located in the periplasms.  $\text{Hg}^{2+}$  is then transferred to MerT, a cytoplasmic membrane protein, and finally to an active site of MerA (mercuric reductase). Next,  $\text{Hg}^{2+}$  is then released into the cytoplasm and volatilizes from the cell (Figure 10). It is not known whether all genus of SRB carry *mer* operon genes although not all genus of SRB are capable of methylating mercury.



**Figure 10.** Transport and reduction mechanism of  $\text{Hg}^{2+}$  (Figure is taken with permission from Madigan and Martinko (2005))

It is important to note that the potential of the *mer* system for the bioremediation of mercury has been recognized (Barkay and Wagner-Doebler, 2005), and may possibly contribute to demethylation process, a subject which will be covered in the subsequent chapter of this thesis.

### 3.2.3.1. Sulfate-reducing bacteria

Sulfate-reducing bacteria (SRB) are important regulators of a variety of processes in wetland soils, including organic matter turnover, biodegradation of chlorinated aromatic pollutants in anaerobic soils and sediments, and mercury methylation (Castro *et al.*, 2000). SRB occur naturally in surface waters, including sea waters. Generally they require a complete absence of oxygen and a highly reduced environment to function efficiently. SRB also play a primary role in the sulfur cycle in the aquatic systems by

serving as the primary means by which sulfate ( $\text{SO}_4^{2-}$ ) is reduced to sulfide ( $\text{S}^{2-}$ ) (King, 1999).

Castro *et al.* (2000) suggested that analysis of rRNA sequences has allowed organization of the various sulfate-reducing bacteria species into four distinct groups: Gram-negative mesophilic SRB; Gram-positive spore forming SRB; thermophilic bacterial SRB; and thermophilic archaeal SRB. The terms mesophilic and thermophilic are related to the optimum growth temperatures of each group. Mesophilic refers to growth that occurs at approximately 10°C to approx. 45°C with optimal growth occurs at 30-40°C. Thermophilic requires a warmer temperature to grow, at approximately 40°C to 75°C, with optimal growth occurring at 55-70°C. Thermophilic archaeal exhibits growth temperatures above 80°C (Castro *et al.*, 2000).

In addition to its growth temperature, each group of SRB has important characters that focus on its GC content (%) - the proportion of Guanine-Cytosine-base pairs in the DNA molecule, the presence of desulfovirdin and cytochromes, and its ability to oxidize acetate (Table 9). Depending on the species, organic substrates are oxidized incompletely to acetate or completely to  $\text{CO}_2$ . In other words, some species oxidize their electron donors to the level of acetate and excrete this fatty acid as an end product while the rest oxidize fatty acids (including acetate), lactate, and succinate completely to  $\text{CO}_2$  (Madigan and Martinko, 2005).

**Table 9**Important characteristics in the classification of representative sulfate-reducing bacteria<sup>a</sup>

	Shape	Motility	GC content (%) <sup>b</sup>	Desulfovirdin <sup>c</sup>	Cytochromes	Oxidation of acetate	Growth temp. (°C)
<b>Gram-negative mesophilic</b>							
Desulfobulbus	lemon to rod	-/+	59-60	-	b, c, c3	I <sup>d</sup>	25-40
Desulfomicrobium	ovoid to rod	+/-	52-67	-	b, c	I	25-40
Desulfomonas	rod	-	66	+	c	I	30-40
Desulfovibrio	spiral to vibrioid	+	49-66	+/-	c3, b, c	I	25-40
Desulfobacter	oval to rod	+/-	44-46	-		C <sup>e</sup>	20-33
Desulfobacterium	oval to rod	+/-	41-52	-	b, c	C	20-35
Desulfococcus	spherical to lemon	-/+	46-57	+/-	b, c	C	28-35
Desulfomonile	rod	-	49	+	c3	C	37
Desulfonema	filaments	gliding	35-42	+/-	b, c	C	28-32
Desulfosarcina	oval rods or coccoid, packages	+/-	51	-	b, c	C	33
<b>Gram-positive spore-forming</b>							
Desulfotomaculum	straight to curved rods	+	48-52	-	b, c	I/C	most 25-40, some 40-65
<b>Bacterial thermophilic</b>							
Thermodesulfobacterium	vibrioid to rod	-/+	30-38	-	c3, c	I	65-70
<b>Archaeal thermophilic</b>							
Archaeoglobus	coccoid	+/-	41-46	-	<sup>f</sup> n.r.c	I	64-92

<sup>a</sup> Table is adapted from Castro *et. al* (2000)<sup>b</sup> The proportion of guanine-cytosine (GC)-base pairs in the DNA molecule<sup>c</sup> The typical pigment of the genus *Desulfovibrio* (Biebl and Pfennig, 1977)<sup>d</sup> I - incomplete<sup>e</sup> C - complete<sup>f</sup> n.r. - not reported

### 3.2.3.1.1. Biochemical pathway

Sulfate-reducing bacteria couple the oxidation of organic compounds or molecular H<sub>2</sub> with the reduction of sulfate as an external electron acceptor under

anaerobic conditions, a process known as dissimilatory sulfate reduction (Singleton Jr., 1993; Tebo and Obraztsova, 1998). The biochemistry of sulfate-reducing bacteria is described by King (1999) with sulfate as a terminal electron acceptor under anaerobic conditions. The study focuses on marine sediments as the habitat of sulfate reducers.

The reduction of sulfate ( $\text{SO}_4^{2-}$ ) to sulfide ( $\text{S}^{2-}$ ) is an 8-electron reduction. Because the sulfate anion is a relatively stable anion, it must first be coupled to the “high energy” molecule of ATP prior to being reduced. An enzyme known as ATP sulfurylase catalyzes the reaction. In this process, two phosphates are released in the triphosphate molecule producing adenosine-5' phosphosulfate (APS). In dissimilative sulfate reduction, the addition of two electrons to the APS molecule results in the formation of sulfite ( $\text{SO}_3^{2-}$ ). When sulfite is formed, the subsequent 6-electron reduction of sulfite proceeds readily to sulfide (Madigan and Martinko, 2005). Sulfate-reducing bacteria reduce sulfate through a cytochrome-based electron transport process. The cytochrome molecules that compose the electron transport chain of sulfate reducing bacteria are ferredoxin, flavodoxin, and cytochrome  $\text{C}_3$ . Cytochrome  $\text{C}_3$  is a low energy cytochrome molecule that is unique to the organisms that utilize sulfate as a terminal electron acceptor (Madigan and Martinko, 2005). Species of sulfate reducers capable of reducing acetate and fatty acids also contain cytochrome b. For example, the oxidation of lactate to pyruvate is facilitated by the lactate dehydrogenase enzyme. This results in the release and transport of  $\text{H}_2$  across the plasma membrane. A hydrogenase enzyme located in the plasma membrane oxidizes the  $\text{H}_2$  to  $2\text{H}^+$  atoms. The two electrons (see Figure 10) are subsequently transported down the electron transport chain that terminates with APS. It should be noted that sulfite can also accept electrons through the same process (Madigan

and Martinko, 2005). The  $H^+$  ions located on the exterior of the plasma membrane can be utilized in the production of ATP energy molecules. Because of the concentration gradient and proton motive force which occurs due to the  $H^+$  difference on the plasma membrane,  $H^+$  ions can be funneled through an ATPase enzyme which utilizes the proton motive force to synthesize ATP (Madigan and Martinko, 2005). Growth yield studies with SRB have been utilized in order to determine the stoichiometry between ATP production and sulfate reduction. Studies suggest that three ATP molecules are produced per sulfate reduced to sulfide (Madigan and Martinko, 2005). However, the net ATP produced is only one molecule given that the conversion of sulfate to sulfite is associated with two high energy bond equivalents being consumed (King, 1999).

Sulfate reduction is a true respiration process that can accomplish net energy conservation.  $H_2$  is oxidized in a single step that does not allow substrate-level phosphorylation. Therefore, growth with  $H_2$  as the only electron donor must be coupled with chemiosmotic energy conservation during reduction of the electron acceptor (Cypionka, 1995). While the end product of aerobic respiration is water, sulfate reduction produces hydrogen sulfide ( $H_2S$ ), which is not only malodorous, but toxic, even for SRB at concentrations above 5 mM (Cypionka, 1995). Sulfate reduction also tends to change the pH since protons are consumed by a formation of  $H_2S$  from the sulfuric acid. Hydrogen sulfide is a weak acid only partially dissociated at neutral pH. Because the first dissociation constant ( $pK_1$ ) of  $H_2S$  is about 7.0,  $H_2S$  and  $HS^-$  are both present within the pH range where SRB are active. The second dissociation constant ( $pK_2$ ) of  $H_2S$  is around 17 to 19, meaning that free  $S^{2-}$  cannot exist in aqueous solutions because an  $S^{2-}$

ion, even in strongly alkaline solution, immediately deprotonizes water to form  $\text{OH}^-$  and  $\text{HS}^-$  (Cypionka, 1995).

SRB are not restricted to energy conservation by sulfate reduction. They are also capable of fermentative growth or utilization of other electron acceptors (Sorensen *et al.*, 1981; Devereux *et al.*, 1989; Berman *et al.*, 1990; Cypionka, 1995; Vainshtein, 1996; King, 1999). Some species are also capable to utilize ethanols and sugars as energy source, for example *Desulfotomaculum nigrificans* and a second thermophilic spore-forming species, *Dtm. geothermicum* are reported to oxidize fructose (Hansen, 1993) as energy substrates. The diversity of SRB in terms of their metabolic abilities translate to their habitats, which includes not only marine and freshwater sediments, but also rice paddies, freshwater lakes, and anaerobic digester (King, 1999). Lactate is one of the widely used substrates for cultivating SRB in biochemical studies (Sorensen *et al.*, 1981; Hansen, 1993).

Tebo and Obratzsova (1998) studied a spore-forming sulfate-reducing bacterium *Desulfotomaculum reducens* sp. nov. strain MI-1 that was isolated from heavy metal contaminated estuarine sediments. The result demonstrates that SRB can grow by coupling the oxidation of organic compounds (i.e. lactate) with the reduction of Cr(VI) to Cr(III), Mn(IV) to Mn(II), Fe(III) to Fe(II) or U(VI) to U(IV).

