NIMD

PROCEEDINGS OF

NIMD FORUM 2001

—Mercury Research: Today and Tomorrow—

Date: March 19-20, 2001

Venue: National Institute for Minamata Disease
Moyai Center

Minamata City, Japan

Organized by National Institute for Minamata Disease

PROCEEDINGS OF

NIMD FORUM 2001

－Mercury Research: Today and Tomorrow－

Date: March 19-20, 2001

Venue: National Institute for Minamata Disease
Moyai Center

Minamata City, Japan

Organized by National Institute for Minamata Disease

Contents

Preface

The NIMD Forum 2001 of the International Scientific Meeting was held in the National Institute for Minamata Disease, Minamata, on March 19 and 20, 2001. The aims of this forum are to review the current status, future needs, and directions for mercury research in various fields. These proceedings consist of speeches given at the meeting and written contributions submitted by the speakers in charge of the International Steering Committee for members of the 6th International Conference on Mercury as a Global Pollutant.

Present knowledge tells us that the most immediate hazard to humans is created by methylmercury. The primary facts determined were: (1) mercury amplification in food chains, and (2) transformation of methylmercury. The dominant source of methylmercury accumulation in man is usually food. Other forms of mercury in aquatic ecosystems including inorganic mercury can be converted into methylmercury that can be absorbed and concentrated by aquatic species. Then, it is further concentrated along the food chain involving higher organisms including human beings.

We are very pleased to have been exposed to such a wide variety of viewpoints during the meeting. The NIMD forum has been extremely successful in bringing together scientists who have a common interest. We had the good fortune of personally getting to know many of the active and experienced scientists, researchers, and workers in this field. My heartfelt thanks are due to everyone who has performed the difficult and time-consuming tasks of submitting their manuscripts.

Intensive discussion of the impacts of various research efforts will improve the quality of environmental health projects and will encourage the future development of international cooperative research.

March 22, 2001

Yukio Takizawa, M.D.
Director-General
National Institute for Minamata Disease

Welcome Address

It's our great pleasure to hold the third forum of National Institute for Minamata Disease, the NIMD Forum 2001, here in Minamata. I'd like to especially express our sincere appreciation to the participants from abroad who have joined us here. Over forty years have passed since the first case of the Minamata Disease was reported in this region. The outbreak of Minamata Disease is one of the most serious forms of pollution. It originated from the discharge of wastewater containing harmful substances from chemical plants.

Since then, many efforts have been made to solve the problem. Information from abroad has also made us recognize that there are various forms of mercury contamination including those arising from gold mining and coal burning. Given these circumstances, when we investigate the health effects of mercury contamination, we also have to consider signs and symptoms caused by the direct inhalation of inorganic mercury as well as by organic mercury that are caused by the consumption of methylmercury contaminated fish. Therefore, it will be important to compare the types of mercury contamination and the adverse health effects in various countries and at various sites from now on.

We hope that NIMD will collect and summarize the information, and then disseminate it all over the world in order that the tragedy we experienced in Japan will never occur again anywhere in the world. Finally, we hope that this forum and the 6th International Conference on Mercury as a Global Pollutant will provide useful opportunities to discuss these matters.

Thank you for your attention.

Ryo Takagi, M.D.
Section Chief
Special Environmental Diseases Office
Ministry of the Environment

Organizing Committee

Tazusa Arakaki
Social Sciences Section
Department of International Affairs and Environmental Sciences

Yoshihisa Fujita
General Affairs Section
Department of Administration Division

Kiyoka Miyamoto
Clinical Medicine Section
Department of Clinical Medicine

Kuniko Sato
International Affairs and Information Section
Department of International Affairs and Environmental Sciences

◎ Megumi Yamamoto
Physiology Section
Department of Basic Medical Sciences

Editorial Board

Agenda

Proceedings

Affiliation

1. Hirokatsu Akagi (Japan)
Dept. of International Affairs and Environmental Sciences
National Institute for Minamata Disease

2. Milena Horvat (Slovenia)
Dept. of Environmental Sciences, Institute Jožef Stefan

3. Monica Costa (Brazil)
Dept. of Oceanography, Federal University of Pernambuco

4. Justinian R. Ikingura (Tanzania)
Dept. of Geology, University of Dar es Salaam

5. John H. Munthe (Sweden)
Dept. of Atmospheric Chemistry and Environmental Effects
IVL Swedish Environmental Research Institute Ltd

6. Steven E. Lindberg (USA)
Dept. of Environmental Sciences Division, Oak Ridge National Laboratory

7. Ricardo Melamed (Brazil)
Dept. of Coordination of Mineral Processing, CETEM-Center for Mineral Technology

8. Marcello M. Veiga (Canada)
Dept. of Mining and Mineral Process Engineering, University of British Columbia

9. Makoto Futatsuka (Japan)
Dept. of Public Health, Kumamoto University School of Medicine

10. Kimiyoshi Arimura (Japan)
The Third department of Internal Medicine, Faculty of Medicine, Kagoshima University

11. Hiroshi Satoh (Japan)
Dept. of Environmental Health Sciences, Tohoku University Graduate School of Medicine

12. Ronald E. Wyzga (USA)
Dept. of Environment, Electric Power Research Institute

13. Rudi Rajar (Slovenia)
Factory of Civil Engineering, University of Ljubljana

14. Ralf Ebinghaus (Germany)
Dept. for Organic Trace Analysis, GKSS Forschungszentrum Geesthacht

15. Olaf Malm (Brazil)
Program of Environmental Biophysics and Radioisotopes Laboratory
Biophysics Institute Carlos Chagas Filho

Analytical Technique for the Evaluation of the Environmental Factors influencing the Dynamics of Mercury in Aquatic Systems with particular Emphasis on Tropical Areas

Hirokatsu Akagi* and Justinian R. Ikingura**
*National Institute for Minamata Disease, Hama 4058-18, Minamata,
Kumamoto 867-0008, Japan
** University of Dar Es Salaam, P.O. Box 35052, Dar Es Salaam, Tanzania

Abstract

Environmental mercury contamination is an issue of deep concern in tropical and other regions. In particular, a number of countries in the tropical belt of South America, Africa and Southeast Asia have experienced tremendous increase in uncontrolled small-scale gold mining using mercury amalgamation since early 1980s, which is likely to amplify adverse effects on the contaminated ecosystems and cause health hazards.

Ecologically, the most critical but complex part of the mercury pollution problem is that concerned with the transformation of inorganic mercury into more toxic mono-methylmercury that is more biologically available for aquatic organisms. Although increasing literature dealing with mercury contamination levels in human populations and in aquatic ecosystems exists, we are still unable to make predictions of the behavior of mercury in the aquatic systems, particularly under the tropical conditions. Thus, it is imperative to obtain basic data concerning the overall dynamics of methylmercury production, as well as the environmental factors influencing mercury methylation and the partitioning of mercury in the aquatic systems in order to understand and predict the cycling and bio-accumulation of mercury.

To facilitate studies on the dynamics of mercury in the aquatic systems, we have developed highly sensitive and systematic methods for the analysis of total mercury and methylmercury in a wide range of biological and environmental materials containing mercury down to background levels. With these methods, it is now possible to make quantitative measurements of the equilibrium distribution or partitioning of methylmercury produced in sediments in a model of aquatic systems under various environmental conditions similar to those found in the fields. Such an approach should undoubtedly help elucidate the mechanisms of mercury pollution in the tropical ecosystems and promote efforts in mercury pollution prevention.

Key words: Analysis, Mercury methylation, Aquatic systems, Environmental factors,

1. Introduction

The knowledge of mercury cycling in the aquatic systems has improved significantly in recent years because of the development of highly systematic and sensitive analytical techniques. The improvement of analytical methods and renewed interest in environmental mercury research triggered by the discovery of high mercury levels in fish from pristine remote lakes in US, Canada and Scandinavia (Stephens, 1995; Andersson et al., 1995) and the increase of mercury pollution from gold mining activities in developing countries, has made it necessary to re-evaluate the existing data on the biogeochemistry of mercury.

A better understanding of mercury distribution in aquatic systems not only requires knowledge of total mercury, but also the amount of mercury that is in methylated form and more available for bioaccumulation. The conversion of inorganic mercury into methylmercury has long been recognized as a critical step in the environmental behavior of this metal however, the mechanism of the synthesis of methylmercury is not very well understood. Moreover, it is important to know the chemical forms of mercury in the sediment, since organic mercury is water-soluble and easily taken up by aquatic organisms to be biomagnified up through the aquatic food chain. The determination of the methylation sites and processes and methylmercury pathways and fluxes in the environment are therefore essential in the understanding and prediction of mercury migration in the environment since a major source of human exposure to methylmercury is through the consumption of fish and fish products.

Previous studies have used radiochemical methods to investigate the speciation and distribution of mercury in aquatic systems (Kudo, et al., 1977; Akagi, et al, 1979; Guimarães et al., 2000). Inorganic mercury and methyl mercury in sediment and water were extracted in dithizone-benzene and measured after separation by thin layer chromatography. Other researchers have used analytical methods based on the Westöö (1968) procedure to extract mercury from environmental samples followed by the analysis of methylmercury by gas chromatography with electron capture detection (GC-ECD). In most of these and earlier studies, methylmercury extraction efficiency and the results of the certified reference materials were not reported. Also the presence of interfering organic compounds reduced the accuracy of the methylmercury analyses. This limited the reliability of methylmercury data for the evaluation of methylmercury production and partitioning in various compartments of aquatic systems. In the efforts to overcome the above shortcomings, Akagi and Nishimura (1991) developed highly sensitive and reliable analytical methods for the determination of total mercury and methylmercury in a wide range of environmental and human samples. Recently, the refinement of those analytical methods at the National Institute for Minamata Diseases (NIMD, Japan) has simplified the analytical procedure and enabled rapid and accurate determination of total mercury (T-Hg) and methylmercury (MeHg) in various samples, including highly contaminated sediment and fish from gold mining areas in tropical regions.

The methods described in this paper have been repeatedly verified for reliability, accuracy and precision through inter-laboratory comparison (Matsuo et al.; 1989, Kehrig et al., 1998; Ikingura et al., 1999) and the analysis of certified reference materials (e.g. TORT) for T-Hg and MeHg. Those methods have been used also in the certification of the reference materials from the International Atomic Energy Agency (IAEA, Horvat et al., 1997). The detection limits are 5 ng/g for T-Hg and 5 ng/g for Me-Hg in biological and sediment samples and 0.5 ng/L for T-Hg and 0.1 ng/L for Me-Hg in water samples.

2. Materials and Methods

2.1 Reagents

Analytical grade mercury-free reagents are used in the preparation of different solutions for the digestion and extraction of mercury and methyl mercury from the samples. Mercury-bearing reagents are used only in the preparation of internal or calibration standards. The reagents generally include toluene, ethanol, hexane, acetone, sulfuric acid, nitric acid, perchloric acid, hydrochloric acid, potassium hydroxide, sodium hydroxide, potassium permanganate, dithizone ($C_6H_5N{:}NCSNHNHC_6H_5$), L-cysteine hydrochloride monohydrate($HSCH_2CH(NH_2)COOH \cdot HCl \cdot H_2O$), ethylenediaminetetraacetic acid tetrasodium salt tetrahydrate (EDTA-4Na), methylmercuric chloride (CH_3HgCl), sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$), hydroxylamine hydrochloride ($NH_2OH \cdot HCl$), stannous chloride ($SnCl_2$), sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$), anhydrous sodium sulfate (Na_2SO_4), Florisil (60-100 mesh). Other consumable materials include pure N_2 gas.

2.2 Total Mercury Analysis

The analytical method for total mercury is based on cold vapor atomic absorption spectrometry using a semi-automated mercury analyzer (Sanso Seisakusho Co. Ltd., Japan). This method involves reducing mercury ions (Hg^{2+}) in the sample solution using stannous (II) chloride to generate mercury vapor (Hg^0). The sample is then aerated in order to purge Hg vapor into the atomic absorption cell. Mercury absorbance is then measured at 253.7nm. The Hg vapor purging system consists of a semi-automated air circulation pump connected by combined glass-teflon tubing to the sample reaction vessel, an acidic gas trap, and a four-way stop cock. The total volume of the solution needed for T-Hg analysis is 10 ml.

Before analyzing X ml of a blank sample, standard sample or the actual sample solution for T-Hg, a calibrated dispenser is attached to the reaction vessel ready to dispense a known volume of distilled water. Gently add X ml (solution + water, maximum of 10ml) of each solution to a stoppered reaction vessel then 1 ml of stannous (II) chloride solution is injected from the accessory syringe. Press the start button of the analyzer. The diaphragm pump will operate and the Hg vapor generated from the sample will be circulated through the four-way cock between the reaction vessel and acidic gas collection bottle. During the circulation, acidic gases leaving the sample solution are removed by an acidic gas trap containing 5 N NaOH. After 30 seconds, when the concentration of Hg comes to equilibrium the vapor is automatically introduced into the absorption cell by turning the 4-way stopcock by 90°. When the maximum peak height has been recorded, the sample from the reaction vessel is discarded into the liquid waste trap and the vessel purged with air to remove the residual Hg vapor. The reset button is then pressed to start the next measurement.

2.2.1 Preparation of standards and sample solution

All glasswares should be cleaned with acidic $KMnO_4$ solution (0.5% $KMnO_4$ in 1 N H_2SO_4) and Hg-free water prior to sample preparation.

Hg standard solution

Methylmercury standard solution in toluene

Dissolve 12.5mg of CH_3HgCl in toluene to make a final volume of 100ml. Dilute this solution 100 times using toluene to prepare a solution containing 1 ppm MeHgCl in toluene. 1ml of this solution contains 1.0μg of Hg (1000ng Hg).

Methylmercury-cysteine solution

Mix 0.5ml of 1ppm MeHgCl in toluene and 5ml of 0.1% L-cysteine in 0.1N NaOH solution into a 10ml Pyrex glass test tube. Shake for 3 minutes. Centrifuge at 1000 rpm for 3 minutes and discard the organic phase (top layer). Seal the tube, and store in a cool, dark place. 1ml of this solution contains 0.1μg (100ng) of Hg. Prepare a fresh solution every month.

Sediment or fish samples

Weigh accurately the sample (0.5g or less wet weight) into a digestion flask, add 1ml of distilled water and mix with 2ml of nitric acid – perchloric acid (1:1), and 5ml of sulfuric acid. Add boiling chips. Wipe the flask with paper towel to ensure that no spilled chemicals onto the surface. Heat the flask on a hot plate at 200 ±5°C for 30 minutes. Allow to cool and then add Hg-free water to obtain a fixed volume. Use the resulting solution for Hg analysis of the sample. Simultaneously prepare a blank solution by adding the same reagents into an empty clean digestion flask and carrying the reagents through the digestion procedure outlined above. The standard solution is prepared by measuring 1ml (corresponding to 0.10μg of Hg) of MeHg-cysteine solution (0.10μg Hg/ml) into the digestion flask. Then follow the same procedure indicated above for the preparation of the sample solution.