According to Reis *et al.* (1992), who conducts a study on SRB with inoculum isolated from an anaerobic digester, only a small amount of the hydrogen sulfide formed by dissimilatory sulfate reduction is assimilated by organisms for cell synthesis, as these bacteria have relatively low cell yields; thus almost all the hydrogen sulfide formed is

released into the environment. The culture in this study belongs to the genera *Desulfovibrio* and it is not able to grow on lactate in the absence of sulfate or on acetate in the presence of sulfate. The study demonstrates that hydrogen sulfide has been found to inhibit SRB. The inhibition may be the result of an intrinsic toxicity of H<sub>2</sub>S to living systems or it may be due to indirect toxicity by rendering the iron insoluble as FeS. Iron is needed for cell constituents such as ferredoxin and cytochrome C. Complete inhibition of SRB is observed for an H<sub>2</sub>S concentration of 547 mg/L (16.1 mM). The highest growth rate of the SRB culture used in this experiment was observed at pH 6.7. The author also concluded that as the pH increases, there is a shift in the relative importance of the inhibitory effects on growth due to hydrogen sulfide and acetic acid. At low pH, acetic acid is found to be more inhibitory but at higher pH hydrogen sulfide prevails as the inhibitor of concern. Therefore, fermentations carried out at near neutral pH will be mainly affected by the hydrogen sulfide produced and to a lesser extent by acetic acid concentration.

Recent study demonstrates iron-reducing bacteria as mercury methylators in riverine sediments which contain dissolved iron (Fleming *et al.*, 2006). The sediments continue to produce methylmercury even in the presence of molybdate concentrations sufficient to fully inhibit sulfate reduction. Molybdate acts as a competitive inhibitor for sulfate in the “sulfate activation” step of dissimilatory sulfate reduction, which is catalyzed by the enzyme ATP sulfurylase. The resultant molecule of adenosine-5'-phosphomolybdate, the synthesis of which consumes an ATP molecule, is unstable, and repeated production and breakdown of this intermediate can lead to a targeted “energy uncoupling” of sulfate-reducing bacteria (Wilson and Bandurski, 1958; Fleming *et al.*,

2006). The mechanism of methylation by iron-reducing bacteria is facilitated by sediments bioturbation which makes mercury available at sediment depths of 10 to 30 cm, while in neutral-pH freshwater sediments, sulfate reduction is confined to the upper 5 to 10 cm. The study suggests that ferric oxyhydroxides (FeOOH) are at least as effective as soluble ferric iron at promoting methylation in pure cultures. Iron-reducing bacteria directly reduce the mineral surface and may give direct access to sorbed mercury for methylation independent of the requirement for soluble mercury (Wilson and Bandurski, 1958; Fleming *et al.*, 2006).

#### **3.2.3.1.2. Methylation by SRB**

An earlier work on mercury methylation by (Choi *et al.*, 1994) confirmed that the process by the sulfate reducers is catalyzed by enzyme. The study proved that mercury methylation is tied exclusively to the activity of sulfate reducers. The authors added that should mercury methylation be nonenzymatic, homoacetogens or methanogens might be methylators of mercury as well because they often contained cobalamin in concentrations several orders of magnitude higher than reported for sulfate reducers (Choi *et al.*, 1994). In this pathway, the methyl group originated either from C-3 serine or formate via the acetylcoenzyme A (acetyl-CoA) pathway, implying involvement of carbon monoxide dehydrogenase enzyme by a transfer of a methyl group from CH<sub>3</sub>-tetrahydrofolate to a corrinoid-containing protein, followed by enzymatic methylation in crude cell extracts (Choi *et al.*, 1994; Choi *et al.*, 1994; Barkay and Wagner-Dobler, 2005). The optimal growth conditions were pH 6.5 and temperature 35°C and that the activity decreased in the presence of air (Chemaly, 2002), which shows that methylation occurs predominantly

under anaerobic conditions, therefore wetlands, aquatic sediments, and temporarily saturated soils are the main sites of production in watersheds (Krabbenhoft *et al.*, 2005).

Another pathway of mercury methylation is through the acetate metabolic pathway using methyltransferase enzymes (King *et al.*, 2000). The study focused on marine sediments, utilizing pure cultures representing five genera of the SRB (*Desulfobulbus propionicus*, *Desulfovibrio desulfuricans*, *Desulfococcus multivorans*, *Desulfobacter* sp. strain BG-8, and *Desulfobacterium* sp. strain BG-33) that were grown in a strictly anoxic, minimal medium that received a dose of inorganic mercury 120 h after inoculation. The findings showed that the mercury methylation rates normalized per cell were up to 3 orders of magnitude higher in pure cultures of members of SRB groups capable of acetate utilization than in pure cultures of members of groups that are not able to use acetate. SRB utilize acetate by completely oxidizing the acetyl group of the acetyl coenzyme A to CO<sub>2</sub> by two mechanisms. *Desulfobacter* strains employ the citric acid cycle, while *Desulfobacterium* strains utilize the carbon monoxide dehydrogenase pathway. According to King *et al.* (2000), since the carbon monoxide dehydrogenase pathway includes several tetrahydrofolate enzymes, induction of these enzymes may be responsible for rapid mercury methylation rates observed in *Desulfobacterium* pure cultures. In addition, little or no mercury methylation was observed in cultures of *Desulfobacterium* or *Desulfovibrio* strains in the absence of sulfate, indicating that mercury methylation was coupled to respiration in these strains.

Siciliano and Lean (2002) proposed methyltransferase pathway for mercury methylation. Methyltransferase activity can happen by a cobalamin-dependent or -

independent process. Cobalamin-dependent methyltransferase requires *S*-adenosylmethionine (SAM), one of three major biological methylating agents, and vice versa. The study simulated mercury methylation in wetland sediments with addition of four different amounts (0, 382, 1,910, and 3,820 µg) of crude enzyme extract from a cobalamin-dependent methionine synthase producer, *Escherichia coli* XL1-Blue/pKF5A. The result demonstrates that the enzyme responsible for methylating mercury also transfers methyl groups from methyltetrahydrofolate to thiols such as homocysteine, supporting the hypothesis that mercury methylation can be considered a mistaken methylation of homocysteine.

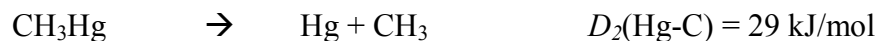
Studies have shown that in pure cultures SRB grown in the absence of sulfate do not generate  $\text{CH}_3\text{Hg}^+$  from available inorganic mercury (Pak and Bartha, 1998). However these studies only utilized primarily one SRB, *Desulfovibrio Desulfuricans*, to determine the mercury methylation potential of the entire SRB population (King *et al.*, 2000). To date, this group of bacteria has been identified to carry out methylation: *Clostridium butyricum*, *Desulfobulbus propionicus*, *Desulfovibrio desulfuricans*, *Desulfococcus multivorans*, *Desulfobacter* sp., *Desulfobacterium* sp. (King *et al.*, 2000; Benoit, Gilmour *et al.*, 2001).

Studies on SRB continue to show the diversity of these bacteria, not only in their biochemical pathway but their ability to survive in different environments by utilizing a variety of substrates. As mercury compounds are widely distributed, understanding the role of SRB in mercury methylation will play a key role in assessment of mercury cycle as well as demethylation process in the environment.

## CHAPTER 4

### DEMETHYLATION OF MERCURY

Methylmercury ( $\text{CH}_3\text{Hg}^+$ ), dimethylmercury ( $(\text{CH}_3)_2\text{Hg}$ ), and their complexes such as  $\text{CH}_3\text{HgCl}$ ,  $\text{CH}_3\text{HgOH}$ , to name a few, are organometallic molecules formed in aqueous solutions. They are very stable in the environment and based on their enthalpies of formation ( $\Delta H_f^0$ ) shown below, their degradation is an endothermic reaction involving the breaking of the metal-carbon bond (Craig *et al.*, 2003):



where  $D_1$  and  $D_2$  denote stepwise dissociation energies.

Like methylation, demethylation of mercury can also occur through biotic and abiotic process. Biotic process involves decomposition of methylmercury performed by bacteria while abiotic process occurs kinetically when environmental conditions are favorable.

#### 4.1. Biotic demethylation process

The decomposition of methylmercury performed by bacteria has been identified as having two different pathways: 1) reductive, and 2) oxidative demethylation (Barkay and Wagner-Doebler, 2005). Reductive demethylation (RD) is performed by mercury resistance bacteria, which is conferred by the *mer* operon genes. The degradation pathway reduces methylmercury to volatile elemental mercury ( $\text{Hg}^0$ ) and methane ( $\text{CH}_4$ ). Oxidative demethylation (OD) results in the production of  $\text{Hg}^{2+}$  and  $\text{CO}_2$ , depending on

the type of bacteria that carry out the process. Demethylation studies have identified two factors that affect the choice between the reductive and oxidative pathways - redox and levels of mercury concentration (Barkay and Wagner-Doebler, 2005). According to the authors, reductive demethylation is favored at high mercury concentrations and oxic conditions, whereas oxidative demethylation dominates at low mercury concentrations and anoxic conditions (Table 10). Oxidative demethylation pathway will result in  $Hg^{2+}$  which, given the right environmental conditions, can be subsequently methylated again.

Table 10 - Effect of redox and mercury concentration on methylmercury degradation pathway<sup>a</sup>

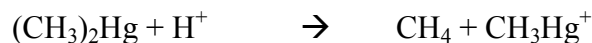
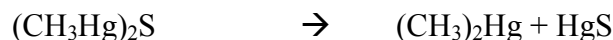
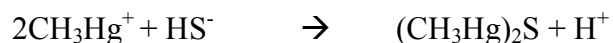
	Redox	Hg <sub>T</sub> ng/g sediment <sup>b</sup>	% CH <sub>4</sub> end-product	Methylmercury degraded by
Everglades (FL)	Oxic	70-320	0-25%	OD
	Anoxic	70-320	31-52%	OD
San Fransisco Bay salt marsh (CA)	Oxic	~100-1300	100%	RD
	Anoxic	~100-1300	25%	OD
San Carlos Creek (CA)	Oxic	4,500-21,000	>99%	RD
	Anoxic	4,500-21,000	>99%	RD
Almadén/calclines	Oxic	160-34,000	100%	RD
Almadén/sediments	Anoxic	3-2,300	7.5-22%	OD
Almadén/soils	Oxic	6-8,889	100%	RD
Northern Adriatic Sea	Anoxic	1-32	<10%	OD

<sup>a</sup>Table is adapted from Barkay and Wagner-Doebler (2005)

<sup>b</sup>Concentration of total Hg per g sediment (dry weight)

The above table, however, does not show the percentage of  $\text{Hg}^{2+}$  presents in total mercury concentrations, which subsequently will translate to the amount of methylmercury concentrations that are degraded through each pathway.

Another methylmercury degradation pathway has been studied and demonstrated for sulfate-reducing bacteria *Desulfovibrio desulfuricans*, which is not mediated by the *mer* genes (Baldi *et al.*, 1993; Marvin-DiPasquale *et al.*, 2000; Mason and Benoit, 2003). The result of the study (Baldi *et al.*, 1993; Marvin-DiPasquale *et al.*, 2000; Mason and Benoit, 2003) demonstrates that methylmercury is less toxic for sulfate-reducing bacteria than for aerobic broad-spectrum mercury-resistant *Pseudomonas putida* FB-1, which is known to degrade methylmercury, ethylmercury, and phenylmercury by means of organomercurial lyase. The methylmercury resistance in *D. desulfuricans* is due to the transformation of methylmercury to insoluble dimethylmercury sulfide, which reacts with  $\text{H}_2\text{S}$  from the dissimilative reduction of sulfate to produce dimethylmercury, mercury, and metacinnabar as well as low concentration of methane ( $< 0.4\mu\text{g/mL}$ ) (Baldi *et al.*, 1993). The proposed biological and chemical reactions of this degradation pathway are as follows:



Methylmercury is formed back at the end of the pathway shown above, however the bacteria in this study are able to keep methylmercury concentration at low level ( $0.9\mu\text{g/mg}$  dry weight) by continuous production of  $\text{H}_2\text{S}$  and then by rapid precipitation out of the cells as dimethylmercury sulfides, which later decomposes further to other

products (Baldi *et al.*, 1993). Although it was argued that the experiment was carried out at conditions (i.e. high sulfide and methylmercury concentrations) that would thermodynamically favor the abiotic formation of dimethylmercury (Mason and Benoit, 2003).

#### **4.1.1. Reductive Demethylation**

Early work on demethylation demonstrates bacterial degradation of methylmercury in lake sediments (Spangler *et al.*, 1973). As mentioned in methylation chapter, methylation process occurs in parallel with demethylation, which results in the difficulty to find trace amounts of methylmercury in sediments or in the aquatic environment. The study was carried out on the biomethylation of  $\text{Hg}^{2+}$  in sediments taken from the delta area of the St. Clair River, Michigan (Spangler *et al.*, 1973). It offered evidence that the failure to find methylmercury in sediments is due, at least in part, to the presence of bacteria capable of degrading methylmercury. The result demonstrates that as the amount of methylmercury shows a rapid decrease, there is a concomitant rapid increase in the amount of  $\text{Hg}^0$  volatilized to the trap that was attached to the flask to collect any volatile mercury species in the effluent flushing gas. The study also found that there were no organomercurials found in the trap contents, indicating that the methylmercury produced has been degraded and that the volatile species was trapped as mercury vapor (Spangler *et al.*, 1973). There was a possibility that dimethylmercury ( $(\text{CH}_3)_2\text{Hg}$ ) was formed from methylmercury and was volatilized to the trap. In addition, this reductive demethylation reaction also resulted in the formation of methane ( $\text{CH}_4$ ). The demethylation process in this study was carried out by facultative organisms that

readily carried out the degradation under both aerobic and anaerobic conditions. This biotic demethylation occurs through the *mer* pathway, which involves mercury resistance in bacteria carrying genes of *mer* operon (Oremland *et al.*, 1991; Marvin-DiPasquale and Oremland, 1998; Marvin-DiPasquale *et al.*, 2000; Barkay and Wagner-Dobler, 2005).

#### 4.1.2. *mer* Operon

The presence of *mer* operon has been found for both gram negative and gram positive bacteria and under aerobic and anaerobic conditions (Foster, 1987; Marvin-DiPasquale *et al.*, 2000). This clustered genes allows bacteria to detoxify  $\text{Hg}^{2+}$  into volatile metallic mercury ( $\text{Hg}^0$ ) by enzymatic reduction (Summers, 1986; Nascimento and Chartone-Souza, 2003). The enzyme that catalyzes the reduction of  $\text{Hg}^{2+}$  to  $\text{Hg}^0$  is the intracellular, FAD-containing mercuric reductase (Foster, 1987). There are two main *mer* determinant types: narrow-spectrum *mer* determinants confer resistance to inorganic mercury salts only, whereas broad-spectrum *mer* determinants confer resistance to organomercurials such as methylmercury and phenylmercury, as well as to inorganic mercury salts. The broad-spectrum *mer* determinant carries *merB* genes, in addition to genes that encode the functional proteins for regulation (*merR*), transport (*merT*, *merP* and/or *merC*, *merF*) and reduction (*merA*).

The enzyme mercuric reductase is a flavoprotein, protein containing a derivative of riboflavin which functions as electron carrier in the electron transport system (Madigan and Martinko, 2005). Mercuric reductase is found intracellularly and catalyzes the NADPH-dependent reduction of  $\text{Hg}^{2+}$  to  $\text{Hg}^0$  (Nascimento and Chartone-Souza, 2003). The *merB* gene, exists only in broad-spectrum resistance, encodes for the

organomercurial-lyase enzyme, which cleaves methylmercury, forming methane (CH<sub>4</sub>) and Hg<sup>2+</sup> as end products. The associated *merA* gene, common in both resistance types, produces the enzyme mercuric reductase, which further reduces Hg<sup>2+</sup> to volatile elemental mercury (Hg<sup>0</sup>) (Summers, 1986; Foster, 1987; Marvin-DiPasquale *et al.*, 2000; Nascimento and Chartone-Souza, 2003).

The mechanism of mercury resistance is described by Nascimento and Chartone-Souza (2003), who proposed that Hg<sup>2+</sup> diffuses across the outer membrane. Mercuric ions are transported outside the cell by a series of transporter proteins. This mechanism involves the binding of Hg<sup>2+</sup> by a pair of cysteine residue on the *merP* protein located in the periplasm. Hg<sup>2+</sup> is then transferred to a pair of cysteine residues on *merT*, a cytoplasmic membrane protein, and finally to a cysteine pair at the active site of *merA*. Hg<sup>2+</sup> is then reduced to Hg<sup>0</sup> in an NADPH-dependent reaction. Finally, Hg<sup>0</sup> is then released into the cytoplasm and volatilizes from the cell. Figure 10 illustrates this process.

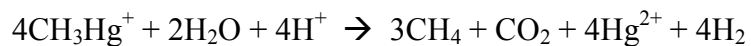
#### **4.1.3. Oxidative demethylation**

Oxidative demethylation is the product of biochemical pathways, most likely those of carbon-one (C1) metabolism of both methanogens and sulfate-reducing bacteria (Oremland *et al.*, 1991; Barkay and Wagner-Doebler, 2005). Reductive demethylation is commonly assumed to account for methylmercury demethylation, however CO<sub>2</sub> has been observed as the major methylmercury demethylation product in anoxic freshwater lake sediments (Oremland *et al.*, 1991; Barkay and Wagner-Doebler, 2005). The study on oxidative demethylation pathway is carried out in estuarine salt marsh sediment with

addition of inhibitors or substrates that affect sulfate reduction or methanogenesis. The result demonstrates that demethylation is more rapid and extensive under aerobic than anaerobic conditions. The product of aerobic demethylation is  $\text{CH}_4$  while anaerobic demethylation product is predominantly  $\text{CO}_2$  (Oremland *et al.*, 1991; Barkay and Wagner-Doebler, 2005). In addition, molybdate, an inhibitor of dissimilatory sulfate-reducing bacteria, stimulates  $\text{CH}_4$  production, but prevents the formation of  $\text{CO}_2$  and  $\text{CH}_4$  under anaerobic conditions while having no effect on  $\text{CH}_4$  under aerobic conditions. The authors also suggest that methanogens are not involved in demethylation in estuarine sediments. The study (Oremland *et al.*, 1991) also carried out experiments in freshwater sediments. The result demonstrates that aerobic demethylation produces both  $\text{CO}_2$  and  $\text{CH}_4$ . The addition of molybdate inhibits  $\text{CO}_2$  production but enhances production of  $\text{CH}_4$ . It is concluded that sulfate reducers are important methylators in both estuarine and freshwater sediments. Both methanogens and sulfate reducers compete for electron donors in both environments, however only in freshwater sediments do they compete in demethylating methylmercury (Oremland *et al.*, 1991).

Methylmercury degradation was also investigated along an eutrophication gradient in the Florida Everglades (Marvin-DiPasquale and Oremland, 1998). The purpose of this study was to determine the OD pathway in anaerobic sediments. The results demonstrate that the additions of phosphate ( $\text{PO}_4^{3-}$ ) and nitrate ( $\text{NO}_3^-$ ) have no effect on methylmercury degradation, indicating that the bacteria involved in demethylation were not phosphate limited neither they were nitrate respiring bacteria. However, the authors (Marvin-DiPasquale and Oremland, 1998) found that in environments where  $\text{NO}_3^-$  concentrations are high, it may exert a secondary influence on methylmercury

degradation by inhibiting methanogens and possibly sulfate reducers. As a result, the following reaction pathway has been proposed:



The study assumes that both methanogens and SRB can simultaneously degrade methylmercury through OD although SRB will outcompete methanogens for acetate and H<sub>2</sub>. Since Hg<sup>2+</sup> is the end product of OD, it reiterates the fact that methylation and demethylation occurs in parallel, which may effectively maintain a methylmercury pool that is continually available for bioaccumulation.

#### **4.2. Abiotic demethylation**

Abiotic demethylation includes the chemical transformation of various methylmercury species. Based on the thermodynamic stability of methylmercury species, CH<sub>3</sub>HgOH is the preferred species in freshwater environment. In estuarine environments CH<sub>3</sub>HgCl is preferred (Stumm and Morgan, 1996). However, at low pH, decomposition of mercury and methyl group is thermodynamically favored in both environments, and dimethylmercury will always be dissociated below pH 7.5.

Abiotic process also includes photodegradation of methylmercury in lake surface waters (Sellers *et al.*, 1996; Mason and Benoit, 2003). This study (Sellers *et al.*, 1996) found that methylmercury concentration decreased in the sunlight but not in the dark. The kinetics of methylmercury degradation were first order with respect to methylmercury concentration and light intensity. *In situ* incubations also showed that methylmercury photodegradation rates decreased with depth below the lake surface, and corresponded to the exponential decrease in light intensity with depth (Sellers *et al.*,

1996). The conditions of photodegradation were most favorable for oligotrophic freshwater with low pH and low DOC (Mason and Benoit, 2003).

Methylmercury degradation mechanisms are as complex as its methylation process because it is necessary to fully understand mercury biogeochemistry and to accurately measure bioavailability of  $\text{Hg}^{2+}$ , the mercury species that can be methylated given the right environmental conditions. According to Barkay and Wagner-Dobler (2005), there are two important matters lacking: 1) true rates of microbial methylation in the environments, and 2) how environmental factors affect methylation and  $\text{Hg}^{2+}$  reduction - information that is critical for mercury or methylmercury remediation in the environment.

With a lack of understanding of methylmercury degradation, monitoring of mercury to identify the sources and to quantify the input to the environment becomes a difficult task. However, several monitoring programs on different environment medium have already been in place.

## CHAPTER 5

### MONITORING MERCURY

Mercury monitoring is difficult due to the wide transport and distribution of mercury through all environmental mediums. In order to quantify the amount of mercury in the environment, samples must be taken from combustion sources, the aquatic environment, and the background environment.