Water samples

Transfer 1L of water sample into a separatory funnel and then add 5ml of 20N H_2SO_4 and 2ml of 0.5% $KMnO_4$ solution. Mix the sample and reagents by shaking vigorously and let stand for 5 minutes. Neutralize using 10ml of 10N NaOH and mix with 2ml of 10% hydroxylamine-HCl solution. Allow to stand for 20 minutes. Mix with 2ml of 10% EDTA and then add 5ml of purified 0.01% dithizone-toluene (Dz-Tol) solution. Shake vigorously and let settle to allow complete separation of the aqueous and organic layers. Discard the aqueous layer (lower phase). Transfer 4ml of the organic layer to a sample digestion flask. Using a rotary evaporator, immerse the flask in water bath at 60° C and evaporate to dryness. Reconstitute the residue by following the procedures indicated above for fish/sediment samples. Prepare a separate 1L Hg free water as the blank sample and another 1L Hg-free water spiked with 200μl (corresponding to 20ng as Hg) of Me-Hg cysteine solution (0.10 μg Hg/ml) as the standard. Follow the above procedures and use the resulting solution for T-Hg analysis.

2.3 Methylmercury Analysis

A Gas Chromatograph with electron capture detector (GC-ECD) is often used for the determination of methylmercury and other organomercury compounds. This method provides good separation and superior sensitivity for the determination of halogenated organomercury compounds. Conventionally, it has been widely used for the quantification of MeHg in various types of biological and environmental samples.

2.3.1 Gas Chromatographic Conditions

Column: Use a glass column (3mm × 0.75~1.0m) packed with Hg-20A-Uniport HP (GL Science, 60 ~ 80 mesh) or 10% KOCL-Hg-Chromosorb W (AW/DMCS, Yanaco, 60 ~ 80 mesh). At the injection port, pack 2 ~ 3 centimeter of NaCl previously heated at 500°C for 2 ~ 3 hours

Temperature: Column oven: 140 ~ 160°C, injection port: 180°C, Detector oven: 200°C

Carrier gas: N_2, 30 ~ 40ml/min.

2.3.2 Preparation of standards

Preparation procedures of MeHg standard solutions are the same as described in 2.2.1.

2.3.3 Preparation purified 0.01% dithizone in toluene

Dissolve 0.011g of dithizone in 100ml of pure toluene in a 200ml separatory funnel. Add 50ml of 0.1N NaOH and shake to extract the dithizone to the aqueous layer (bottom phase). After separation, transfer the bottom phase to a stoppered glass container and add ca. 5 ml of 1N hydrochloric acid to make the solution slightly acidic (black colored crystals will precipitate). Add 100ml of pure toluene and mix to obtain a purified 0.01% dithizone solution. Allow to settle, discard the bottom phase, seal, and store in a cool, dark place (prepare at the time of use).

2.3.4 Pre-Analytical Run

Perform a trial run on toluene by gas chromatography to ensure that no other peak co-elutes with the expected retention time for MeHg.

2.3.4 Methylmercury extraction

Fish samples

Weigh X g of homogenized sample (0.5g or less as wet weight, approximately 0.1g for dry samples) in a 50ml screw-capped conical centrifuge tube. Add 10ml of 1N KOH – EtOH (1:1) solution. Seal tightly and heat at 100°C in an isothermal bath for 1 hour with occasional mixing. Allow to cool it down. Add 10ml of 1N HCl and 5ml of hexane and shake for 3 minutes (to remove fats) using a recipro-type shaker. Centrifuge at 2000 rpm for 3 minutes then suck off and discard the hexane (upper layer). Add 2ml of 20% EDTA and 5 ml of purified 0.01% Dz-Tol. Shake to extract the methylmercury as dithizonate complex in the toluene layer (upper layer). Centrifuge at 2000 rpm for 3 minutes then suck off and discard the lower layer.

Clean-up of MeHg-dithizonate extract

Add 3 ml of 1N NaOH to the toluene layer, shake (to remove excess dithizone) and centrifuge at 2000 rpm for 3 minutes. Suck off and discard the lower layer (aqueous layer). Repeat the procedure for the clean-up. Let the solution settle for a while, remove the lower layer, and centrifuge again at 2000 rpm for 3 minutes to obtain a clear toluene layer. Transfer a fixed volume of the toluene layer (normally 3 ml) to a 10ml glass-stoppered conical centrifuge tube. Add 2ml of alkaline Na_2S solution, and shake to back-extract the MeHg into the aqueous layer. Centrifuge at 1000 rpm for 3 minutes then suck off and discard the upper toluene layer. Wash the aqueous layer with 2ml of toluene, shake for 2-3 minutes and centrifuge at 1000 rpm for 3 minutes. Suck off and discard the toluene layer (upper layer). Acidify with 1N HCl (3 ~ 4 drops). Bubble the solution with N_2 gas gently at a flow rate of 50ml/min for 3 minutes. Re-extract the MeHg with 2ml of Walpole's buffer solution and purified 0.01% dithizone solution (0.2 ~ 1.0ml, normally 0.5ml). Shake for 2-3 minutes and centrifuge at 1000 rpm for 3 minutes, suck off and discard the lower aqueous layer. Add 3ml of 1N NaOH to the toluene layer then shake. Let the solution settle and then suck off and remove the aqueous layer (lower layer). Centrifuge at 1000 rpm for 3 minutes. Suck off and discard the lower layer as much as possible. Acidify with 2 drops of 1N HCl. Then place the solution on a vortex mixer to mix and use the resulting solution as the sample for GC-ECD analysis. This clean up procedure removes the organic materials in the sample that may otherwise interfere with MeHg analysis by GC-ECD. Perform the sample solution preparation protocol for the standard using 0.10ml (corresponding to 0.010μg of Hg) of MeHg-cysteine. Use the resulting sample as the MeHg standard.

Sediment samples

For MeHg analysis in sediment, the sample (1 g or less) is first shaken with 10 ml of 1N KOH solution in ethanol (1N KOH-EtOH) for 10 minutes. The treated sample is slightly acidified with 1 N HCl and bubbled with N_2 through the sample solution for 5 minutes at a flow rate of 100 ml/min. The sample is then mixed with 2 ml of 20% hydroxylamine-HCl and 2ml of 20% EDTA-4Na and MeHg in the sample is extracted with 5 ml of purified 0.01% Dz-Tol solution. This is followed by the clean up of the Dz-Tol extract. In the clean up of the Dz-Tol extract of a sediment sample the extract is passed through a Florisil column (0.5 g Florisil, topped with 0.5g Na_2SO_4) and washed twice with I N NaOH before the extract is subjected to back extraction with Na_2S following the procedure outlined above for fish samples.

Water samples

For MeHg analysis in water, the same extraction procedure as that for T-Hg extraction in the water is followed. The Dz-Tol extract of the water sample is carried through the same clean up procedure as for the fish sample, finally re-extracted with 0.2 ml of 0.01% Dz-Tol and followed by MeHg analysis by GC-ECD. Perform the sample solution preparation protocol for the standard using 20μl (corresponding to 2.0ng of Hg) of MeHg-cysteine. Use the resulting sample as the MeHg standard.

3. Sample Analysis and Data Processing

3.1 Total Mercury

The detection and measurement of T-Hg in the sample solution is accomplished using a semi-automated analyzer. After dispensing a fixed volume (up to 10ml, normally 5ml) of the blank, T-Hg standard, or actual sample (or actual sample diluted with blank solution). The peak heights (mm) thus obtained are labeled, respectively, as Pbl, Pstd, and Ps. The

T-Hg concentration in the sample can then be calculated as follows:

For fish or sediment samples:
T-Hg concentration in the sample (μg/g) = 0.1μg × (Ps-Pb1)/(Pstd-Pb1) × dilution factor × 1/Sample weight (g)

For water samples:
T-Hg concentration in original water sample (ng/L) = 20ng × (Ps - Pb1)/(Pstd - Pb1) × sample extract dilution factor × 1/Water sample volume (L).

3.2 Methyl Mercury

Inject a fixed volume (normally 5μl) of the blank, MeHg standard sample, or actual sample into the gas chromatograph. Label the peak heights thus obtained as Pb1, Pstd, and Ps, respectively.

For fish or sediment samples:
MeHg concentration in the sample (μg/g) = 0.020(μg) × Ps-Pb1/Pstd-Pb1 × dilution factor × 1/Sample weight (g wet wt or dry wt).

For water samples:
MeHg concentration in water sample (ng/L) = 2.0ng × Ps-Pb1/Pstd-Pb1 × dilution factor × 1/Water sample(L)

4. Discussion and Conclusion

A number of wet digestion procedures for total mercury analysis have been proposed and used but most of them involve time consuming operations and require considerable number of reagents and careful handling during digestion. For accurate analysis of T-Hg, complete digestion of organic materials in the samples is essential. Various combinations of acids and oxidizing agents have been used since a major problem in the sample preparation procedure is mercury loss during the digestion process. Our laboratory studies at NIMD have shown that the presence of oxidizing agents $HClO_4$ or 5% $KMnO_4$ prevents the loss of Hg completely even under extreme heating conditions. Of these two oxidizing agents, $HClO_4$ was selected because the use of $KMnO_4$ will require another reagent. In normal H_2SO_4 – HNO_3 digestion, mercury may vaporize during the reaction process. However, in the presence of a strong oxidizing agent ($HClO_4$) Hg vaporization under severe heating conditions can be completely prevented. The use of a long neck (10 cm or more)-thick walled flask as the digestion container prevents further the Hg loss even with heating at 200 ~ 250°C.

In water samples the concentration of Hg is extremely low and its measurement requires some pre-concentration. Of the methods reported, the dithizone extraction technique described by Chau and Saitoh (1970) was found to be applicable for this purpose. In addition, pretreatment with H_2SO_4 and $KMnO_4$ was found to be essential to obtain satisfactory recoveries. The reason for this is presumably due to the release of ionized Hg compounds from binding sites on organic material and particulate matter in water samples by oxidation. When preparing water samples for analysis, the addition of hydroxylamine-HCl neutralizes the strong oxidizing property of $KMnO_4$ and the addition of EDTA prevents the interference caused by other metals in the sample. Both are therefore added to protect dithizone from oxidation and unnecessary cross-reactions with other metal ions.

In the case of MeHg, the widely used benzene extraction method developed by Westöö(1966) for analysis of environmental samples such as fish and sediment often results in poor extraction efficiency and requires longer time for extraction. In order to overcome the shortcomings in the Westöö procedure, Akagi and Nishimura (1991) developed a highly precise and efficient method for the determination MeHg in wide range of samples. More recently, the refinement of the latter method has enabled accurate and rapid determination of MeHg even at very low background concentrations encountered in water samples.

Pretreatment of biological samples by KOH in C_2H_5OH has been found to be very effective in extracting Hg with Dz–Tol after acidification. Alkaline digestion is advantageous for analysis of MeHg particularly in biological samples since the digests are clear and do not form any emulsion on initial solvent extraction. This can be attributed to the fact that proteinaceous materials are broken down in the sample matrices during digestion. This alkaline treatment can also be applicable to the pretreatment of sediment samples since they contain humic substances and sulfide ions having a great affinity for Hg compounds. The humic substances are soluble in alkaline solution and thus MeHg is released and sulfide ions can be removed by N_2 bubbling.

Westöö reported gas chromatographic methods for estimating MeHg in fish and showed that MeHg dithizonate has the same retention time for chloride or bromide homologues. This finding strongly suggests that the determination of MeHg in various samples by the combination of dithizone extraction and gas chromatographic separation is possible. This was validated by gas chromatography-mass spectrometry method indicating the immediate conversion of MeHg dithizonate into its chloride form. Freshly prepared and purified dithizone solution should be used for the extraction since diphenylthiocarbadiazone, the oxidized form of dithizone, may interfere with the determination of MeHg in the GC-ECD analysis. Furthermore, toluene is preferred over benzene, for health and safety reasons.

The methods described in this paper represent significant advancement over the existing methods for total mercury and methyl mercury determination in environmental and human samples. Whatever methods are used in mercury analysis, the implementation of quality assurance and quality control procedures should be undertaken regularly. The regular use of the appropriate reference materials such as IAEA-085, IAEA-086, and IAEA-142 and other standard materials is recommended. Currently, several standard reference materials are available commercially through the National Institute of Standards and Technology (NIST), the International Atomic Energy Agency (IAEA), and the National Research Council of Canada (NRCC). However, for routine analysis reagent grade Hg compounds may be used to prepare internal standards. The internal standards should, however, be calibrated against reference standard materials to ensure the reliability of the results.

Scientists from tropical and other regions have used successfully the methods described here in the studies of mercury pollution and human exposure to methyl mercury. More research efforts using those analytical methods would facilitate better understanding of the dynamics of mercury pollution in tropical ecosystems. These in turn will help in the formulation of mercury pollution mitigation measures.

References

Akagi, H. Mortimer, D.C. and Miller, D.R. (1979) Mercury Methylation and partitioning in aquatic systems. *Bull. Contami. Toxicol.*, **23**, 372-376.

Akagi, H. and Nishimura, H. (1991), Mercury speciation in the environment, *In* "Advances in Mercury Toxicology" (T. Suzuki, N. Imura and T.W. Clarkson ed.), pp.53-76, Plenum Press, USA.

Akagi, H., Malm,O., Branches, F.J.P., Kinjo,Y., Kashima,Y., Guimarães, J.R.D., Oliveira, R.B., Pfeiffer, W.C., Takizawa Y. and Kato, H. (1995) Human exposure to mercury due to gold Mining in the Tapajós river basin, Amazon, Brazil: Speciation of mercury in human hair, blood and urine, *Water, Air and Soil Pollut.,* **80**, 85-94.

Akagi, H. (1997) Analytical methods for evaluating human exposure to mercury due to gold mining. Proceedings Intern. Workshop: Health and Environmental Effects of Mercury due to Mining Operations. Manila, Philippines: National Institute for Minamata Disease, Japan, pp.131-141.

Andersson, P., Borg, H., and Karrhage, P. (1995) Mercury in fish muscle in acidified and limed lakes. *Water, air and Soil Pollut.,* **80**, 889-892.

Chau Y. K. and Saitoh, H. (1970) Determination of sub-microgram quantities of mercury in lake water. *Environ. Sci. Technol.,* **4,** 839

Guimarães, J.R.D. Ikingura, J.R. and Akagi, H. (2000) Methylmercury production and distribution in river water-sediment systems investigated through radiochemical techniques. *Water, air and Soil Pollut.,* **124**, 113-124.

Horvat, M., Liang, L., Azemard, S., Mandic, V., Villeneuve, J.-P. and Coquery, M. (1997) Certification of total mercury and methylmercury concentrations in mussel homogenate(Mytilus edulis) reference material, IAEA-142. *Fresenius J. Anal. Chem.* **358**, 411-418.

Ikingura, J.R. and Akagi, H. (2000) Methylmercury production and distribution in aquatic systems. *Sci. Total Environ.,* **234**, 109-118.

Kehrig H.A., Malm, O., Akagi, H., Guimarães, J.R.D. and Torres, J.P.M. (1998) Methylmercury in Fish and Hair from Balbina Reservoir, Brazilian Amazon. *Environ. Res.,* **77**, 84-90 (1998)

Kudo, A., Akagi, H., Mortimer, D.C. and Miller, D.R. (1977) Equilibrium concentrations of methylmercury in Ottawa Riber sediments. Nature, 220, 419-420.

Matsuo, N., Suzuki, T. and Akagi, H. (1989) Mercury concentration in organs of contemporary Japanese. *Arch. Environ. Health,* **44**(5), 298-303.

Stephens, G.R. (1995) Mercury concentrations in a remote Canadian Arctic Lake. *Water, Air and soil pollut.,* **80**, 633-636.

Westöö, G. (1966) Determination of methylmercury compounds in foodstuffs. *Acta Chem. Scand.,* **20**, 2131-2137.

Westöö, G. (1968) Determination of methylmercury salts in various kinds of biological materials. *Acta Chem. Scand.,* **22**, 2277-2280.

The Impact of Mercury Mining on the Environment and Human Health

Milena Horvat[1], Qu Liya[2], Vesna Jereb[1], Martina Logar[1], Nataša Nolde[1], Vesna Fajon[1]

[1]Department of Environmental Sciences, Institute Jožef Stefan, Jamova 39, Ljubljana, Slovenia
[2]Guizhou Institute of Environmental Protection, Guizhou Province, Guiyang, China

Abstract

A short review of the mercury research activities carried out in two case studies will be presented: closed mercury mine in Idrija, Slovenia and active mercury mining activity in Guizhou, China. These examples are representative of different environmental conditions. In both of them only a very limited number of studies have been conducted, although, serious pollution with Hg has been present for a long period of time.

Key words: Mercury mining, mercury speciation, and human exposure

1. Introduction

Past and current mercury mines are located along the geological belts naturally enriched with Hg. Mercuriferous belts include East Pacific Rise, Circum Pacific and the Mediterranean-Himalayan belts (Figure 1). The number of active mercury mines has decreased in recent years and only a few are still operational. Historic and current mercury mining represents an important source of mercury in the environment and it should be taken into consideration in regional and global mercury budgets. In this paper preliminary investigations in two case studies will be presented indicating the importance of mercury behaviour in order to properly mange contaminated sites.

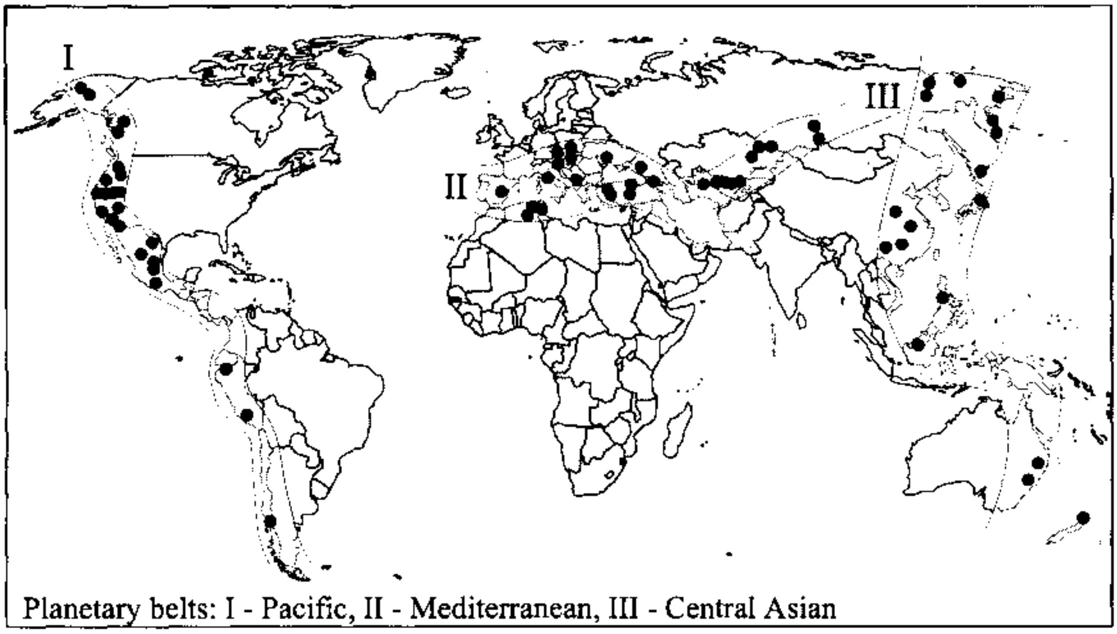


Figure 1. Mercuriferous belts in the world. Black dots represent naturally enriched Hg areas.

Mercury released from mercury mining areas is mostly in inorganic form, either as elemental mercury (Hg^0) or as a cinnabar. The form in which Hg is introduced into the environment dictates its biogeochemical behaviour in various environmental compartments. The most important is the pollution of the atmosphere, which is due to smelting of mercury ore, evaporation of Hg from contaminated soils and active transport of aerosols enriched with Hg. In addition, Hg is transported from waste disposal sites by leaching and by erosion phenomena. Due to these facts the level of contamination in these areas is rather high and widespread. Concentrations of Hg in different environmental samples is very high, in most cases several orders of magnitudes higher tcompared to "clean" areas. During active production of Hg, the main problem is related to professional exposure of workers, mainly in smelting facilities, and inhabitants living in the vicinity. A number of studies conducted in mercury-polluted areas suggest that Hg biogeochemical cycling is different compared to less contaminated areas, due to the induction of mercury detoxification mechanisms. It is therefore important to understand Hg cycling in these environmental conditions in order to properly manage the remediation actions, if necessary.

2. Case study 1: Mercury mine in Idrija, Slovenia

The Idrija mercury mine in Slovenia (Figure 2) is the site of the second largest Hg mine in the world, which was in operation continually for 500 years until about 20 years ago. Over five million metric tones of Hg ore was mined, and much of the residues were spread around the town and its vicinity. It has been estimated that 73% of the Hg mined was recovered (107.000 t), and the remaining 17% dissipated into the environment (Miklavèiè, 1996). One unfortunate outcome of the centuries of Hg mining activity has been the constant exposure of the inhabitants of the area to Hg poisoning, including high Hg levels in miners (Kobal et al 1999).

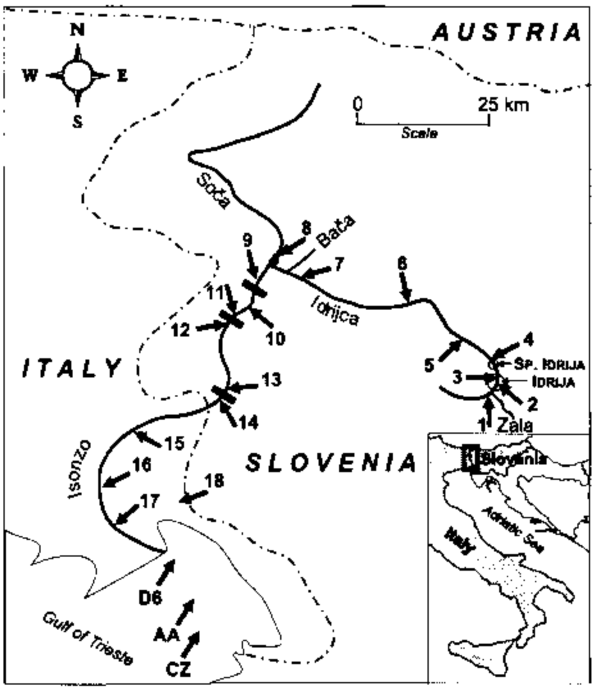


Figure 3. Sampling locations during a sampling period in August 1999 (Horvat et al., 2001)

Most of the airborne Hg was deposited in the Idrija basin and caused elevated Hg levels in the surficial soil layers. Weathering of Hg bearing bedrock and erosion of contaminated soils and dumps of ore residues and mining wastes supply the Idrijca/Soèa river system and ultimately the

Gulf of Trieste with Hg (Covelli et al. 1999, Gosar *et al,* 1996; Pelinkaš *et al,* 1995; Gnamuš *et al,* 1999).

2.1. Mercury speciation in soil, stream sediments and water

Mercury and methylmercury were measured in various environmental compartments during the period 1995-2000. Some recent measurements of mercury in water, sediments and soil are shown in Figure 3. Total Hg in the Idrijca river water increased by a factor of 4 downstream of the mine, with methyl Hg (MeHg) accounting for 1.5 % above the town of Idrija, 0.2 to 0.7 % after Idrija, 2-3% in river reservoirs and only about 0.2% in marine waters. This clearly shows the important role of reservoirs in MeHg dynamics. Concentrations of Hg in sediments and flood plain soils also increased by several fold downstream, with MeHg ranging from 0.01 to 0.1% in riverine and marine sediments, and less than 0.01% of MeHg in flood plain soil.

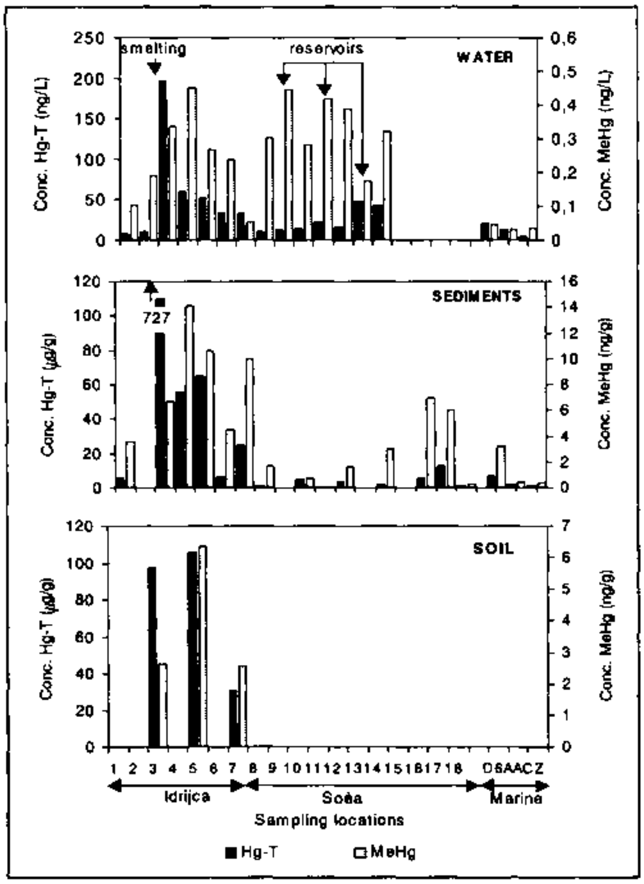


Figure 3. Comparative presentation of the concentrations of Hg in water, sediments (clay/silt fraction) and surficial soil (Horvat et al., 2001)

Concentrations of MeHg in soil samples are lower than in stream sediments and show irregular concentration profiles with depth (Figure 4), most probably reflecting complex hydrological conditions in the region. It is interesting to note that MeHg concentrations in forest soil close to the smelting facilities, about 50m above the river bank, are much higher (65 – 97 ng/g) (Gnamuš *et al.,* 2000) as compared to alluvial ground (2-3 ng/g). This may be related to the different origin of the mercury. Mercury in forest soil close to the smelting house mainly originates from atmospheric deposition of Hg, while in the alluvial ground Hg originates from deposition of particles, in which Hg is present as Hg sulphide (BIESTER *et al.,* 2000). The reactivity of mercury deposited from the atmosphere seems to be much higher than that of unreactive cinnabar particles close to the river bad, resulting in about 20 times higher concentrations of MeHg in soil samples. This is of course important, as during heavy rain Hg and MeHg may be released from contaminated forest soils (less binding capacity than for inorganic Hg) and contribute to the mass of MeHg transported into rivers and later into the sea.

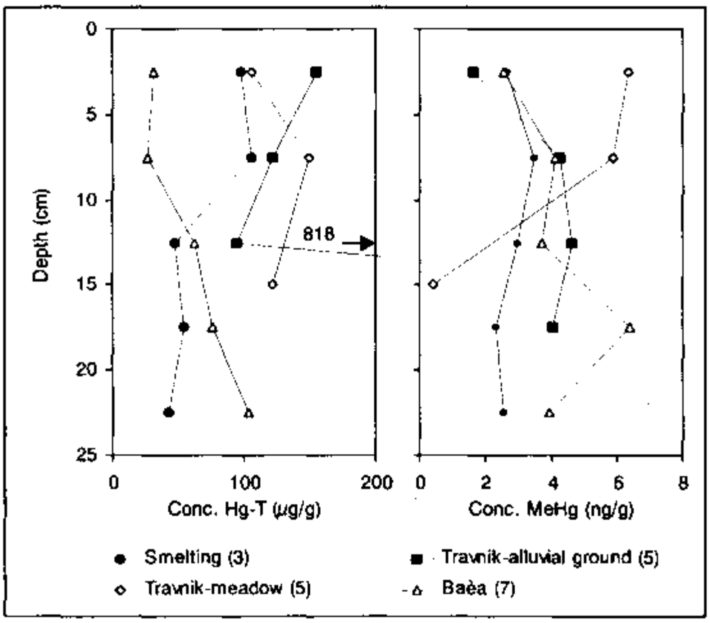


Figure 4. Concentrations of total Hg and Me Hg in the Idrijca alluvial soil profiles (Horvat et al., 2001)

Beside soils that are naturally elevated in Hg due to the high levels of Hg in geological materials in the Idrija district, there are also Hg-laden material and tailings that are continuously eroded and serve as an important additional source for the river, flood plains and the Gulf of Trieste. During such a transport important transformation mechanisms take place, which is evidenced from the presence of MeHg in the various samples analysed. The percentage of Hg as MeHg is highest in water samples, followed by sediments and soil (Figure 5). These data suggests the importance of the water compartment for transformation processes, as it supplies reactive Hg that triggers further transformations of Hg (methylation, demethylation and reduction to Hg). In the town of Idrija, which is highly contaminated with Hg, atmospheric Hg deposition may still be very important. Future studies should carefully address fluxes of Hg from contaminated soil into the atmosphere, its re-deposition on soil and water and its reactivity for further transformation.

This study also reveals that reservoirs play an important role in mercury transformation and transport processes. Further studies should be conducted in order to understand the biogeochemistry of Hg in artificial lakes.

Mercury transport in the study area is mainly driven by hydro meteorological conditions and therefore a careful measurement campaign is necessary to asses accurately Hg transport for mass balance calculations in the Gulf of Trieste and to estimate the temporal variability of Hg transported after closure of active Hg mining.

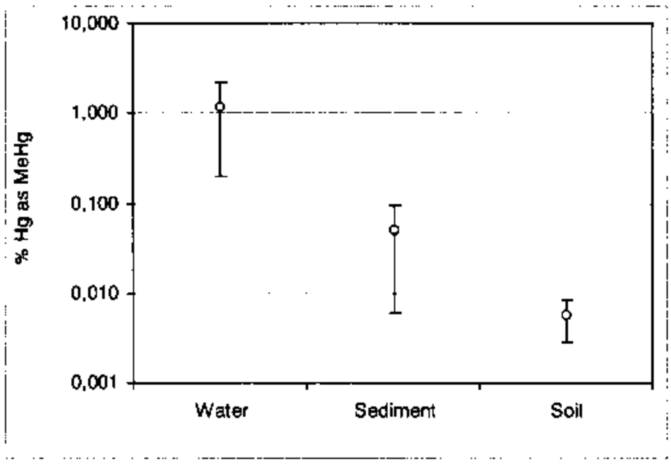


Figure 5. Relative percentage of Hg as MeHg in water, stream sediment and riverbank soil in the Idrija area. Results are presented as mean values and the error bar represents the range (min-max).

Although mining operations in Idrija ceased in 1994 and the mine is currently in the phase of gradual total shutdown, a considerable amount of Hg is still transported from contaminated sites to the Gulf of Trieste, where active transformation of Hg takes place, resulting in elevated Hg levels in fish, consumed by the local population. (Horvat *et al,* 1999; Hines *et al,* 2000; Biester *et al;* 2000). It was found that the most important area for methylation of Hg is the Gulf sediment, while in bank soil demethylation prevails (Hines et al. 2000).