#### 5.1. Emission Monitoring

According to EPA estimates, approximately 75 tons of mercury are found in the coal delivered to power plants each year and about two thirds of this mercury is emitted to the air, resulting in about 50 tons being emitted annually. Three different species of mercury are released from power plants: reactive gaseous mercury (RGM) or  $\text{Hg}^{2+}$ , elemental mercury ( $\text{Hg}^0$ ), and particulate mercury (TPM). The fractions of each species depend heavily on conditions of combustion, control technologies, and the type of coal being burned.

When the type of coal being burned is bituminous, approximately two-thirds of the mercury released is RGM, one-third  $\text{Hg}^0$ , and a small fraction of TPM (Edgerton, 2004). When the fuel is subbituminous or lignite coal, the primary form of mercury released is  $\text{Hg}^0$  (EPA 2004). Another factor that can greatly affect the speciation of mercury emissions is the chlorine content of the coal. When there is less than 700 micrograms of chlorine per gram of coal, the mercury will be primarily  $\text{Hg}^0$  with RGM and TPM at about 8%. When the chlorine content of coal is greater than 700 micrograms

chlorine per gram of coal, the amount of RGM and TPM increases to 50% (Lauda *et al.*, 2003).

Studies have shown that mercury speciation can change after it is released from the stack. When a plume reaches a rural testing site anywhere from 25 to 125 kilometers away, approximately 86% of the mercury is in the elemental form and only 11% is in the reactive form. Since a power plant burning bituminous coal releases mercury primarily in the form of RGM, the ending concentration of 11% lends to the conclusion that the reactive mercury is reduced to elemental mercury while in the plume (Edgerton, 2004). Other testing sites in urban and suburban areas also show a large decrease in the fraction of reactive mercury and an increase in elemental mercury. The mechanism for the reduction of the reactive mercury to elemental mercury is still unknown (Edgerton, 2004).

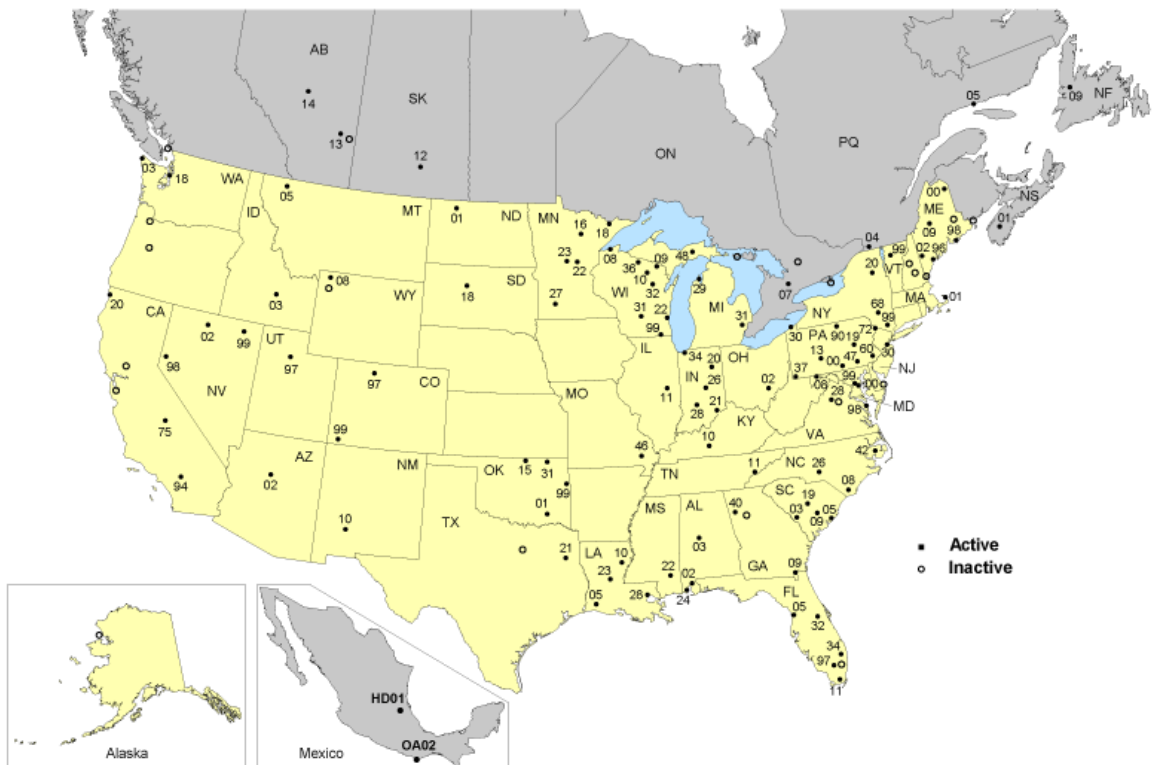
Emissions monitoring quantifies different species of mercury through methods such as stack testing and Continuous Emissions Monitoring System (CEMS). Stack tests consist of taking quantitative air samples from exhaust stacks and analyzing these samples in a laboratory to determine pollutant concentrations (i.e. mercury). The mercury emission rate established by a source test must be less than the allowable rate specified in the facility's permit to operate. Testing serves a two-fold purpose: to determine compliance with emission rates listed in permits and to establish permit terms and conditions; to set operating parameters for the source and air pollution control equipment. According to EPA websites, CEMS is the total equipment necessary for the determination of a gas or particulate matter concentration or emission rate using pollutant analyzer measurements and a conversion equation, graph, or computer program to

produce results in units of the applicable emission limitation or standard. CEMS are required under some of the EPA regulations for either continual compliance determinations or determination of exceedances of the standards. The individual subparts of the EPA rules specify the reference methods that are used to substantiate the accuracy and precision of the CEMS. Performance Specifications are used for evaluating the acceptability of the CEMS at the time of or soon after installation and whenever specified in the regulations. As part of Clean Air Mercury Rule, stack testing and CEMS are part of monitoring options that power plants can adopt to meet standard compliance set forth in the regulations.

## **5.2. Mercury Deposition Network (MDN)**

The Mercury Deposition Network is a nationwide network for mercury sampling in precipitation. It is part of National Atmospheric Deposition Program (NADP), which was organized in 1977 by the U.S. State Agricultural Experiment Stations. The NADP is a cooperative monitoring program comprised of federal and state agencies, academic institutions, Native American tribal governments, and private organizations. The MDN began measuring total mercury in precipitation in 1996 and now has more than 88 sites (Figure 11).

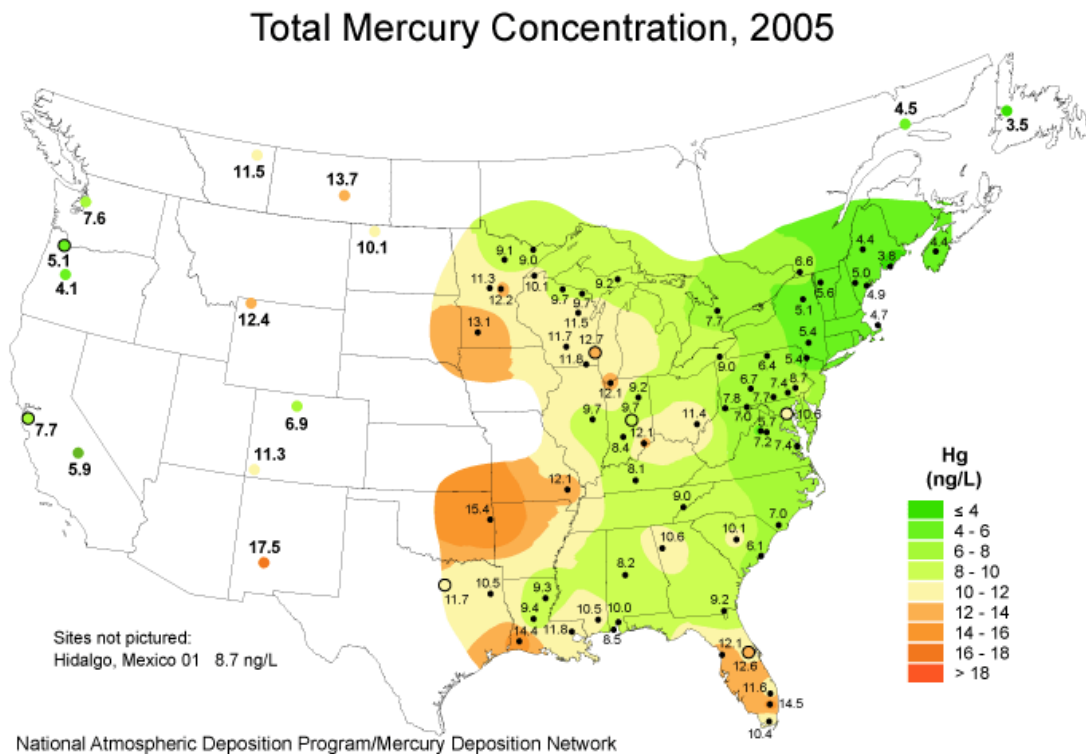
## National Atmospheric Deposition Program Mercury Deposition Network



**Figure 11.** The MDN map showing all the 88 participating sites where weekly samples are collected and analyzed. The numbers on the map are the site ID number assigned by MDN. Picture is taken from MDN website (<http://nadp.sws.uiuc.edu/mdn/>).

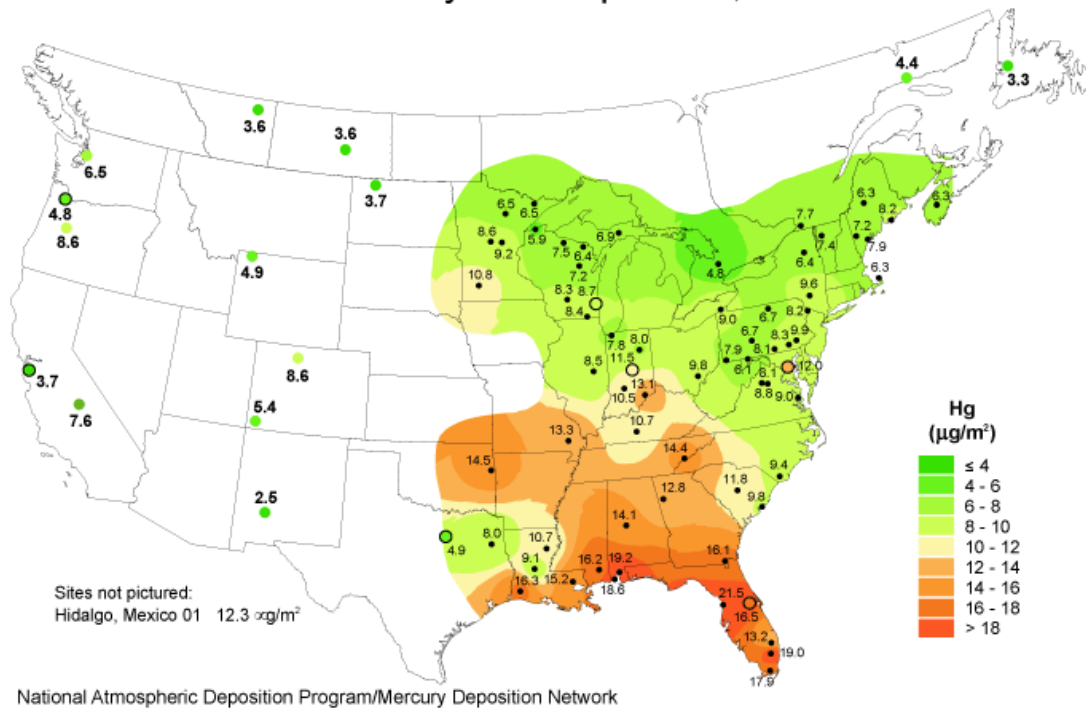
The MDN is also a cooperative effort among several groups, including state agricultural experiment stations, the U.S. Geological Survey, and the U.S. Department of Agriculture. It collects data to compare regions and looks for trends in deposition and correlations between deposition and emissions. Currently, in Georgia, there are two network sites, one in Okefenokee National Wildlife Refuge with U.S. Fish and Wildlife Service as its operating agency and the other one in Yorkville with Atmospheric Research and Analysis Inc., an air quality consulting firm, as its operating agency.