2.2. *Air*

Mercury concentrations in air in the town of Idrija are still very high. They vary from 0.01 to about 0.490 $\mu g.m^{-3}$ and did not show a significant drop during the last ten years, however they are much lower than during the active period of the mining (0.1 to 10 $\mu g.g^{-1}$). The air concentration depends up-on the weather conditions, direction of winds, and sampling locations. There are two major sources: one is close to former smelter and the other is close to the ventilation shaft from the mercury mine. Biomonitoring of Hg air contamination using epyphitic lychens was developed and is succesfully used to estimate average air mercury concentrations in the study area (Lupšina et al. 1993, Horvat et al. 2000).

2.3. *Terrestrial ecosystem*

Mercury distribution and uptake by plants, including mushrooms, and various organisms of the contaminated Idrija region and control areas in Slovenia has been the subject of various studies (Horvat et al. 2000, Byrne et al. 1970, 1995). The results confirmed the effective transfer of Hg from soil (and air) to vegetation, herbivores and carnivores further up the food web. Interestingly, a higher accumulation of MeHg was observed in those environments polluted with high concentrations of inorganic mercury compared to less contaminated and control areas (Gnamuš et al., 1999, 2000).

2.4. *Drinking water and food*

Mercury concentrations in drinking water (surface and groundwater) are below 0.1 $\mu g.l^{-1}$ and are even lower away from Idrija region (Miklavèiè, 1996, Doctor et al. 2000). These values are far below the permissable level of 1 $\mu g.l^{-1}$. This also indicates that total intake of Hg by humans through water consumption is low. The mercury concentration in foodstuffs in the Idrija region varies considerably depending on location and type (Kosta et al. 1974, Stegnar et al. 1973, Miklavèiè, 1999). However, Hg is elevated in almost all food products including meat. The highest concentration were found in fish caught in the Idrijca and Soèa rivers and vary from 1.07 to 1.87 mg/kg, f.w., where the percentage of MeHg in fish increases with distance from the town of Idrija (Miklavèiè, 1996).

2.5. *Health related studies*

There are two major exposure pathways for the local population. One is the exposure to atmospheric Hg in Idrija and nearby surroundings and the other is exposure through food consumption, including fish in the coastal area of the Gulf of Trieste. Today the highest values of Hg in air are still close to the US EPA reference concentrations for chronic inhalation exposure of 0.4 $\mu g.m^{-3}$. This indicates that some inhabitants in is more exposed areas of the town of Idrija are potentially at risk due to exposure to elemental mercury through inhalation. Exposure to inorganic Hg through food consumption other than fish is not considered to pose significant problems. On the contrary, the coastal population shows increased values of Hg in hair (Ingrao et al., 1993) as an indication of exposure to MeHg due to fish consumption. Further comprehensive studies conducted in the region will provide more information about possible health effects.

3. Case study 2: Mercury mining in Guizhou Province, China

3.1. *Mercury contamination in the Province Guizhou, China*

The province of Guizhou (N24°30'-29°13', E103°1'-109°30', 1100 m above sea level, subtropical humid climate) in South-western China is one of the world's most important mercury production areas. The cinnabar deposits in Guizhou, approximately 80,000 tons, represents about 70% of the total in China. Emissions of mercury from the province to the global atmosphere have been estimated to be about 12% of the world total anthropogenic emissions (Nriagu, 1979). Guizhou has produced mercury for 600 years, and the total output was reported to be 26,000 tons from 1949 to 1981, and the peak annual mercury emission from mining and refining to the atmosphere has reached 11 tons (Tan et al., 1997a). In addition, Guizhou is one of the major coal production provinces in China. About 80% of the total energy consumption by a population of 34 million (Tan et al., 1997b) results from coal combustion. In recent years annual coal burning was above $8x10^6$ tons without flue gas cleaning or emission control actions. The concentrations of mercury in coal vary from 0.255 – 0.552 mg/kg (Xinbin et al. 1997).

An additional source of environmental Hg is emission Hg from chemical industries where Hg is used as catilysts for the production of acetaldehyd and other organic compounds. Hg is also used in agriculture, medicine, the electrical industry, extraction of gold and in traditional uses such as red pigments, in medicine, etc. (Jingyi, 1997).

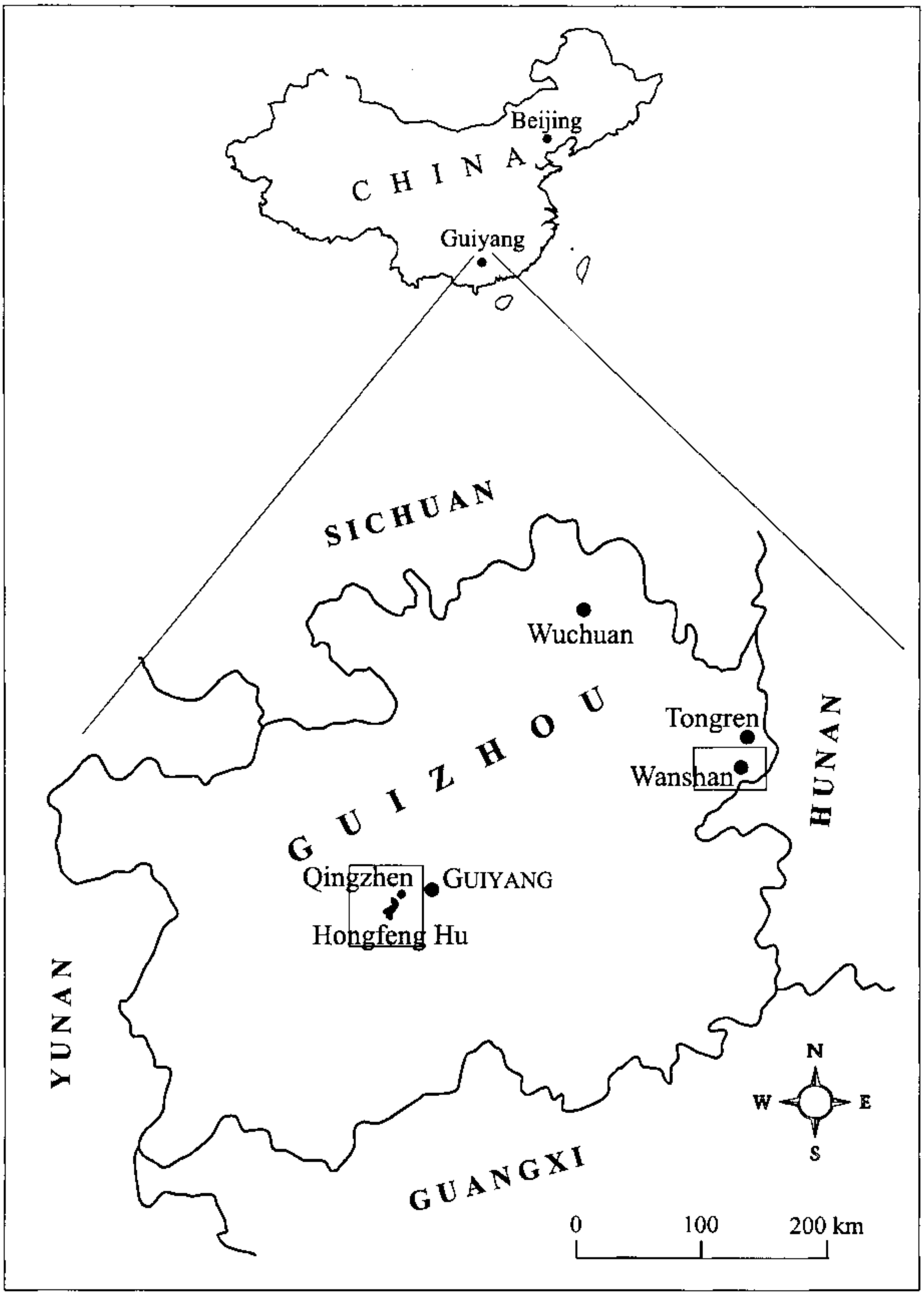


Figure 6: Map of Guizhou province with study areas identified

An inventory of anthropogenic mercury emissions from Guizhou and the atmospheric mercury deposition on Fanjing Mountain Nature Reserve of the province (Tan et al., 1997b, Lindquist et al., 1998, Xiao et al., 1998, He et al., 1997) have been reported. Previous research data show the seriousness of pollution, and strongly suggests much future research work is needed in order to understand Hg transport and transformation mechanisms in this karstic environment and in particular the possible health effects of Hg and its compounds.

Exposure of the local population to Hg may occur due to inhalation of Hg present in air and consumption of Hg contaminated food and water. In the air, the major form of Hg is volatile elemental Hg. The highest value of Hg in air in the city of Guiyang was reported to be 0.566 $\mu g/m^3$ (Liya in Hunyuan, 1999), while the concentrations of Hg in air in mercury mining area are even much higher. These values considerably exceed the USA EPA Reference Concentration (RfC) for chronic Hg exposure (RfC is 0.0004 mg/m^3). These values also exceed the Minimal Risk Level (MRL) as defined by the AT SDR (*Agency of Toxic Substances and Disease Registry*, 1999). Intake of Hg through food consumption is also an important route of Hg exposure. Major sources of Hg in human diet in the region are rice and fish consumption. For example, Hg concentrations in rice in the contaminated area of Wanshan may exceed 0.3 μg T-Hg/g. With a daily consumption of 400 g of contaminated rice, the daily dose for 60 kg person would mean 2 μg T-Hg/kg body weight (it is assumed that 100% is retained in the body), which also exceeds the international recommendations for daily intake of Hg (Liya in Hunyuan, 1999).

3.2. Sampling area

In the framework of the Slovenian-Chinese collaboration sampling was conducted in the period from 21. to 23. September, 2000 in areas presented in Figure 1 and 2. The study area is located in typical karst areas with all its characteristics. Wanshan is a hilly karstic site about 700 m above sea level. Sampling was performed during the rice harvesting period.

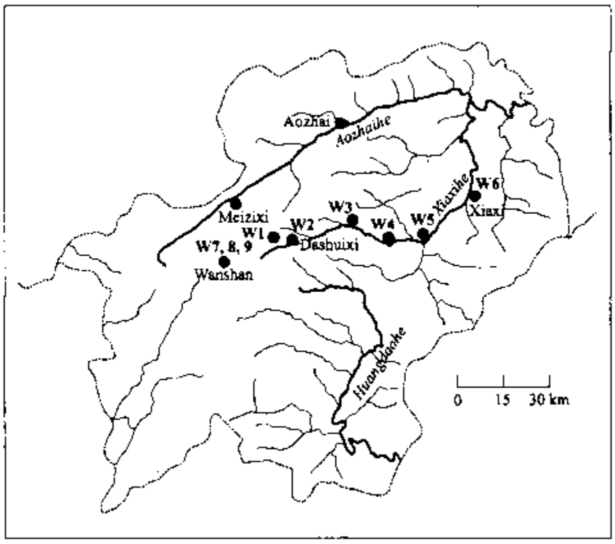


Figure 7: Sampling sites in Wanshan. Sampling sites W7, W8 and W9 are located in the vicinity of the mine, W1 on the mine residues, while the rest of the stations are located along the river Xiaxihe (Horvat et al., 2000).

Three stations were located in the area of the city of Wanshan (W7 – mining and floatation facilities, W8 – smelting facilities, W9 – former waste disposal site). The remaining stations were located in the valley of the river Xiaxihe, into which waters from mining and ignition residues disposal sites are leached. The river Xiaxihe confluence with the river Jin Jiang at Yangtou. Some 25 km upstream the river Jin Jiang another active Hg mine, Tongren, is located. River Jin Jiang is a tributary of the Yuan Jiang river, which flows into a lake Dongting Hu (some 250 km distant from Wanshan). This complex hydrological system is finally connected with the Jangze Kiang River.

3.3. Mercury in soils, sediment and mine residues

Data for total and MeHg are presented graphically in Figure 8. In general there is no correlation between concentrations for total Hg and MeHg, which reflects the well-known fact that the total mercury concentration is not the only factor influencing transformation mechanisms of Hg in different environmental conditions. According to literature data, the % of MeHg in soils very rarely exceeds 1 %.

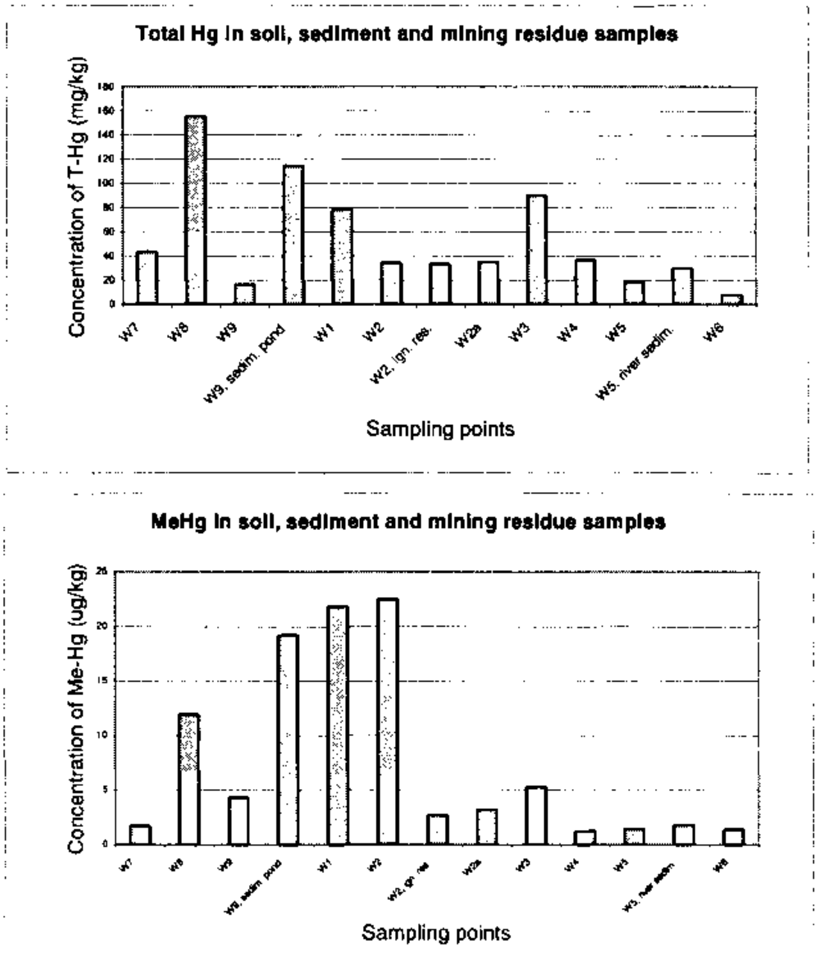


Figure 8: Concentrations of total Hg and MeHg in soil, sediments and mining residue samples (Horvat et al., 2000)

Closer observation of the data in the area of Wanshan reveals some interesting features. The concentrations of total Hg in soils from the rice fields are very high in the vicinity of smelting facilities (W8), where values of over 150 mg/kg were recorded. This value is comparable with values found in contaminated soil near the smelting house in the former Hg mine in Slovenia (Gnamuš et al., 2000). It is assumed that most Hg in this area is deposited from the atmosphere in the form of Hg enriched aerosols. Interestingly, concentrations of Hg in soil close to the mercury mine (W7) are more than three times lower. The sampling point W7 represents an area close to the mercury mine and facilities where Hg enriched particles are separated by the process of floatation. The wastes from this process (mining residues) are then disposed in the vicinity of the mine or elsewhere in the area. This indicates that smelting is a much more active way of contamination of local environment with mercury. It is also interesting to note that the values of MeHg in the smelting area are much higher compared to the area impacted by mining activity alone. This may be explained by the lower reactivity of Hg at station W7, where Hg is present as unreactive HgS.

Sampling area W9 is located close to the city of Wanshan. Until 1960 most of the mining and ignition residues were dispersed in this area. The total Hg concentration in this sample is lower than expected; however the sediment of the fishing pond showed much higher values (over 100 mg/kg). This suggests that the sample taken from the field may be not representative of the whole area. Relatively high concentrations of MeHg were found in pond sediments as compared to MeHg values found in other sampling points of this area indicating a much more favourable conditions for mercury transformation in the aquatic environment as compared to the soil.

Sampling stations from W1 to W6 represent soil samples taken along the river Xiaxihe. The upper stations W1 and W2, where mining and ignition residues are disposed, represent the major sources of Hg in the area. Evidently, the concentrations of total mercury decreased for a factor of 10 at station W6 as compared to station W1. A surprisingly, high value of 90 mg/kg in soil was found at station W3. This sampling station is located on one of the biggest flood plains along the river Xiaxihe and therefore serves as an accumulation area for mercury-enriched particles transferred from contaminated upstream areas during heavy rain events. However, the concentration of MeHg in this area is rather low, as compared to stations W1 and W2, indicating different behaviour of Hg in this rice field, possibly favouring demethylation over methylation processes.

3.4. Mercury in water samples

Water samples from 10 stations were taken and the results are graphically presented in Figure 9. It is well known that water samples represent only information under the particular hydrological conditions during the time of sampling and the data are therefore of limited value only. The data confirm the well-known fact that mercury tends to be bound to particulate matter in water samples (much higher concentrations in non-filtered water samples). There seems to be very little correlation between total and MeHg concentrations in water samples, reflecting environmental differences affecting transformation of Hg in various water environments.

In the area of Wanshan the highest concentrations were found at station W2 where water leached from the mining and ignition residues enters the river Xiaxihe. The dissolved fraction of Hg is also relatively high, indicating high solubility of Hg in leached water at this high pH. Relatively high concentrations of MeHg (0.4 ng/l) were also recorded in this sample. The less polluted Xiaxihe River at station W2a quickly dilutes this concentration. The concentrations at other stations decreased with the distance from the source of pollution; however the values remain elevated even at station W5, some 20 km downstream.

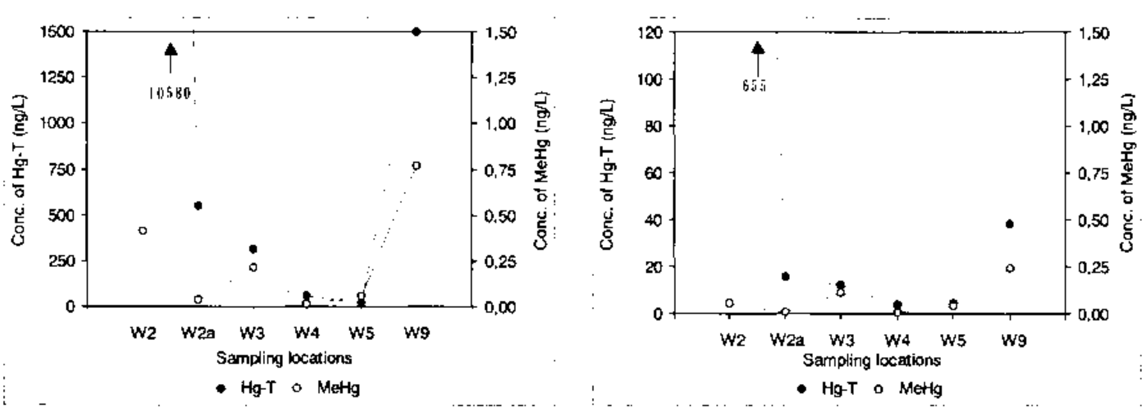


Figure 9: Concentrations of total Hg and MeHg in nonfiltered (left) and filtered (right) water samples

The water in the fish pond (W9) contains highest concentrations of MeHg (0.77 ng/l). It is generally known that in stagnant waters mercury is more easily methylated than in well-aerated river waters. This may well explain MeHg values in the waters of this fishpond. Unfortunately, no fish samples were analysed.

3.5. *Mercury in rice*

Concentrations of mercury and MeHg in rice are presented graphically in Figures 10. At each sampling station the sample was divided into two portions. The hull was removed from the grain (*without hull – brown rice*), as to represent rice as consumed by the local population. Rice grains with hull (*with hull*) was also analysed in order to account for surface contamination, particularly in areas where atmospheric Hg is high. In general, the concentrations for MeHg in rice with and without hull are similar at all stations, probably indicating that atmospheric (or external) source of MeHg are minimal. Evidently, the difference between total Hg in rice with and without hull samples is significant at following stations:

- W8: Close to the smelting house, where the concentrations of Hg in air can be very high and consequently leading to surface contamination of the rice seeds.
- W7: Below the mercury mine where the elevated concentrations in air are mostly due to Hg bound to aerosols as HgS.

The general observation is that the concentrations of total and MeHg decrease with distance from the source of contamination. Most probably Hg in rice in this area reflects combined uptake of Hg from the soil and from the atmosphere. It is difficult to interpret the concentrations in rice vs. Hg in soil. There is no correlation between total or MeHg in soil and in rice. This clearly indicates that the uptake and retention of Hg in rice is influenced by a number of factors, of which the concentration of total Hg in soil may only be one of the variables. One of the stations in the Wanshan area, W2, deserves a very spatial attention. The concentrations in soil are between 33 to 37 mg/kg, but concentrations in rice seeds (rice without hull) are relatively high at 569 ng/g total Hg and 144 ng/g of MeHg.

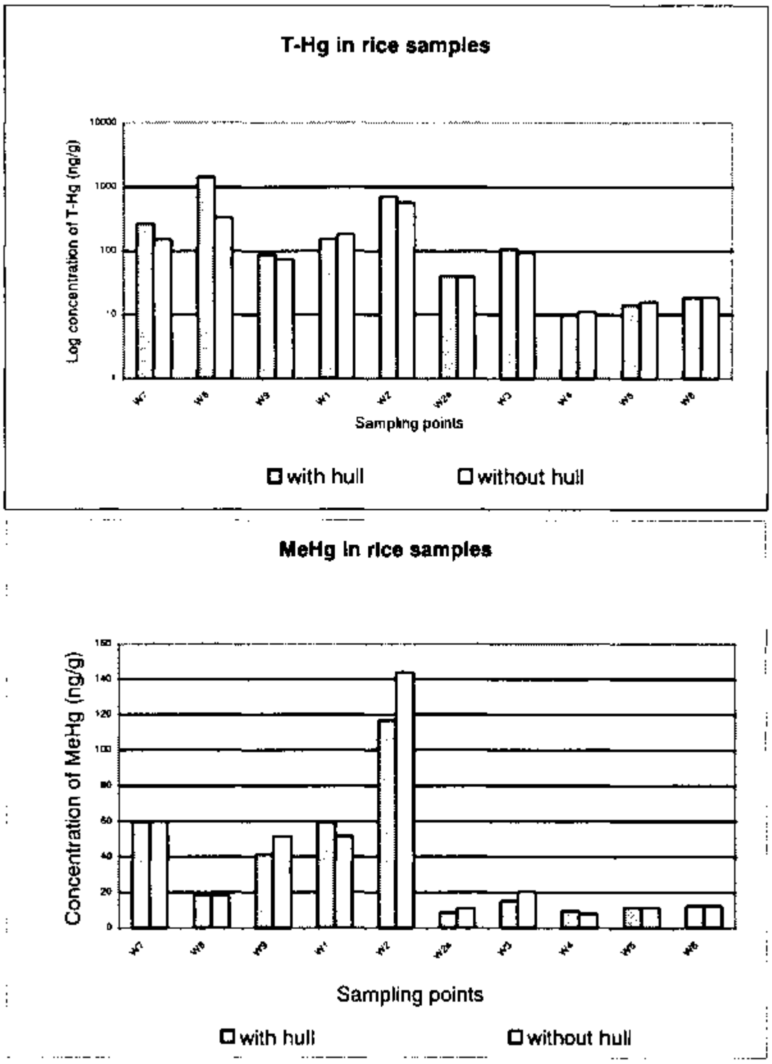


Figure 10: Concentrations of total Hg (left) and MeHg (right) in rice

3.6. Mercury and man

Estimated daily intakes of mercury in the Wanshan area is presented in Table 1. Concentrations of Hg in air fluctuate considerably depending on the temperature, wind direction, humidity, vicinity of the sources of Hg, etc. of a particular area. For the calculation of exposure through the inhalation route, average Hg concentrations in air were used. It should be emphasized that the real body uptake is smaller than presented in Table 1 due to limited absorption of elemental Hg in the lung. Only approximately 80 % of inhaled elemental Hg is retained in the human lung.

The same applies to the estimation of the daily uptake of Hg through food consumption, where the limited absorption of inorganic Hg (difference between total and MeHg concentrations) should be taken into account. In man this proportion is about 8% of inorganic Hg and 100% for MeHg.

According to the recommendations of FAO/WHO the daily intakes are considerably exceeded for MeHg in the area of Wanshan. A significant portion to the total daily intake is also due to inhaled Hg. Populations heavily exposed to elemental Hg through inhalation and MeHg through food consumption are close to the smelting facilities (W8), mining activities (W7) and areas close to the disposal sites for mining residues (W1 and W2) in the Wanshan area. Population groups along the river Xiaxihe (W3-W6) are not heavily exposed to Hg and are not considered as a population at risk.

The Minimal Risk Level (MRL) for chronic exposure to Hg^0 is $0.2\mu g/m^3$, while the LOAEL was found to be $26\mu g/m^3$. Taking these values into consideration, the population in Wanshan considerably exceeds MRL concentrations for Hg^0 are considerably exceeded in Wanshan. The same applies to the area in Quingzhen in the vicinity of thermal power plant where concentrations of elemental Hg may reach up to $0.5\ \mu g/m^3$.

Table 1: Estimated total daily intakes of total Hg and MeHg in adult population in the area of Wanshan (calculated as μg/kg body weigh of 60 kg person (Horvat et al., 2000)

	WANSHAN	
	Inorganic Hg*	MeHg
Rice (400g per day)	**0,003-0.227**	**0,05-0,96**
Fish (100 g per day)	No data	No data
	Elemental Hg**	MeHg
Air	**0,13-0,40**	Not significant

* Conc. of inorganic Hg = (conc. of total Hg - conc. of MeHg). The intake values are corrected for the low absorption of inorganic Hg (cc. 8%)

** For calculation of daily intake by inhalation we assumed that the levels of Hg in air in Wanshan are in the range from 500 to 1500 ng T-Hg/m^3, and in the area of Quingzhen from 5-500 ng T-Hg/m^3.

An interesting outcome of this study is the observation that the percentage of MeHg in rice is rather high and represents the major source of this toxic Hg form to the local population.

The estimated daily intakes for the adult population in the areas studies revealed interesting conclusions. It was assumed that the major routes of exposure are consumption of contaminated food (mainly rice and fish) and through inhalation of air contaminated with Hg. Daily intakes through food consumption are much higher than inhalation in both studied areas. In the area of Wanshan daily intakes for MeHg are considerably exceed the FAO/WHO recommendations.

The third example represents active mining activity in the karstic environment of the Province Guizhou, China. An interesting outcome of this study is the observation that the percentage of MeHg in rice is rather high and represents the major source of this toxic Hg form to the local population. Daily doses of inorganic and organic Hg in this population significantly exceed the international recommendations.

Acknowledgements: This work was conducted in the framework of Scientific and Technological Cooperation between the Republic of Slovenia and the People's Republic of China, Project Number 03-3-01 and the support through the programme activity P531 funded by the Ministry of education, Science and Sport of the republic of Slovenia.

4. References

Barkay T. 1992. Mercury cycle, Vol. 3., Academic Press, 65-74.

Biester, H., Gosar, M., Covelli, S.: *Environ. Sci. Technol.*. **34**, 3329-3336, (2000).

Byrne AR, Kosta L (1970) *Vestn. Slov. Kem. Druš.* **17**: 5-11.

Byrne AR, Skreblin M, Falnoga I, Al-Sabti K, Horvat M, (1995) Acta Chim. Slov. 42/2, 175-198

Byrne, A.R., Kosta, L., Stegnar, P. (1975). *Environ. Lett.,* **8**, 2, p.147-155.

Covelli S, Faganelli F, Horvat M, Brambati A (1999) Estuar. Coast. Shelf Sci., **48(4)**, 415-428.

Doctor DH, Lojen S, Horvat M (2000)*Acta Carsi*col. **29/1**, 79-92.

EPA Mercury Study Report to the Congress, EPA 452/R-97-0003, EPA, USA, December 1997.

Falnoga I, Kregar I, Stegnar P, Tušek- nidariè M (1997) in Proc. of the Ninth Int. Symp. on Trace Elements in Man and Animals- TEMA-9, NRC Press, Ottava, Canada, pp. 489-490.

Gnamuš A, Horvat M (1999). *in Mercury Contaminated Sites*, eds. R. Ebinghouse, R.R. Turner, L.D. de Lacerda, O. Vasiliev, W. Solomons, pp.281-320, Springer-Verlag Berlin, Heildelberg, 1999.

Gnamuš A, Horvat M (1999) In Mercury Contaminated Sites : Characterization, Risk Assessment and Remediation, (Environmental Science). Berlin: Springer, 1999, 281-320.

Gnamuš, A., Byrne, A.R., Horvat, M. (2000). *Environ. Sci. Technol.*, **34**, 3337-3345.

Gosar, M., Pirc, S., Bidovec, M.(1996): Mercury in sediments of River Idrijca In: Book of Abstracts: Idrija as Natural and Antropogenic Laboratory, 22-26 Idrija, May 1996.

Gray, J.E., Theodorakios, P.M., Bailey, E.A., Turner R.R.: *Sci. Tot. Environ.* **260**, 21-33 (2000).

He J, Tan H, Zhou Y, Yao S, Xiao Z, Lindquvist O, and Sammer J. (1997). *Acta Scientae Circumstantiae* **19**, 164-169.

He, J., Tan, H. (1995). *Pedosphere*, **5**, p. 379-381

He, J., Tan, H., Zhao, Y., Yao, S., Xiao, Z., Lindquvist, O., Sammer, J. (1999). *Acta Scientiae Circumstantiae*, **19**, 164-169.

Hines, M.E., Horvat, M., Faganeli, J., Bonzongo, J.-C.J., Barkey, T., Major, E.B., Scott, K.J., Bailey, E.A., Warwick, J.J., Lyons, W.B. (2000) *Env. Res.* **83,** 129-139.

Horvat M, Covelli S, Faganeli J, Logar M, MandiæV, Rajar R, Širca A, agar D (1999) *Sci. Total Environ.*, **237/238**: 43-56.