The MDN maps portray spatial variability in the concentration (Figure 12) and estimated deposition of mercury (Figure 13). The concentrations are precipitation-weighted averages. According to the MDN brochure posted on its website, estimates suggest mercury wet deposition accounts for 50-90 percent of the mercury load to many inland U.S., water bodies and estuaries. Currently the estimated cost for individual sites to participate in the network is about \$11,000 which covers equipment costs for year 1 only. Composite of weekly methylmercury analysis are available for an added charge.



**Figure 12.** The spatial map for total mercury concentration for 2005. Black dots marked site locations. The numbers represent the annual concentration for each site. Picture is taken from MDN website (<http://nadp.sws.uiuc.edu/mdn/>).

## Total Mercury Wet Deposition, 2005



**Figure 13.** The 2005 MDN map for mercury wet deposition. Notice how the orange color concentrates along or on the Georgia coast. Picture is taken from MDN website (<http://nadp.sws.uiuc.edu/mdn/>).

Concentration and deposition data resulting from the individual analysis results of the weekly and event samples from all MDN sites are readily accessible to the mercury depositions research community and the general public at the MDN site. The MDN is a long-term monitoring program. Data acquired through uninterrupted long-term operation of this network will enable the examination of local and regional scale problems and the evaluation of control efficacy. MDN data can be utilized to establish and verify relationships between emissions and effects to sensitive receptors of mercury contamination although this is yet to be accomplished for Georgia because both sites are not maintained and operated by the same agency.

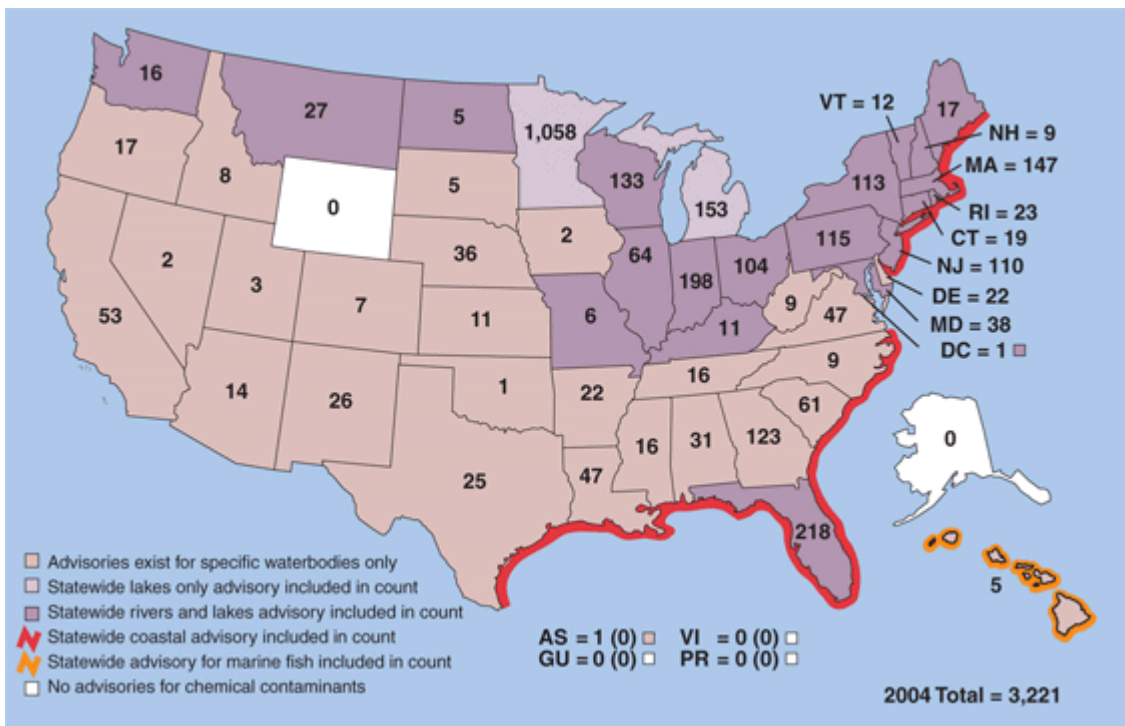
### **5.3. NATIONAL LISTING OF FISH ADVISORIES**

The National Listing of Fish Advisories consist a listing of information on locally issued fish advisories and safe eating guidelines, which is accessible to the public through EPA website (<http://www.epa.gov/waterscience/fish>). The fish consumption advisories and safe eating guidelines are meant to inform people about the recommended level of consumption for fish caught in local waters. EPA provides an annual summary of fish advisory information submitted by states because the states have developed their own fish advisory programs over the years, and as a result there is variability among states in the scope and extent of monitoring, in how frequently previously tested waters are samples, in how decisions are made to place water under advisory, and in the specific advice that is provided when contamination is found in fish. The website includes:

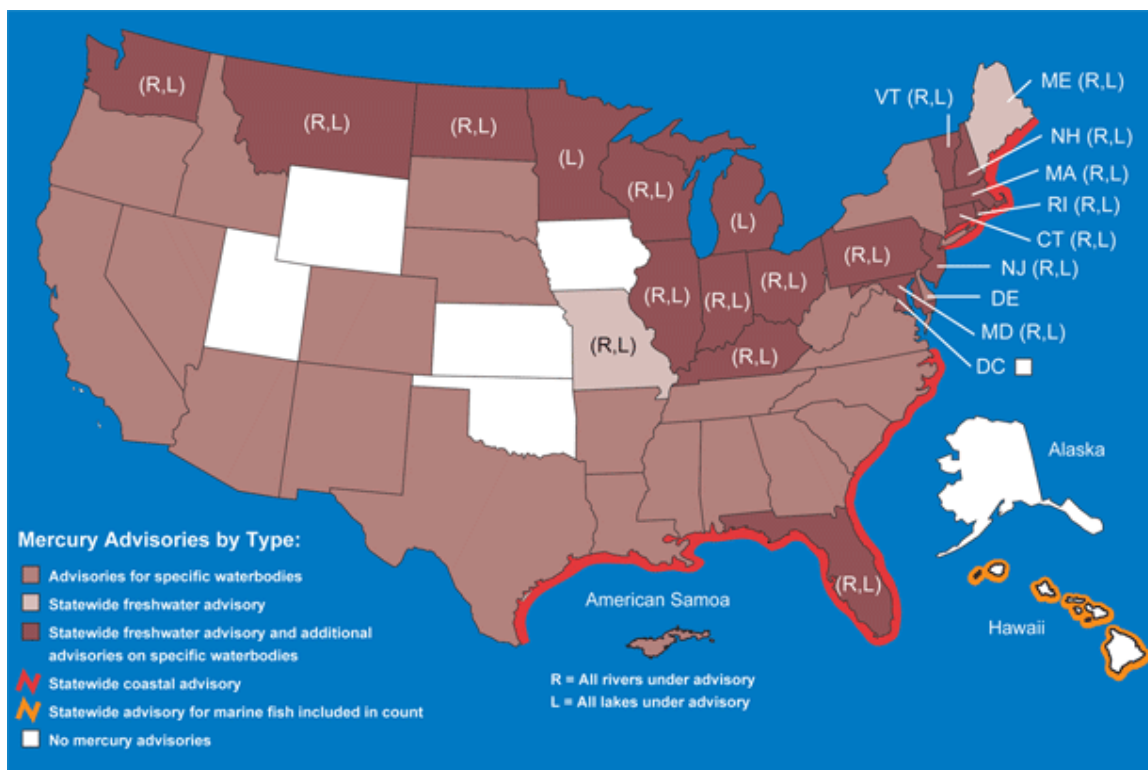
- Information on species and size of fish or water-dependent wildlife under advisory
- Chemical contaminants identified in the advisory
- Geographic location of the waterbody
- Lake acreage or river miles under advisory
- Population for whom the advisory was issued
- Meal size and meal frequency (number of meals per week or month) by advisory
- Data on the concentrations of contaminants in fish tissue for 48 states and the District of Columbia
- States and tribal contact information

Currently, the advisories in the national listing (Figures 14 and 15) represent 35% of the nation's total lake acreage and 24% of the nation's total river miles. Almost 65% of the

coastline of the United States (excluding Alaska, which has no advisories) is also under advisory. Based on coastal size estimates from the National Oceanic and Atmospheric Administration, 92% of the Atlantic coast and 100% of the Gulf coast were under advisory in 2004. The Atlantic coast advisories have been issued for a wide variety of chemical contaminants, including mercury. All of the Gulf coast advisories have been issued for mercury. Hawaii has a statewide advisory in effect for mercury in several marine fish species.



**Figure 14.** Total Number of Fish Consumption Advisories - 2004 (figure is taken from EPA website). Notice the red line along the coast which represents statewide coastal advisory. Picture is taken from MDN website (<http://nadp.sws.uiuc.edu/mdn/>).



**Figure 15.** Mercury Advisories for 2004 as issued and posted on EPA website. The red line marks the coastal area. Picture is taken from MDN website (<http://nadp.sws.uiuc.edu/mdn/>).