Horvat M, Jeran Z, Špiriæ Z, JaæimoviæR, Miklavèiè V (2000) J. Environ. Monitor. 2, 139-144.

Horvat M, Nolde N, Fajon V, Jereb V, Logar M, Lojen S, Jaèimoviæ J, Qu Liya, Drobne D, Faganeli J. (2000) Mercury and other elemnts in Guizhou Province, China, IJS-DP 8294, Decembre 2000, (71 pages).

Horvat M, Jereb V, Fajon V, Logar M., Faganeli J, Hines ME., Bonzongo J.-C. (2001) Mercury distribution in water, sediment and soil in the Idrijca and Soèa river systems, Submitted to *Materials and Geosciences*, 2001.

Ingrao G, Belloni P, Santorini GP (1993) Convegno Il Mercurio Neelle Lagune di Grado e Marano, Aspeti Igienico-Sanitari, 1993, Udine, pp-68-75.

Jingyi, L. (1997). Introductory remarks on mercury pollution in China. V: SCOPE Workshop on mercury pollution in Asia, Chagchun, Jilin, China, September 16.-20., 1997, p. 21-23.

Kobal A, Nanut E, Horvat M, Stegnar P, Sabadin A (1999) In Mercury Contaminated Sites: Characterization, Risk Assessment and Remediation, (Environmental Science). Berlin, Springer, 1999, 271-279.

Kosta L, Byrne AR, Zelenko V, Stegnar P, Dermelj M, Ravnik V (1974) *Vestn. Slov. Kem. Drus.*, **21,** 49-76.

Kosta L., Byrne A.R., Stegnar P., Zelenko V. (1973). *Talanta,* **28**, 115-123.

Kosta, L., Ravnik, V., Byrne, A.R., Štirn, J., Dermelj, M., Stegnar, P. (1978). Some trace elements in the waters, marine organisms and sediments of the Adriatic by neutron activation analysis. *J. Radioanal. Chem.* **44**, 317-332.

Lindqvist O, Wei S, Tan H. (1998). Inventory of anthropogenic mercury emission from Guizhou province, China. Book of Abstracts, the Fourth International Conference on Mercury as a Global Pollutant, August 4-8, 1996, Hamburg.

Liya, Q., Hunyan, H. (1999). The status of mercury pollution during resources development in Guizhou Province, China. Personal communication.

Liya, Q., Huiyan, H. 1997. Mercury polution and resource developement in Guizhou, China. V: SCOPE Workshop on mercury pollution in Asia, Chagchun, Jilin, China, September 16.-20., 1997, p. 24.

Lupšina V, Horvat M, Jeran Z, Stegnar P (1992) Analyst, **117,** 673-675.

Miklavèiè V.(1999) Mercury in the town of Idrija (Slovenia) after 500 years of mining and smelting. In. Mercury in Contaminated Sites, Characterisation, Risk Assessment and Remediation (Eds.R. Ebinghaus, R.R. Turner, L.D. de Lacerda, O. Vasiliev, W. Salomons) pp.259-269. Springer-Verlag Berlin Heidelberg New York, 1999.

Nriagu JO. (1979). The biogeochemistry of mercury in the environment. Topics in environmental health, vol. 3. Elsevier/North-Holland biomedical press, Amsterdam, 1979.

Palinkaš, L.A., Pirc, S., Miko S.F., Durn, G., Namjesnik, K., Kapelj, S. (1995): The Idrija Mercury Mine, Slovenia. A semi-millenium of continuos operation: an ecological impact. In: Environmental Toxicology Assessment /ed. M. Richardson, Taylor&Francis Ltd. 317-339.

Širca A, Horvat M, Rajar R, Covelli S, agar D, Faganelli J (1999) Acta Adriat. 40/2, 75-85.

Stegnar P, Kosta L, Byrne AR, Ravnik V (1973), *Chemosphere*, **2**, 57-63.

Tan H, He J, Lindqvist o and Xiao Z. (1997a) Mercury emission from its production in Guizhou province, China. *Guizhou Science* 15, 112-117 (in Chinese, with English abstract).

Tan H, He J, He T. (1997b). Dry and wet deposition of elemental mercury by moss bag near a mercury mine. *Environ. Sci.* **18,** 71-72.

Tan H., He, J., Liang L., Lazoff S., Sommer J., Xiao, Z. F., Lindqvist, O. (2000). Atmospheric mercury deposition in Guizhou of China. *Sci. Tot. Env.,* submitted.

Tan H., He, J., Lindquvist O., Xiao, Z. (1997). Mercury emission from its production in Guizhou province, China. *Guizhou Science,* China,. **15(2),** 112-117.

Xiao Z, Sommar J and Lindqvist O, Tan H, and He J. (1998). Atmospheric mercury deposition on Fanjing Mountain Nature Reserve, Guizhou, China. *Chemosphere* **36,** 2191-2200.

Xinbin F., Yetang H., Bing H., Jianyu, N., Yongxuan, Z. (1997). Forms of Mercury in Some Coals of Guizhou, China. V: SCOPE Workshop on Mercury Pollution in Asia, Chagchun, Jilin, China, September 16.-20., 1997, 17-18.

 agar D, Rajar R, Horvat M, Širca A, Covelli S (2000) *Sci. Tot. Environ.,* **260**, 109-123.

A REVIEW ON THE INVESTIGATIONS OF MERCURY CONTAMINATION USING BIOLOGICAL INDICATORS ALONG THE BRAZILIAN COAST.

Costa, Monica[1] & Kehrig, Helena do A.[2]

1 Departamento de Oceanografia da Universidade Federal de Pernambuco. Cidade Universitária, Recife, PE, 50740-550, Brazil; e-mail: mfc@npd.ufpe.br;
2 Laboratório de Radioisótopos Eduardo Penna Franca, IBCCF, CCS, UFRJ, Rio de Janeiro, RJ, 21949-900, Brazil; e-mail: kehrig@biof.ufrj.br

ABSTRACT

The study of mercury speciation and its cycling in the marine environment has quickly evolved in the last ten years. It remained of paramount importance the role of mercury contamination assessment on coastal environments through biological indicators. The use of biological indicators in the assessment of mercury contamination in tropical coastal environments is still somewhat meager. This sort of work still faces a number of difficulties in developing countries. Mercury analysis requires the establishment of special laboratory setups. In Brazil, these investigations are made by a small number of laboratories. In this review, we will focus on the biomonitoring work done along the Brazilian coast for the last 20 years. The published studies have been scarce, concentrating mainly on Guanabara Bay and other heavily contaminated estuaries. The two types of biomonitors preferred are fish muscle tissue (of various feeding habits) and homogenates of the soft tissues of whole bivalve individuals (mussels and oysters). Some other animal groups (prawns and mammals) have been tested and reported. All the studies available at the recognised sources of literature will be commented here. FAPERJ, PRONEX/CNPq, UFRJ, UFPE.

Keywords: total mercury, methylmercury, marine and estuarine biota, Brazilian coast

1. Introduction

The study of mercury speciation and its cycling in the marine environment has quickly evolved in the last ten years. Sea surface interaction with the atmosphere through a number of processes has been the highlight of these progresses. Those are very important informations of mainly geochemical importance.

However, it remains of paramount importance the role of mercury contamination assessment on coastal environments through biological indicators. The use of biological indicators in the assessment of mercury contamination in tropical coastal environments is still somewhat meager. This sort of work still faces a number of difficulties in developing countries. Mercury analysis requires the establishment of special laboratory setups and training and is therefore, often rated as a secondary priority in detriment of the other metals and contaminants in general. However, only the analysis of biological tissues or organisms as indicators of mercury contamination will be able to generate information on bioavailability and risks for the human health. The intricate behavior of this metal, once in the trophic web, is a complex issue which needs to be addressed when monitoring actions are suggested. Not all the laboratories concentrate the necessary expertise, equipment and funds for a total approach of the problem of mercury contamination in coastal environments. Many more studies could have been done if the Brazilian institutions worked in an articulated fashion, which unfortunately is not always true. The union of efforts around a single important theme is a hard exercise that we are still to learn. This review is a very modest try to bring together institutions from afar.

Mercury contamination is a somewhat punctual type of pollution to the Brazilian coastal environments, being considerable almost only in the vicinities of chlor-alkali plants, where it may be severe. In contrast, knowledge about the contamination resulting form other metals have more successfully developed due to the fast development of multielemental methods and techniques.

In Brazil, these investigations are made by a small number of laboratories. Other institutions, which are analytically competent in mercury analysis on animal or plant tissues, dedicate themselves to problems related to human health, the mining activities and its consequent environmental contamination in the mainland. In Brazil, coastal areas are much less studied concerning mercury contamination than the Amazon region, and other gold mining regions in the country, due to the artizanal goldmining activity being one of the most important sources of mercury to the environment in this country.

Along the Brazilian coastal zone, the easily identified mercury point sources are mainly chlor-alkali plants. However, there are also cases of areas contaminated by agricultural run-off, from sugar-cane plantations for example, which used mercury compounds as chemical defensives in the form of phenylmercury. The published studies have been scarce, concentrating mainly on Guanabara Bay and other heavily contaminated estuaries. A larger number of works have been done on estuarine and coastal sediments contamination by metals, including mercury along the Brazilian coast. These works are skillfully reviewed by subject and geographical region in Niencheski & Baumgarten (1999). Sediments are somewhat easier to sample, to store and report. They don't need to be taxonomically identified, have a somewhat lesser degree of risk concerning rapid biological deterioration and call the attention of researchers mainly due to the cumulative character of the information they provide.

Unfortunately, the majority of the works are still written in Portuguese, reaching a somewhat limited number of readers. Some authors have only recently started to publish their works in the national press and international scientific literature.

In the present review the works concerning mercury studies in the estuarine, coastal and marine biota in Brazil are chronologically listed and described divided in two main phases. The first, more preliminary phase, goes from the beginning of the 1980s up to the first years of the 1990s. This period is characterised by preliminary studies on total mercury concentration. The second phase encompasses the largest portion of the 1990s decade until the year 2000. The second period focus on the aquatic environmental mercury speciation through the estuarine, coastal and marine foodchain. The two types of biomonitors preferred are fish muscle tissue (of various feeding habits) and homogenates of the soft tissues of whole bivalve individuals (both mussels and oysters, depending on the region). Some other animal groups (prawns and mammals) have been tested and reported, however with much smaller frequency. Figure 1 situates the different studies along the Brazilian coast exploited by 22 workers and involving 46 marine or estuarine species.

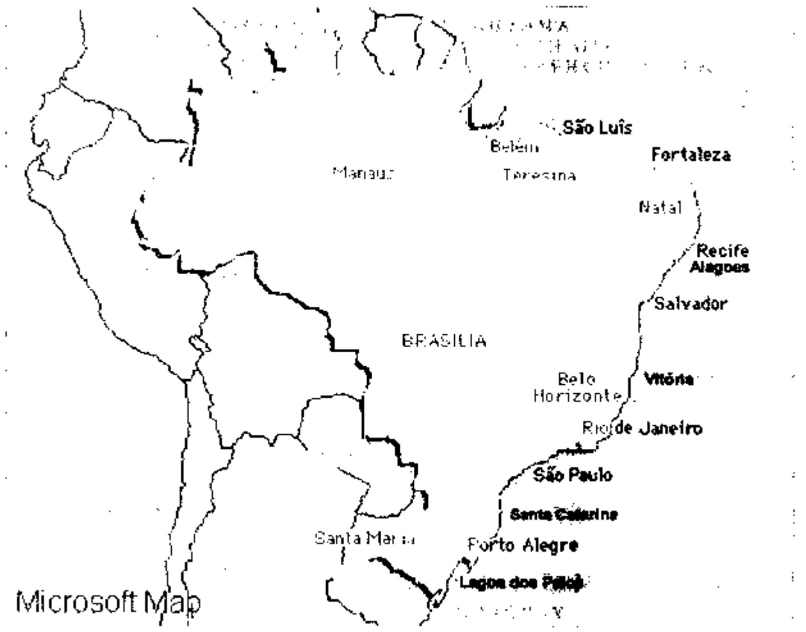


Figure 1: Studied sites on the Brazilian coast. Lagoa dos Patos-Rio Grande do Sul; Lagoa da Conceição-Santa Catarina; Baixada Santista-São Paulo; Waters adjacent to the Paraíba do Sul River Estuary, Baia da Guanabara, Baía de Sepetiba, Baía da Ilha Grande-Rio de Janeiro; Vitória-Espírito Santo; Baia de Todos os Santos-Bahia (Salvador); Lagoa de Mundaú-Alagoas; Canal de Santa Cruz-Recife; Baia de São Marcos-Maranhão (São Luis).

2. The preliminary phase

The earliest published works about coastal or estuarine mercury contamination in Brazil available in the literature date from 1982. The first of these works by Seeliger & Knak (1982) approaches the combined mercury and copper contamination of the Patos Lagoon, at the extreme south of Brazil. At that time their intention was to contribute with a metal contamination baseline study, which would later facilitate monitoring through the use of benthic estuarine algal tissues (*Enteromorpha*). The area was then an expanding industrial district and superport, and on the other hand also a breeding and nursery ground for much of the coastal fauna which migrates through the canal and still represents a significant percentage of the national fishery resources which contamination by mercury would lead to far-reaching biological and economic implications. Tissue levels of mercury in *Enteromorpha* were then already significantly higher nearer Rio Grande in relation to the control stations north and south from that point. Concentrations of bioaccumulated mercury were found to be a linear function of total dissolved metal in the water column, but could be considered low, compared to other estuaries, reflecting low dissolved mercury concentrations in the water. The authors have also pointed to an inverse relationship between particulate mercury in water and algal tissues, causing higher mercury concentrations in *Enteromorpha* when desorption processes associated to salinity changes occur. Their hope was that continuous and frequent monitoring of water and algal tissues could eventually establish a high degree of confidence on the biomonitor response to the water quality, and that future monitoring could be satisfies by analysis of algal samples alone.

The next work, done by Tavares (1982) ecologically characterizes Todos os Santos Bay, in Bahia State (Brazilian Northeast) under the heavy metals (Pb, Cd, Hg) contamination stress. The experimental work was done from April 1980 up to December 1981 and used bivalves as the biomonitors. The abundance, biomass and diversity of bivalves was also measured, as well as the metal content of other compartments for correlation with this portion of the biota. *Anomalocardia brasiliana* was the species with better adaptation to the wide variety of environments in the bay. The mercury concentrations in tissues of bivalves were reported to be below the EPA limit of 1ppm (w.w.). Cd was found to be a more serious problem during this study, showing high levels in *Pitar fulminata*, with the consumption of ~50g exceeding the maximum tolerance limit of 400-500 ppm weekly intake recommended by the WHO.

The second study focus on the inhibition of embryonic development of *Crassostrea rhizophorae* (mangrove oyster) by heavy metals (Cu, Zn, Pd, Cd, Hg), also in Todos os Santos Bay (Nascimento, 1982). This work calls attention to the fact that larvae and embryos of numerous estuarine and coastal animals live in the bay, and that they would be directly exposed to heavy metals pollution provenient from the expanding industrial park, therefore compromising the recruitment of their species. The oyster is a valuable fisheries resource for Bahia. Abnormalities were observed at different concentrations of inorganic salts of mercury and other metals. The calculated EC_{50} concentration at a 95% confidence levels was established as being 0.08-8.73 (x = 2.65) $\mu gHg.L^{-1}$.

Also in 1982, Santos, in her Masters Dissertation at the Federal University of Bahia, made an interesting comparative study of different methods of preservation and mineralization of soft bivalves tissues for mercury analysis.

Later, Moreira and her group, from the Chemistry Department at the Catholic University in Rio de Janeiro, made their first lot of collaborations with three small works done in Guanabara Bay and the Mundaú-Manguaba lagunar complex in Alagoas state (Moreira & Pinto, 1989; Moreira & Amaral, 1990; Moreira & Pinto, 1991). In the first work (Moreira & Pinto, 1989), five different fish species from Guanabara Bay were studied for their total mercury content in the muscle tissues. They choose the fish species based on their feeding habits and their ingestion by the population. All the samples present total mercury concentration below 260 $\mu g.kg^{-1}$ w.w. Only two fish species studied presented a direct relationship between the fish size and the total mercury.

Moreira & Amaral (1990) investigated the mercury concentration in sediment cores and estuarine bivalves from the Mandaú-Manguaba lagunar complex in Alagoas state, Brazilian Northeast. The bivalve specie studied was *Mytella falcata*, which is a valuable local economic resource. The mercury concentration in the sediment ranged from 74 $\mu g.kg^{-1}$ to 398 $\mu g.kg^{-1}$. The average mercury concentration in the soft tissue of *M. falcata* was 15 $\mu g.kg^{-1}$ w.w. in the wet season and 25 $\mu g.kg^{-1}$ w.w. in the dry season.

In Moreira & Pinto (1991) two different fish species (*Micropoganias furnieri* and *Orthopristis ruber*) and one species of crab (*Callinectes* sp.) were chosen based on their different feeding habits and preference for consumption by the local population. *O. ruber* mercury concentration in the muscle tissue (N=22) ranged from 82.8-319.1 $\mu gHg.kg^{-1}$ (w.w.) and; *M. furnieri* (N= 23)varied from 23.0 to 194.8 $\mu gHg.kg^{-1}$ (w.w.). *Callinectes* sp. (N=10) showed concentrations of 34.9–65.8 $\mu gHg.kg^{-1}$ (w.w.) in its edible parts. *M. furnieri* was the only species to show a relationship between mercury concentration and age (in this case calculated based on the individual total length).

The first large, and still very important, publication on mercury done in Brazil was a book edited by S. Hacon and her collaborators in 1990. This book includes some chapters on biomonitoring in estuarine and coastal areas of Maranhão (São Marcos Bay), Rio de Janeiro (Guanabara Bay) and São Paulo states (Boldrini, 1990; Cavalcante et al., 1990; Eysink, 1990; Pinto et al., 1990). The works were presented during a national seminar, held in Brasília in September 1989, on the risks and consequences of mercury use for the natural environment.

Maranhão State had a suspect of mercury contamination due to the installation of a number of mining and other heavy industries in the region. A group was designated to investigate the case and found that, in spite of the accelerated industrialization of São Marcos Bay surroundings and the runoff of the Mearim and Pindaré rivers, the innermost parts of the bay were still uncontaminated by mercury (Cavalcante et al., 1990). The specimens of *Mytella falcata* and *Crassostrea rhizophorae* analyzed showed concentrations in the range of <0.02-0.04 ppm and <0.02ppm respectively, being compatible with previous studies by Juras (1988).

The work presented in the book concerning Guanabara Bay made use of *Perna perna* mussels as bioindicators for mercury contamination during a one year (1988) sampling effort (N=55) (Pinto et al., 1990). Both sampling points used are still frequently harvested by mussel pickers for their living. The concentrations were found to be relatively low, ranging from 0.014-0.049 $\mu g.g^{-1}$. The same sampling sites were later revisited (Costa et al., 2000).

The works of Eysink (1990) and Boldrini (1990) focus on São Paulo state littoral, and were both results from a long term study conduced by the state's environmental protection agency (CETESB). The first study describes the results of 8 amongst 10 large projects of the agency, including a number of aquatic ecosystems in the state, freshwater reservoirs and rivers, as well as estuaries and coastal lagoons. In this case it was possible to observe that mercury was present in the physical compartments within the minimum criteria for the preservation of aquatic life (0.05 $\mu g.L^{-1}$ and 0.2 $\mu g.L^{-1}$), in spite of eventual high concentrations in the biota (maximum 0.5 $\mu g.g^{-1}$ for human consumption, according to the Brazilian laws at the time). It was also possible to measure larger concentrations of mercury in the muscles and edible parts of the species analysed than in their digestive tract, as well as a direct relationship between size and mercury content for various species. Aquatic organisms were then pointed out as excellent monitoring tools to indicate the un-natural presence of mercury in the water, and that, in general, concentrations were higher at the end of the trophic web.

As a general approach it could be said that in the aquatic environments of São Paulo state, only carnivorous fish could, exceptionally, be unfit for human consumption. Amongst the fish, prawns, oysters and crabs analysed none presented mercury levels systematically above the 0.5 $\mu g.g^{-1}$ maximum limit. Slightly high values were detected in the muscle and stomach contents of *Sphoeroides testudineus* (0.4 and 0.12 $\mu g.g^{-1}$ respectively) and *Centropomus spp.* (0.34 and 0.10 $\mu g.g^{-1}$ respectively). The species of economic interest in the estuarine region of Iguape and Cananéia were represented in this study by *Lycengraulis grosidens*, *Anchoviella lepidentostoli*, *Mugil curema* and *Mugil liza*. The highest mercury content amongst these species was detected in *L. grossidens* (0.26 $\mu g.g^{-1}$ in the muscle), and in the others the 0.5 $\mu g.g^{-1}$ maximum limit was never exceeded.

The second study in São Paulo state (Boldrini, 1990) is a much wider and longer one. She studied in detail the evolution of mercury contamination in the different environmental compartments along the Baixada Santista, a highly impacted area, which encompasses the Cubatão region. This study from CETESB dates from 1974, and focus on the Santos and São Vicente estuaries and bay. Cubatão industrial estate was, and still is, responsible for the discharge of numerous and complexes liquid effluents in the region, being followed by port activities, domestic sewage and the Billings reservoir discharges as the responsible for the degradation of water quality there. Associated to this point sources there are agricultural runoffs and the permanent risk of oil spills.

From 1974 to 1978 an early study indicated that the region's aquatic systems were widely contaminated by mercury, above the levels recommended for the protection of aquatic life (0.1 $\mu g.L^{-1}$). In 1975 the first evaluation of the biota was done, aiming to detect possible risks to human health. Fish, mollusks and crustacean were analysed. The maximum value found was 0.795 $\mu g.g^{-1}$, and 16.7% of the samples were above the recommended 0.5 $\mu g.g^{-1}$ limit. Following these results a more consistent monitoring program was undertaken from June 1979 up to march 1980. Eventually high levels were observed in Santos and São Vicente estuaries in the stomach contents of *Mugil liza* (0.98 $\mu g.g^{-1}$), *Achirus* sp. (0.56 $\mu g.g^{-1}$) and in the muscle tissue of *Eucinostomus gula* (0.73 $\mu g.g^{-1}$).

In Santos Bay high levels were observed in all sampling campaigns for the species *Arius spixii* (up to 1 μg.g^{-1}), *Micropoganias furnieri* (up to 4.80 ug.g^{-1}), *Stellifer rastrifer* (up to 0.9 μg.g^{-1}) and *Netuma barba* (up to 1 μg.g^{-1}). These high levels were more frequently observed in the stomach contents of the fish, mainly in species with bottom feeding habits. A significant mercury bioconcentration factors was observed in the muscles (444 to 5611 times) of *Caranx* sp., *Centropomus undecimalis, Eucinostomus gula, Chaetodipterus faber, Bagre bagre, Arius spixii, Netuma barba, Trichiuris lepturus, Cynoscion virescens* and *Micropogonias furnieri*; and in the stomach contents (167 to 5944 times) of *Caranx* sp., *Mugil brasiliensis, Mugil curema, Bagre bagre, Arius spixii, Netuna barba, Achirus* sp., *Micropogonias furnieri* and *Stellifer rastrifer*. The crab *Callinectes danae* also had occasional mercury contents in their edible parts between 0.69 and 0.76 μg.g^{-1} reflecting the environmental mercury contamination accordingly with their detritivorous feeding habit. All the oyster and mussel samples were below the maximum permitted concentration for human consumption.

At that time the leafs of the mangrove trees were also analysed to evaluate their mercury retention from the contaminated sediments. The values for the three species of trees were between 0.0002–0.42 μg.g^{-1} for *Rhizophorae mangle*; 0.0003-0.16 μg.g^{-1} for *Laguncularia racemosa* and; 0.001-0.54 μg.g^{-1} for *Avicenia schaueriana*. In comparison with mangrove leaves from non-contaminated areas, this region was found to accumulate mercury in the plants tissue up to 105 fold. In face of these results CETESB intensified their efforts with the region's biomonitoring in 1983, starting to include the Cubatão River in the campaigns. For these samples specifically, none of the fish or prawns were above the maximum permitted concentration of 0.5 μg.g^{-1} for human consumption. Nevertheless, mercury was always present at detectable levels, denoting undergoing processes of bioconcentration and biomagnification.

Le Campion (1992) investigated a infaunal mollusk (*Tagelus plebeius*) in a lagunar complex at the Brazilian Northeast, the Mundaú-Manguaba Lagoons, Alagoas State. Although he detect relatively high levels of lead and zinc, mercury had very low, near the detection limit, levels.

The last work of this phase was the Masters Dissertation of Kehrig (1992). This study compared the total mercury levels in fish muscles from four important Brazilian estuaries. The main species used for this work was *M. furnieri*. This widely consumed fish occurs along the Brazilian coast and further down to Argentina, being more abundant in the south of Cabo Frio (Rio de Janeiro). A total of 224 individuals were sampled in three estuaries in Rio de Janeiro State (Guanabara Bay (N=61), Sepetiba Bay (N=63), Ilha Grande Bay (N=57)) and also in the Brazilian south, at Conceição Lagoon (N=43), Santa Catarina State, from the summer of 1990 up to the spring of 1991. The concentrations ranged from 13.8 - 433.7 μgHg.kg^{-1} (w.w.), which were all below the maximum limit of 500 μg.kg^{-1} (w.w.) permitted by the Brazilian legislation for food at that time (Brasil, 1975). This species is exclusively carnivore. The other fish species approached was *Macrodon ancylodon*, also consumed by the population, but of a different feeding habit, being an herbivore. The mercury content in the muscle of *M. ancylodon* sampled in Sepetiba bay in the autumn 1991 ranged from 10.1-130.0 μgHg.kg^{-1} (w.w.). Both species presented direct relationships between age (represented by their size and weight separately) and the mercury content in their muscle tissues. It was also possible to observe seasonal variations in the age-normalized mercury concentrations for *M. furnieri*, showing that the physico-chemical characteristics of the water have an influence on the assimilation of the metal by this species. This work was later widely published in the national press (Kehrig et al., 1997) and international literature (Kehrig et al., 1998).

3. The second phase

The second phase of the works on mercury contamination of estuarine and coastal environments in Brazil using biological indicators may be characterised by some new progresses and approaches. First, it is possible to observe the analytical improvement of Brazilian laboratories, result of collaborations with internationally recognised institutions on mercury analysis and environmental diagnosis. Amongst the international institutions which collaborated for the development of Brazilian laboratories NIMD, IJS, IAEA-Monaco and National Research Council – Canada can be cited as the most important and influent. Secondly, it is notable that there was a much larger variety of species and individuals of each species sampled for analysis. The study of different trophic levels, seasonal sampling and increased frequency of sampling in the same environment were also decisive for a better knowledge of mercury dynamics in tropical aquatic systems. Finally, and probably most important of all, the studies done during this second phase started to include mercury speciation and transfer rates and mechanisms to the biological compartment as key features in the diagnosis of estuarine and coastal environments.

The first example of this second phase to approach these advances is a work in Guanabara Bay (Mauro et al., 1997). This work determined the methylation potential of the environment, which was evaluated and rated as low. At the same time, the authors analysed the total and methyl mercury content of four different trophic levels: *Micropogonias furnieri* (carnivorous fish), *Menticirrhus americanus* (detritivorous fish), *Perna perna* (mussel) and *Penaeus brasiliensis* (prawn). The study had also a seasonal characterisation of the mercury content in the edible parts of each species. The levels found were between 17.4-266.8 ng.g^{-1} for *M. furnieri* with 93% of MeHg in average (N=61), 10.5-130.0 ng.g^{-1} for *M. americanus*, 14.0-48.0 ng.g^{-1} for *P. perna*, three times less than in the fish muscle, and <2.8-5.4 ng.g^{-1} for *P. brasiliensis*. If the environmental conditions remain the same, these levels are considered by the authors, as inexpressive regarding human consumption.

In 1998 Pinho, in her Masters Dissertation, made an interesting investigation of total mercury levels in a variety of fish species from the eastern part of the Brazilian ZEE (14-22°S). The mercury concentration in the muscle of 55 samples from four fish species (*Katsuwonus pelamis*, *Lopholatilus villarii*, *Pseudopersis numida* and *Trichiurus lepturus*) had an average concentration of 0.27 ± 0.37 μg.g^{-1} (w.w.). This value was found to be significantly lower than the average value found for the sharks and rays group (1.01 ± 0.94 μg.g^{-1} (w.w.)). She analysed the edible muscle tissue of 160 sharks samples, of different trophic levels, represented by six species (Pinho et al., 1999). The two species of the genus *Mustelus*, which preys mostly on small invertebrates analysed had levels from 0.05-1.54 μg.g^{-1} (w.w.) (average 0.40 ± 0.34 μg.g^{-1}; N=92). The species *Galeocerdo cuvieri* (tiger shark), which is a less selective feeder, had values ranging from 0.17-0.25 μg.g^{-1} (average 0.21 ± 0.04 μg.g^{-1}; N=3). Sharks from the genus *Carcharhinus* (three species) and two species from the genus *Squalus*, which are mainly fish feeders, had concentrations from 0.32-2.57 μg.g^{-1} (average 1.37 ± 0.74 μg.g^{-1}; N=9) and 0.34 – 0.4.06 μg.g^{-1} (average 2.07 ± 0.68 μg.g^{-1}; N=55) respectively. She concluded that higher trophic levels may present a higher risk for human consumption, based on WHO recommendations, especially for those groups with a high shark meat intake in their diet.

Di Beneditto et al. (2000) was the first to investigate levels of mercury contamination in marine mammals in Brazilian waters. Two species of small cetaceans were evaluated in relation to their mercury content in muscles and liver: *Pontoporia blainvillei* and *Sotalia fluviatilis*. *P. blainvillei* (N=7) presented mercury levels of 0.02-0.27 μg.g^{-1} (w.w.) in the muscle tissue and 0.12-2.7 μg.g^{-1} (w.w.) in the liver tissue, being always below the permissible value, according to the Brazilian legislation. Female specimens of *S. fluviatilis* (N=3) presented mercury levels of 0.32-1.40 μg.g^{-1} (w.w.) in the muscle tissue and 1.1-15.8 μg.g^{-1} (w.w.) in the liver tissue. Male specimens of the same species (N=5) presented mercury levels of 0.31-0.91 μg.g^{-1} (w.w.) in the muscle tisue and 1.1-21.7 μg.g^{-1} (w.w.) in the liver tissue. This species presented a higher environmental sensibility in respect to mercury contamination. Both mammals, of restricted migrating habits, occupy the highest trophic level in the food chain around the estuary of Paraiba do Sul River, which is the suspected mercury source for this region of the Brazilian coast. One fish species (*Trichiurus lepturus*) which are preyed by *S. fluviatilis* was also successfully investigated as an attempt to assess the amount of mercury which could be transferred through the food chain.