Currently, Georgia has fish consumption advisories in effect for four different areas of the state for three chemicals. The three chemicals which have been detected in sufficient quantity to trigger advisories are chlordane, PCBs, and mercury. The Georgia Department of Natural Resources (DNR) is responsible of issuing guidelines for fish eating safety. According to its 2007 update of Fish Eating Guidelines, the DNR has evaluated fish tissues since 1970's. The program was significantly expanded in 1990's to support development of risk-based consumption guidelines. The DNR placed high priority for testing on the 26 major reservoirs that make up more than 90% of the total lake acreage, a list of which is included in the guideline. In addition, sampling of fish in rivers and streams downstream of urban and/or industrial areas has been made a high

priority as well. The DNR also focuses attention on areas frequented by a large number of anglers.

The Food and Drugs Administration (FDA) also issues fish consumption advisories based on its action levels or tolerances, data of which is accessible through their website. In addition, signs are posted along waterways to advise the community of fish consumption from that particular waterway (Figure 16).

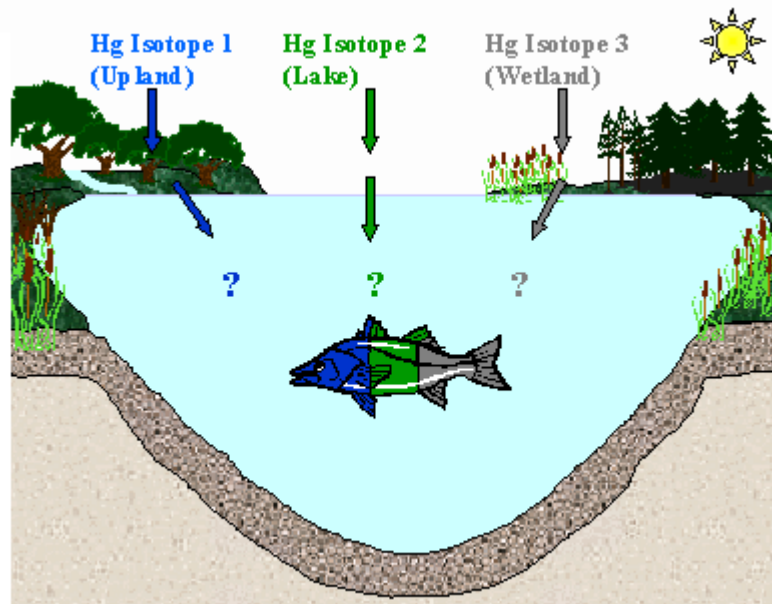


**Figure 16.** Samples of fish consumption advisories that are posted in public places in some states. Pictures are taken from Google (<http://www.google.com/>).

#### 5.4. METAALICUS

Mercury Experiment to Assess Atmospheric Loading in Canada and the United States (METAALICUS) project is a whole-watershed mercury loading experiment being carried out in the Experimental Lakes Area (ELA) in northwestern Ontario (SERC, 2001; EPRI, 2006). The studies began in 2000 and are being carried out in two phases. Phase 1, now complete, involved pilot studies and collection of background information at the site. Phase 2, the artificial loading of mercury isotopes to the whole ecosystem, began in 2001.

The project studies the relationship between atmospheric mercury deposition and bioaccumulation of mercury in food webs, at a watershed scale. A multidisciplinary team of about 50 researchers from the U.S. Canada is involved in the project. The project utilizes two techniques: one, the use of stable mercury isotopes, and two, the manipulation of a whole watershed. By using the stable mercury isotopes, researchers are able to follow the newly deposited mercury separately from background mercury, through time, and across various habitats. Different isotopes were applied to the upland ( $^{200}\text{Hg}$ ), wetland ( $^{198}\text{Hg}$ ), and lake surface ( $^{202}\text{Hg}$ ) to determine how the route of entry of mercury to an ecosystem affects the amount that becomes accumulated in fish (Figure 17). The mercury were applied at intervals to mimic natural seasonal deposition patterns.



**Figure 17.** Different stable mercury isotopes are used to evaluate the contributions of direct deposition, upland runoff and wetland outflow to fish mercury levels (picture is taken from EPRI website (<http://www.epri.com/>)).

The Experimental Lakes Area is remote from human activities and therefore receives relatively low rates of atmospheric mercury deposition, about  $4\mu\text{g}/\text{m}^2\cdot\text{yr}$ . The project adds another  $22\mu\text{g}/\text{m}^2\cdot\text{yr}$ . The study is designed to simulate deposition rates in contaminated areas. Production of methylmercury is being studied in the lake sediments, upland, and wetland, as is the bioaccumulation of methylmercury into benthic organisms, plankton, and fish.

The results from the first year study showed that the newly deposited mercury was more reactive than the native mercury, with respect to volatilization and methylation pathways (Hintelmann *et al.*, 2002). The paper also mentioned that mobility through runoff was very low and strongly decreased with because of a rapid equilibration with the large native pool of mercury. The initial mobility of mercury received through small rain events or dry deposition decreased markedly in a relatively short time period, suggesting that mercury levels in terrestrial runoff may respond slowly to changes in mercury deposition rates. The mercury additions to the Experimental Lakes Area is continued through the year 2006. Results from the continuous addition to the year 2006 have yet been published. According to METAALICUS website, future additions will depend on 2006 results.

### **5.5. The Georgia Oyster Watch (GEOW) Program**

The Georgia Oyster Watch (GEOW) program is a National Oceanic and Atmospheric Administration-funded project administered by the Coastal Management Program and the Coastal Incentive Grants Program of the Georgia Department of Natural Resources (Frischer, 2007). The GEOW project utilizes the Eastern oyster as a

bioindicator of chemical water quality in coastal Georgia. The 3-year project is currently gathering data on oysters from the six coastal counties. The oysters are then quantified for chemical contaminants including polycyclic aromatic hydrocarbons (PAHs), total petroleum hydrocarbons (TPHs), and numerous metals. The data collected are being used to map water quality in Georgia, and will serve as a tool for resource managers to compare contaminant distribution along the coast to land use. Bioindicators like oysters may provide a more realistic assessment of the condition of an area compared to scientific models that can only mimic an environment its processes. Oysters serve as good bioindicators because they are sessile (adult stage) and therefore cannot avoid insult by removing themselves from adverse conditions presented by their environment. Through filtration, they capture particles that are present in the water column including those that may be harmful to their health. Although the program does not include mercury, it can be extended and easily accustomed to monitor mercury concentration along the coastal counties. The program currently runs until October 2007 and no continuing on funding has been established yet (see <http://www.skio.peachnet.edu/research/ecohealth/frischerlab/geow/index.php> for further information).

## **5.6. The International Conference on Mercury as a Global Pollutant**

The International Conference on Mercury as a Global Pollutant, first held in Sweden in 1990 is an international forum for the presentation and discussion of advances in scientific understanding of environmental mercury pollution. The Eighth International Conference was held in Madison, Wisconsin (USA) on August 6 - 11, 2006, which

addressed key questions concerning mercury in the environment. Forty experts, assembled into four panels, began the effort at an international workshop in July 2005, a full year before the conference. The panels addressed a series of key, policy-relevant questions concerning atmospheric sources of mercury, methylmercury exposure and its effects on humans and wildlife, socioeconomic consequences of mercury pollution, and recovery of mercury-contaminated fisheries. The panels presented their findings in four plenary sessions at the conference in Madison.

At the last conference in Madison, Dr. Booth Nathaniel and Dr. David Krabbenhoft of the U.S. Geological Survey and Dr. Tamara Saltman of the U.S. EPA presented their work for long-term monitoring of mercury. According to the abstract, they have identified indicators of methylmercury production potential based on the main chemical and physical factors that contribute to methylmercury production from inorganic mercury inputs to predict the areas where methylmercury production is most likely to be take place assuming a uniform mercury loading. The main water quality parameters that are linked to methylmercury production, which are widely available in USGS databases, include total organic carbon, pH, and sulfate. The abstract also mentioned that landscapes with a prevalence of wetlands, or anaerobic soils, have been correlated with methylmercury production. The study developed a national-scale database for these indicators which used USGS water chemistry data from over 55,000 sites and USDA hydric soils data. At the time of this thesis is written, the paper which documents their work has not been published yet, although should this database becomes available, it may provide a timely opportunity to identify and monitor areas where methylmercury production is expected to be a concern.

**CHAPTER 6**  
**ASSESSING MERCURY LOADING AND IMPACTS**  
**IN COASTAL GEORGIA**

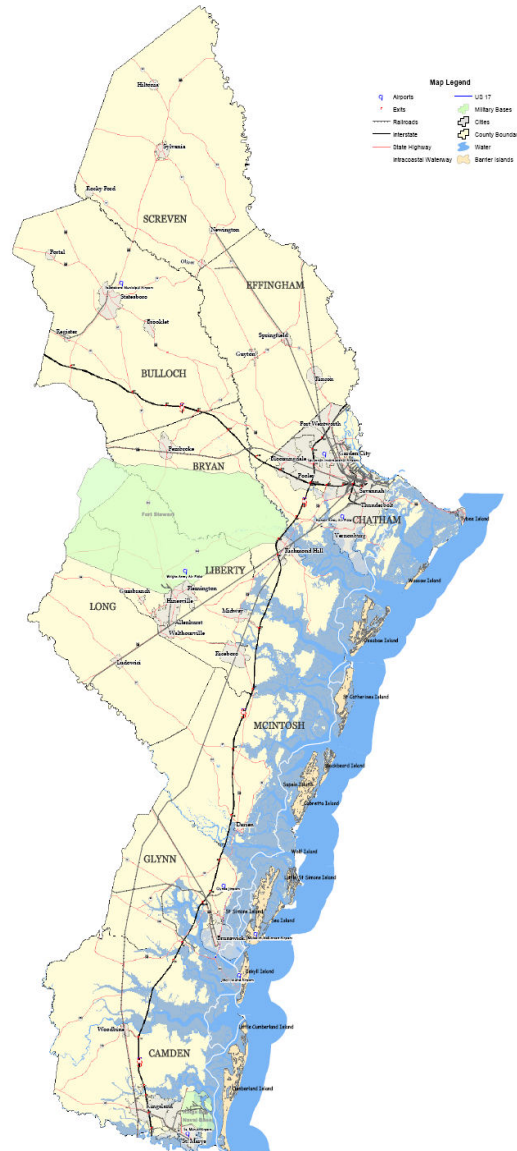
The origin of mercury in the atmosphere is contributed largely by power plant emissions, volcanic releases and municipal/medical waste incineration, with fossil fuel combustion being a major contributor of mercury. Although national rules and regulations have been imposed to limit or minimize mercury release in power plant emissions, correlation of decreasing or increasing mercury concentrations in the environment because of emission reduction has yet to be established. It is generally recognized that projects and initiatives that are already in place to achieve mercury-reduction goals will take many decades before effects will be clearly recognized in food-chain mercury. It is therefore critical that mercury be more intensively monitored and assessed if effective mercury control strategies are to be implemented. An enhanced understanding of mercury flow in the environment is essential because mercury is a global, as well as a local, contaminant. Identifying and implementing effective control strategies should take into consideration this global context of mercury fate and transport in the environment. Finally, mercury effects on human and animal populations are driven by transformation processes that occur in the natural environment. These naturally-driven transformation processes are the major and ultimate determinant in human health effects, therefore control strategies that are currently focused on primary mercury releases become complex as they relate to the control of secondary mercury of food chains.

In the U.S., fossil fuel combustion in power plants contributes about 41% of the total national mercury release per year (Ray, 2005). According to a 2000 report (McClevey, 2002), there are 208 coal-fired power plants in the U.S. with a total of 515 units. Collectively, these plants emitted 96,663 pounds of mercury into the air in 2005 (EPA, 2005). In Georgia, there are 10 coal-fired power plants with a total of 32 units, and in addition to Georgia, there are 22 plants in Alabama, Mississippi, and Florida. All these power plants are owned by Southern Company and in 2003 they ranked second in power plant mercury air emission by company (McClevey, 2002).

In the combustion of coal, biomass or organic wastes, mercury is emitted through smokestacks. Some of the mercury is washed out of the air onto land and into waterways, where it may then be converted into methylmercury. Mercury is also emitted to the atmosphere through sources other than coal combustion. Oil, and to a lesser extent natural gas, combustion also emit mercury to the atmosphere. Various waste combustions contributed by municipal and medical facilities also add mercury loading in the atmosphere. Although these amounts of other emissions do not represent a single major concern, as in mercury emissions from power plants, contributions from these processes are sufficient to add to the pool of mercury in sediments where methylmercury transformation can occur. Sediments have been identified as the primary environmental media for production of methylmercury, therefore efforts in dealing with mercury fate and transport becomes crucial for coastal environment.

Coastal areas have been known to be vulnerable to mercury loading and deposition because of chemical and physical factors that contribute to the production of

methylmercury. The coastal region of Georgia encompasses the six coastal counties and four inland counties, with a total land area of over 5,100 square miles (Figure 18). The region borders South Carolina on the northern boundary and Florida to the south beyond the St. Marys River.



**Figure 18.** Detailed map of the Coastal Georgia region, covering six counties. Picture is taken from Coastal Georgia Regional Development Center website (<http://www.coastalgeorgiarc.org/index.html>)

Six river basins lie completely within Georgia's borders. The Oconee and the Ocmulgee Rivers join to form the Altamaha River, which empties into the Atlantic Ocean. The Ogeechee and Satilla Rivers also flow to the Atlantic. The Flint River joins the Chattahoochee River near the Florida border to form the Apalachicola River, which empties into the Gulf of Mexico. Portions of 8 other river basins lie within the state. The Chattahoochee, Savannah and St. Marys Rivers each form portions of Georgia's border. The lower Chattahoochee River is part of the border between Georgia and Alabama; the Savannah River is the border between Georgia and South Carolina; and the St. Marys River forms part of Georgia's border with Florida.

On the Atlantic coast of Georgia, the Ogeechee River carries a high load of dissolved organic carbon that imparts a 'tea' color to the water. It has a near-neutral pH due to a large input of carbonate-rich water from Magnolia Springs. The Altamaha River is the largest river of the Georgia coast and the second largest basin in the eastern United States. The Satilla River lies entirely within the Coastal Plain. It is a typical 'black water' stream that has a dark color to the water due to inputs of humic material from extensive flood plain swamps of Cypress and black gum bordering the river. Humic material is productive in wetland area and will simply drain to water bodies. In Georgia, total wetland area amounts to 7.7 million acres, covering 20% of the state's landscape (Hefner *et al.*, 1994). In addition, flux of river basins in Georgia discharge to much larger water bodies like a sea which makes the dispersion of mercury, due to its strong interactions with humic material, more troublesome to contain.

Humic substances are ubiquitous in the environment and result from degradation of plant debris and living cells. Their most important function in the environment is their ability to bind heavy metal cations such as mercury. According to (Coates *et al.*, 2002) humic substances play an important role as electron sinks for anaerobic respiratory bacteria and fermentative bacteria, stimulating mineralization of complex organic carbon compounds in the absence of oxygen. They can also serve as suitable electron donors for anaerobic organisms growing on a variety of alternative electron acceptors. In this instance, the organisms simply use the reduced humic substances as an energy source. Their presence in the water column increase mercury bioavailability and persistence in the aquatic phase and decrease settling of mercury to the sediment or volatilization of mercury to the atmosphere.

High loads of dissolved organic carbon in river basins, as well as natural plant debris in productive wetlands, tidal and sub-tidal regions, cause increased growth of fermentative microorganisms. In anaerobic sediments dead plant mass and organic debris is an energy source for fermentative bacteria. Extra cellular enzymatic activity results in solubilization of complex organic matter into proteins, carbohydrates, lipids and other soluble organics. These organics then serve as energy sources for fermentative bacteria. Assimilation of these complex soluble organics results in the production of, for example, a complex array of short-chain fatty acids and alcohols. It is these fermentation end products, derived from the naturally-produced plant mass in rivers and wetlands that provide an energy source for sulfate-reducing bacteria (SRB). Sulfate reducing bacteria have been studied and confirmed as main producers of methylmercury in sediments. They require reducing conditions in anoxic environments such as in the sediments, to

carry out methylmercury transformation. SRB oxidize organic compounds such as acetic acids and utilize sulfate as their final electron acceptor to produce sulfide. SRB are not restricted to utilizing sulfate. They are also capable of fermentative growth and utilization of other electron acceptors such as nitrate. The presence of sulfide in the sediments is evident since sulfide has a distinct malodorous odor which is common in tidal marshes in this coastal region. It is also evident through the deep black color of SRB-active wetland sediments because ferrous iron precipitates with sulfide and forms a black iron-sulfide precipitate. The iron-rich red Georgia clay provides adequate solid-phase iron which results in release of ferrous iron under anaerobic conditions in sediments.

Mercury has also been shown to form strong complexes with organic matter. The presence of sulfate-reducing bacteria in an environment that has a high load of organic matter facilitates the methylation of mercury. The coastal and wetland regions should be a concern because methylmercury production is driven by abundant concentration of organic matter in wetland as well as aquatic system, for which the coastal area is known. It is therefore of interest to examine a coastal region, such as that in Figure 18, to contemplate the overall picture of mercury release, control, fate environmental transformation and effects in a region or state in the US.

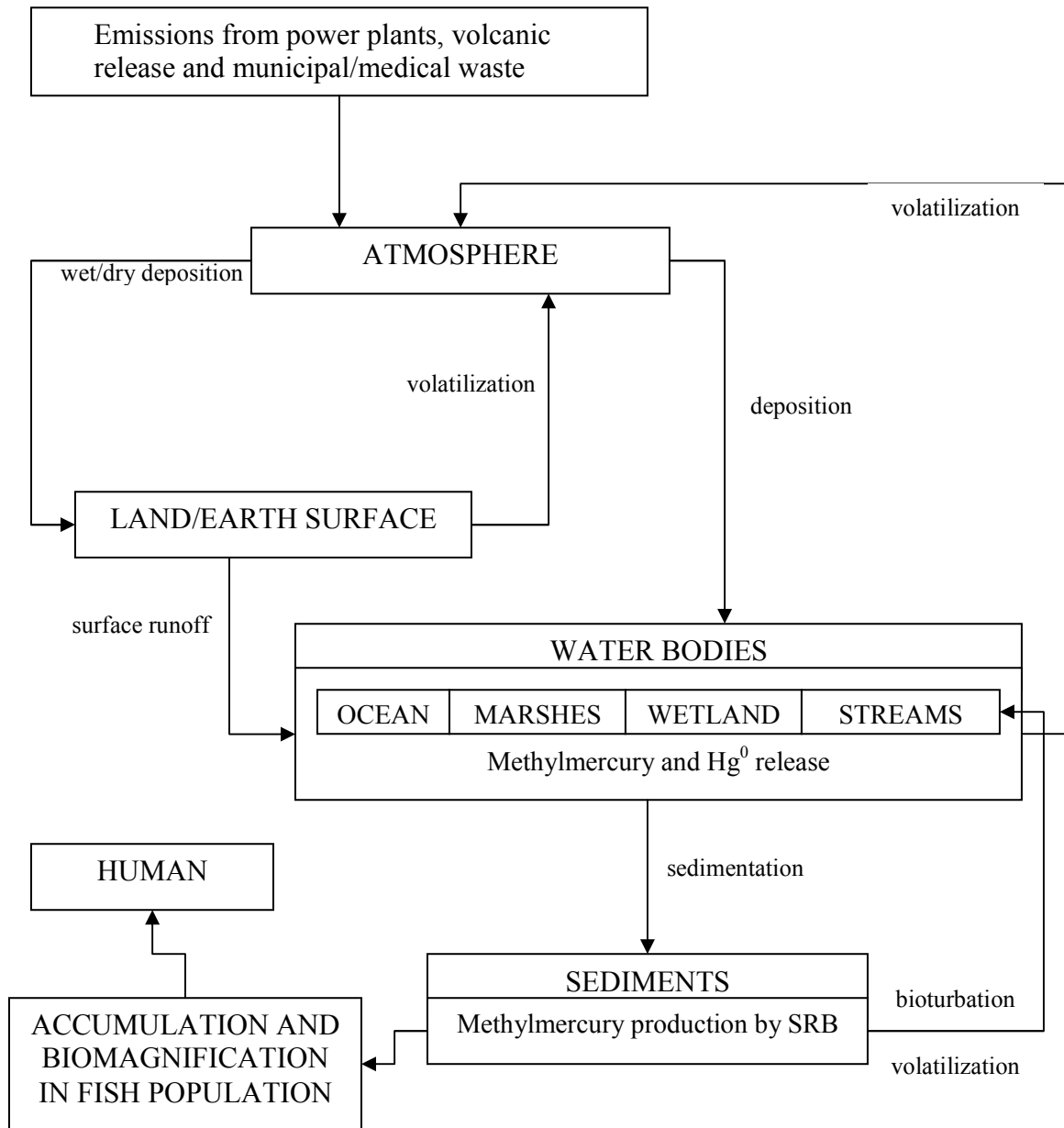
Mercury release and its cycle in the environment involve a series of transformations among environmental media. Contributions of mercury originate from both anthropogenic and natural sources where mercury can subsequently be recycled or deposited into the environment. The cycle of mercury in the environment as shown in

Figure 19 indicates that humans are exposed to the toxicity of methylmercury through consumption of fish and other seafood. Methylmercury is bioaccumulated up the food chain by transfer of residues of methylmercury in smaller organisms that are food for larger organisms in the chain.

Toxicity of methylmercury in the body has been shown to affect the central nervous system. It carries a high risk for a developing fetus, which is why pregnant women have been advised to restrict fish and shellfish intake to within the recommended guidelines published by the Food and Drug Administration. According to EPA, although fish and shellfish are important part of a healthy diet, especially for pregnant women, nearly all fish and shellfish contain traces of mercury.

As shown in Figure 19, accumulation of mercury in sediments originates from numerous sources of water bodies such as ocean, marshes, wetlands, and streams. Through bioturbation, volatilization and natural transport, mercury is mixed and transported back into water bodies, creating a continuous cycle of mercury deposition and release between the water bodies and sediments. Sources of mercury in water bodies are the release from land/earth surfaces through runoff and direct deposition from the atmosphere. Mercury is removed from the atmosphere through wet and dry deposition, which is then transferred to land/earth surface. The direct transfer of mercury species through dry and wet depositions have been acknowledged as important sources of mercury cycle in the environment. The complexity of mercury transformation gives rise to volatilization of mercury from water bodies and land/earth surface back to the atmosphere, a process that has been debated by the scientific community because there is

no definite measure to accurately predict the contribution of this process to mercury concentration in the atmosphere. In addition to anthropogenic sources, mercury is also a naturally occurring element in rocks, air, water, soils, and volcanic dust (Stein *et al.*, 1996). Although the amount is insignificant, it adds up to the burden for nature to sequester this mercury.



**Figure 19.** The box model which details the cycle of mercury distribution in the environment and how it affects human at the end of the cycle.

The process of methylmercury production by SRB in the sediments will take place given the right biogeochemical controls such as organic matter and sulfate deposition. Decay of natural organic matter has been acknowledged to stimulate bacteria activity because of its role as substrate for microorganisms. Sulfate loading in sediments is an important control on methylation of mercury because excess sulfide can create mercury complexes that may temporarily make mercury have limited bioavailability for mercury methylation.

The pool of mercury in sediments and environmental variables that facilitate methylmercury transformation create a problem for the coastal region of Georgia. The vast water line bordering the state of Georgia with its abundant production of fish and shellfish increases the potential exposure of methylmercury toxicity not only to the local population but also to those bordering states which consume fish and shellfish caught within Georgia waterline. To address this problem, we must recognize and understand the processes that facilitate methylmercury production in the environment. Organic matter and sulfate deposition to a coastal region are part of the natural sources and this natural ecology cannot be eliminated. The same goes for sulfate-reducing bacteria (SRB) that play an important function in the sulfur cycle, a continuous geochemical cycle in nature.

The challenge to monitor mercury in order to best identify remediation and/or prevention programs that can reduce the amount of mercury available for methylmercury transformation lies in the difficulty in quantifying mercury that is deposited into the

environment. Mercury concentration that is deposited into the environment may translate to amount of methylmercury produced but this is hugely dependent on natural environment processes which can be unique to each region. Currently the regulations that aim to monitor mercury only focus on the amount released and not the amount deposited in the environment. In addition, not all mercury in the environment will be bioavailable for further transformation because not all mercury species are the precursors for methylmercury.

Speciation of mercury is a complex pathway because methylmercury transformation will only take place if divalent mercury is available for uptake by microorganisms. Atmospheric release of mercury is mostly in the form of elemental vapor, and the remaining balance is made up of particulate-bound divalent mercury and reactive gaseous mercury. Due to its water solubility and high vapor pressure (see section 2.2) elemental mercury will remain in the atmosphere and is transported away from points of release. Under acidic conditions, elemental mercury can be oxidized to divalent mercury, which will have increased solubility as it will complex with hydroxyl or chloride ions. These complex compounds are abundant in natural environment and their complexation will make mercury bioavailable for microorganisms.

Complexation of mercury in sediments where methylmercury transformation takes place has been confirmed through various studies. The anoxic environment of sediments provides an ideal living condition for sulfate-reducing bacteria, the main producer of methylmercury. Complexation also decreases methylmercury production rates which reiterates the fact that speciation of mercury is a complex pathway. King (1999) examined mercury methylation rates by sulfate-reducing bacteria in marine

sediments. The study confirmed that methylation rates varied between 1-100  $\mu\text{mole}$  methylmercury produced by mole of sulfate reduced. This wide variation would potentially indicate that the genus/species of sulfate-reducing bacteria present could determine the relative “hazard” of sediments, even when population density of sulfate-reducing bacteria values were constant across a variety of sediments. These phylogenetic differences, coupled with variations in  $\text{Hg}^{2+}$  availability in sediments, would dramatically influence methylmercury production. Furthermore since sulfate reduction process is driven by the availability of fermentation products (e.g. volatile acids), which subsequently depends on quantity and nature of decaying plant debris, as well as the fermentation and microbe population which produce the primary substrates for SRB. This chain of reaction shows the complexity of mercury transformation pathway in the environment.

Understanding mercury deposition routes and biogeochemical controls is the key to identify necessary measures in mercury monitoring. These measures not only have to target sources of mercury deposition, they also have to target the physical, biological and chemical controls that will stimulate methylmercury production. Efforts must be in place to quantify the activity of mercury production from various delivery points as laid out in Figure 19. Although mercury data collection on fish population is a continuous program of the Department of Wildlife and Fish Services, they are not enough to assess mercury flows because there is no investigation to what causes any increase or decrease, if any, of mercury concentration in fish population. The speciation of mercury contributes to the complexity of utilizing the data of fish population, as not all mercury species can be

transformed to methylmercury. Currently, there is no guideline to predict how much mercury that is detected in the fish population will be transformed to methylmercury.

We should establish a better understanding on where mercury is coming from by addressing the sources of mercury. It is important to note that quantification of mercury should not only focus on the amount of mercury that is being discharged but what is being retained in the environment. This task becomes very challenging because of the complex speciation pathway of mercury. However mercury studies have confirmed several biogeochemical controls that facilitate mercury speciation in the environment such as pH, dissolved organic matter and sulfate deposition. These environmental controls can be utilized as key starting points to identify the necessary control measures that will prevent an increase of methylmercury production in sediments.

Mercury data collection through several monitoring programs that are already in place (see Chapter 5) is currently scattered among different agencies. These scattered data must be streamlined to create a uniform understanding on how methylmercury production can be elevated or minimized. The scope of this effort may be daunting since it involves a network of cooperation from all these agencies for them to reach conformity on how to best utilize the mercury data collection to produce useable data for methylmercury production. The focus of mercury data collection should also be targeted to identifying production rate in sediments. A solid sediment management can be a key control in limiting methylation of mercury.

Mercury introduction into the environment is through fossil fuel combustion and natural emission (e.g. volcano emissions), as well as through runoff processes that move soil-bound mercury into streams and sediments. Mercury methylation is biologically

driven process where transformation of mercury takes place in a complex array of biogeochemical processes in sediments. To address the problem in coastal region, efforts must be focused on inputs of mercury to the environment, the array of land, river, sediments, and characteristic activity of sulfate-reducing bacteria in sediments.

Assessment of mercury needs to be routinely made to get an indication of the real nature of the problem. Responsibilities of tackling mercury in the environment should involve cooperation of agencies and integration of data that can be utilized to determine the best possible measures to prevent, if not, reduce the amount of mercury that can be transformed to methylmercury.

## CONCLUSION

Mercury occurs in solid and dissolved states, as well as in liquid and gaseous phases. Consequently, its biogeochemical cycle involves three phases in nature, encompassing terrestrial, oceanic, and atmospheric processes. Due to its unique physical and chemical properties, mercury has long been applicable in many uses in many fields of industry although its uses are being restricted or minimized because of its toxicity which targets the central nervous system. The use of mercury declined in 1990's as a result of establishment of regulations, following the 1971 milestone when mercury was designated as hazardous pollutant. Nevertheless, in addition to mercury being ubiquitous in nature, mercury waste left behind and released to the environment by industry remains a concern especially in the aquatic system. Mercury can be transformed to a highly toxic form of methylmercury in the aquatic system. Humans are exposed to the toxicity of methylmercury through consumption of fish. Methylmercury is bioaccumulated up the food chain by transfer of residues of methylmercury in smaller organisms that are food for larger organisms in the chain.

Toxicity of mercury depends on its speciation. Elemental mercury ( $\text{Hg}^0$ ), the most common form of mercury found in nature, can be transformed to divalent mercury ( $\text{Hg}^{2+}$ ). Divalent mercury is the necessary precursor for methylmercury formation in the aquatic system. This form of mercury is immobile once deposited, making it available to the biological communities for methylmercury formation, given the right environmental conditions. Both biotic and abiotic processes of methylmercury have been studied and published. Biotic processes are mainly dominated by sulfate-reducing bacteria (SRB) although one study has also confirmed the possibility of other bacteria such as iron-

reducing bacteria can also carry out methylmercury transformation. The biotic formation by SRB is affected by several environmental factors such as temperature (peak during late summer), availability of biodegradable organic carbon, pH, and sulfate deposition. Study on abiotic methylation has shown that methylmercury concentration increases during sunlight hours. This process is believed to be linked to dissolved organic matter and solar radiation although water chemistry that contributes to this abiotic methylation is not clear yet.

Demethylation of mercury can also occur through biotic and abiotic processes. Biotic process involved decomposition of methylmercury performed by bacteria while abiotic process occurs kinetically when environmental conditions are favorable. Biotic demethylation involves reductive and oxidative pathway. Reductive pathway reduces methylmercury to elemental mercury and methane ( $\text{CH}_4$ ). Oxidative pathway will result in the production of  $\text{Hg}^{2+}$  and  $\text{CO}_2$  as well as  $\text{CH}_4$  and sulfide ( $\text{S}^{2-}$ ). This divalent mercury that is produced through this pathway, given the right environmental conditions, can be subsequently methylated again, which further complicates the methylmercury degradation process.

Due to the wide transport and distribution of mercury through the environments, mercury monitoring becomes a difficult task. Several monitoring programs have already been in place to quantify the amount of mercury released. The power plants have been recognized as primary contributors of mercury release to the atmosphere. As a result, the Clean Air Mercury Rule, which requires power plants to implement monitoring programs such as stack testing and continuous emissions monitoring system for compliance set forth in the regulation, was created. A nationwide network called the Mercury

Deposition Network collects data of total mercury in precipitation from different regions with data being accessible to the research community and the general public through its website. The National Listing of Fish Advisories published safe eating guidelines about the recommended level of consumption of fish caught in local waters. Ongoing research such as METAALICUS continues to study mercury loading in watershed. The Georgia Oyster Watch Program shows promise for state-wide mercury monitoring program should funding can be allocated because the program will complete its 3-year funding allocation in October 2007. In addition to these programs, the International Conference on Mercury as a Global Pollutant is held every year to present and discuss advances in scientific understanding of environmental mercury pollution.

This study offers a comprehensive review on chemical and biological processes of mercury transformation in the environment. Although there are numerous similar studies and publications on related topics in literature, this study puts an effort in associating mercury concentration in the environment with its sources as well as monitoring programs that can help formulating control strategies to reduce mercury exposure to the general public.

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