In 2000 Guanabara Bay was again object of three studies using biological indicators for mercury contamination in that environment (Costa et al., 2000; Kehrig et al., 2000 a; Kehrig et al., 2000 b). Costa et al. (2000) is a study which revisits, after ten years, two mussel banks near the mouth of Guanabara Bay. The comparison of total mercury results in soft tissues of *Perna perna* mussels shows no significant difference between 1988 and 1998 for one of the banks and on the second bank a significant increase was observed. This increase was attributed to the rapid variations in water quality to which the mussels respond in a timespan of a few months. According to the authors, in both banks mussels remained safe for human consumption in respect to mercury only.

Some of these data were used by Kehrig et al. (2000a) in a comparison amongst different trophic levels in the bay. In this study, a total of 331 specimens of *Micropogonias furnieri*, *Mugil liza* and *Perna perna* were collected in differents periods between 1988 and 2000 at Guanabara Bay. The total mercury and the methylmercury concentration in the organisms tissues *were* determined. This is the first studied in Brazil with mercury speciation in tropical estuarine organisms. The analysed organisms chosen are widely consumed by the human population around the bay. All organisms presented both low total mercury (THg) and methylmercury (MeHg) concentrations and they were below the maximum limit of 1,000 μg Hg.kg^{-1} w.w. established for human intake of predators fish, by the new Brazilian legislation (Brasil, 1998). *M. furnieri*, a carnivorous fish showed higher THg and MeHg concentration, and also the ratios of MeHg to THg than organisms with other feeding habits and lower trophic levels. MeHg in *M. liza*, a herbivorous fish, was similar to MeHg concentration in *P. perna*, a bivalve, but there was a significant difference in MeHg/THg ratio: carnivorous fish presented higher MeHg percentage (98 %) than detritivorous fish (54 %) and the bivalve (33 %).

The bioaccumulation factor observed in relation to the total mercury accumulation by the bivalves (N=40) from their surrounding water, was in an order of 10^3 (Kehrig et al., 2000b).

Also in the year 2000, Jesus and co-workers have started investigating the problem of other heavy metals and mercury concentrations in the Vitória Island estuary using as bioindicators the bivalves *Perna perna*, *Mytella guyanensis* and *Crassostrea rhizophorae*.

Santa Cruz Channel, in Pernambuco State, Brazilian Northeast, was object of a number of academic studies between 1996 and 2000 (Meyer, 1996; Meyer et al., 1998; Costa et al., 1999; Sant'Anna et al., 2000a; Sant'Anna et al., 2000b).

Meyer (1996) and Meyer et al. (1998) are works from a PhD Thesis developed through a colaboration between the Oceanography department of the Federal University of Pernambuco and with ZMT-Bremen. Meyer conduced a large compartmental study in the channel, and used the oyster *Crassostrea rhizophorae* as the bioindicator. She aimed to know the physiological responses of the mangrove oyster to mercury contamination and depuration through a transplantation experiment. This experiment revealed the capacity of the species to rapidly adapt to new environmental conditions, reflecting satisfactorily the mercury levels between the two sites. Concentrations in the soft tissue of these oysters remained between 0.27-2.21 ppm along the whole study.

Costa et al., 1999; Sant'Anna et al., 2000a; Sant'Anna et al., 2000b are works on a descriptive exposure survey conduced at the channel to assess the possible risk of mercury contamination to which a coastal human population could be exposed through fish consumption. The area was formerly know to be contaminated due to a chlor-alkali plant installed along one of the tributaries of the channel, Botafogo River. The values for total and methylmercury were measured fish (*Mugil* spp.) catch in the area (26.9 ± 26.1 ng Hg-T.g^{-1} and 19.6 ± 16.0 ng Me-Hg.g^{-1} mean values; N=60). The %Me-Hg in the samples showed an average value of 70.2 ± 29.9. Fish muscle presented relatively low values for total and methylmercury concentrations. These works were the result of a colaboration between NIMD and the Oceanography Department of the Federal University of Pernambuco.

There are already records in the literature of successful studies about molecular level damage caused by the presence of heavy metals in the water for *Crassostrea rhizophorae* in Brazilian estuaries (Rebelo et al., 2000). We hope that these efforts will soon be extended to techniques which target mercury as well. The possibility of early warnings of mercury contamination, and risk for human health due to the of ingestion of contaminated fish, may be of importance especially for top carnivores. The detection of molecular level damage and the very early stages of perturbations caused by anthropogenic mercury, coupled to screening techniques, may lie ahead as the next challenge for Brazilian researchers.

4. Final comments

Although the monitoring of mercury in coastal environments in Brazil has suffered of an intermitent character, it has nevertheless successfully established a sort of "baseline" for mercury in marine and estuarine biota. Mercury levels, with very few exceptions, remained relatively low in the 20 years comprised in this review.

A positive aspect in the evolution of the studies is the shift from a large number of species aproach to less, better know and more cosmopolitan species, which may provide the basis for comparisons amongst different coastal environments in Brazil and other adjacent countries.

Aknowledgements

The authors would like to thank their sponsors and respective institutions for their support in writing this review. Dr. I. Moreira, Dr. W.C. Pfeiffer, Dr. O. Malm, Dr. P.S. Liss and Dr. H. Akagi are especialy named for their careful, professional and patient academic and scientific supervision. They have tirelessly supported our work on mercury in the environment since 1986.

References

ANVISA (1998) Portaria no. 685. Agencia Nacional de Vigilância Sanitária, August 27th 1998.

Boldrini, C.V. (1990) Mercúrio na Baixada Santista. In: **Riscos e conseqüências do uso do mercúrio**, p. 161-195. Hacon, S.; Lacerda, L.D.; Pfeiffer, W.C.; Carvalho, D. (Editors), 1990. Rio de Janeiro, 1990. FINEP, IBAMA, Ministério da Saúde e CNPq. In Portuguese.

Brasil (1975) Diário Oficial da União, Resolução no. 18/75.

Brasil (1998) Agência Nacional de Vigilância Sanitária – Portaria n° 685 de 27/08/98

Cavalcante, P.R.S.; Costa, M.L.; Tarouco, J.E.F. (1990) Avaliação preliminar dos níveis de mercúrio da porção interna do Golfão Maranhense. In: **Riscos e conseqüências do uso do mercúrio**, p. 46-53. Hacon, S.; Lacerda, L.D.; Pfeiffer, W.C.; Carvalho, D. (Editors), 1990. Rio de Janeiro, 1990. FINEP, IBAMA, Ministério da Saúde e CNPq. In Portuguese.

Costa, M.; Akagi, H.; Sant'Anna, N.Jr. (1999) Total and methylmercury levels in the hair of a native coastal population in the Brazilian Northeast. In: NIMD Forum'99, p. 1-9. Minamata, Japan.

Costa, M.; Paiva, E.; Moreira, I. (2000) Total mercury in *Perna perna* mussels from Guanabara Bay – 10 years later. *The Science of the Total Environment, vol. 261*, p. 69-73.

Di Beneditto, A.P.M.; Ramos, R.; Souza, C.M^{a}M.; Carvalho, C.E.V.; Rezende, C.E.; Malm, O.; Kehrig, H.A.; Rebelo, M.F.; Pnto, F.N. (1999) Heavy metals concentrations in two species of marine mammals and their preys from the northern coast of Rio de Janeiro State, Brazil. Paper presented at the 25th International Conference on Heavy Metals in the Environment. August 6-10th, 2000, Ann Arbor, Michgan, USA.

Eysink, G.G.J. (1990) A presença de mercúrio nos ecossistemas aquáticos do estado de São Paulo. In: **Riscos e conseqüências do uso do mercúrio**, p. 12-29. Hacon, S.; Lacerda, L.D.; Pfeiffer, W.C.; Carvalho, D. (Editors), 1990. Rio de Janeiro, 1990. FINEP, IBAMA, Ministério da Saúde e CNPq. In Portuguese.

Hacon, S.; Lacerda, L.D.; Pfeiffer, W.C.; Carvalho, D. (Editors) (1990) **Riscos e conseqüências do uso do mercúrio**. Rio de Janeiro, 1990. FINEP, IBAMA, Ministério da Saúde e CNPq. 314 pages. In Portuguese.

Jesus, H.C. & Brandão, G.P. (2000) Avaliação preliminar dos teores de mercúrio em ostras e sururus no estuário de Vitória-ES após acoplamento da técnica do vapor frio com a GF-AAS. ECOTOX – Resumos, p. 103. 3-6th September 2000, São Carlos-SP, Barsil. Abstract in Portuguese.

Juras, A.A. (1988) A preliminary survey of heavy metal concentrations in some estuarine organisms in the litoral zone of São Luiz Island, Maranhão, Brazil. In: Metals in the Coastal Environments of Latin America, p. 16-20. Edited by U. Seeliger; L.D. Lacerda; S.R. Patchineelan. Springer-Verlag, Berlin.

Kehrig, H.A. (1992) Estudo comparativo dos níveis de concentração de mercúrio total em corvinas (Micropogonias furnieri) de quatro estuários brasileiros. Masters Dissertation, 122 pages. Departamento de Química, PUC-RIO, Rio de Janeiro-RJ, Brazil.

Kehrig, H.A.; Malm, O.; Moreira, I. (1997) Mercúrio: uma avaliação na costa brasileira. *Ciência Hoje, vol. 22*, No. 132, p. 20-22, October 1997. In Portuguese.

Kehrig, H.A.; Malm, O.; Moreira, I. (1998) Mercury in a widely consumed fish *Micropoganias furnieri* (Demarest, 1823) from four main Brazilian estuaries. The science of the Total Environment, vol. 213, p. 263-271.

Kehrig, H.A.; Moreira, I.; Malm, O., Pfeiffer, W.C. (2000b) Especiação e acumulação de mercúrio pela biota da Baía de Guanabara. In: **WORKSHOP EFEITOS DE POLUENTES EM ORGANISMOS MARINHO.** *In press*. Villipress editora. In Portuguese.

Kehrig, H.A.; Pinto, F.N.; Costa, M.; Moreira, I.; Malm, O. (2000a). Methylmercury and Total Mercury in Three Different Marine Organisms from Guanabara Bay. ESPR – Special Issue No. 1 on the 7th FECS Conference 2000, p. 24.

Le Campion, G.L. (1992) Níveis de concentração de cobre, chumbo, zinco e mercúrio, em "unha-de-velho" (*Tagelus plebeius*) do canal do Calunga, Alagoas, Brasil. Masters Dissertation. Departamento de Oceanografia, Federal University of Pernambuco, Recife-PE, Brazil. 239 pages. In Portuguese.

Mauro, J.B.N.; Kehrig, H.A.; Guimarães, J.R.D.; Malm, O. (1997) Metilação e incorporação de mercúrio pela biota da baia de Guanabara (RJ) – Brasil. VII COLACMAR, 22-26th September 1997. Resumos Expandidos, Volume II. São Paulo. In Portuguese.

Meyer, U. (1996) On the fate of mercury in the northeastern Brazilian mangrove system Canal de Santa Cruz, Pernambuco. ZMT-Contribution 3. Center for tropical Marine Ecology, Bremen, Germany.

Meyer, U.; Hagen, W.; Medeiros, C. (1998) Mercury in a northeastern Brazilian mangrove area, a case study: potential of the mangrove oyster *Crassostrea rhizophorae* as bioindicator for mercury. *Marine Biology, vol. 131*, p. 113-121.

Moreira, I. & Amaral, O. (1989) Mercury levels in sediments and organismsof Mundaú Lagoon, Brazil. **Heavy Metals in the Environment**, vol. 1, p. 140-143. J.G. Farmer – CEP Consultants LTD (Editor), England.

Moreira, I. & Pinto, A.P. (1991) Mercury in fish and crustacea from a tropical estuary. Heavy Metals in the Environment – International Conference. Editor J.G. Farmer, Volume 2. Edinburgh, September 1991. p. 191-194.

Moreira, I. & Pinto, A.P.F. (1990) Mercury levls in fish from Guanabara Bay, Brazil. **Environmental Contamination**, vol. 1, p. 606-608.

Nascimento, I. A. (1982) Inhibition of embryonic development of the mangrove oyster (*Crassostrea rhizophorae*) by heavy metals. *Atlântica, vol. 5*, No. 2, p. 84. Abstract.

Niencheski, L.F.H. & M.G.Z. Baumgarten (1999) **Oceanografia Química – Levantamento bibliográfico e identificação do estado atual do conhecimento.** MMA, CIRM, FEMAR. 172 pages.

Pinho, A.P. (1998) Mercúrio total em elasmobrânquios e teleosteos da costa leste do Brasil. Masters Dissertation. Instituto de Biofísica Carlos Chagas Filho, Federal University of Rio de Janeiro. Rio de Janeiro, 95 pages. In Portuguese.

Pinho, A.P.; Guimarães, J.R.D.; Costa, P.A.S.; Martins, A.S.; Olavo, G.; Gadig, O.B.F. (1999) Total mercury in edible tissues of sharks from east Brazil offshore waters. Mercury as a Global Pollutant – 5th International Conference Book of Abstracts, p. 214. May 23-28th, Rio de Janeiro, Barzil.

Pinto, A.P.F.; Costa, M.F.; Amaral, O.; Moreira, I. (1990) Mercúrio em mexilhões da Baia da Guanabara. In: **Riscos e conseqüências do uso do mercúrio**, pp. 196-199. Hacon, S.; Lacerda, L.D.; Pfeiffer, W.C.; Carvalho, D. (Editors), 1990. Rio de Janeiro, 1990. FINEP, IBAMA, Ministério da Saúde e CNPq. In Portuguese.

Rebelo, M.F.; Moraes, M.O.; Silva Jr., H.; Sampaio, E.P.; Pfeiffer, W.C. (2000) Detecção e acúmulo do mRNA de metalotioneínas por RT-PCR: biomarcador da contaminação por metal em ostras (*Crassostrea rhizophorae*). ECOTOX – Resumos, p. 85. 3-6th September 2000, São Carlos-SP, Barsil. In Portuguese.

Sant'Anna Jr., N.; Costa, M.; Akagi, H. (2000a) Níveis de mercúrio total e metilmercúrio no cabelo de uma população costeira e peixes do nordeste do Brasil. In: **ECOTOXICOLOGIA – Perspectivas para o século XXI**, p. 229-240. E.L.G. Espíndola et al. (Editors). Rima Ed., São Carlos, SP. In Portuguese.

Sant'Anna Jr., N.; Costa, M.; Akagi, H. (2000b) Total and Methylmercury Levels in Hair of a Coastal Human Population and Fish from the Brazilian Northeast. ESPR – Special Issue No. 1 on the 7th FECS Conference 2000, p. 79.

Santos, V.L.C.S. (1982) Estudo comparativo dos efeitos de diferentes métodos de secagem e mineralização na análise de mercúrio em moluscos. Masters Dissertation, Federal University of bahia, 153 pages. In Portuguese.

Seeliger, U. & Knak, R.B. (1982) Origin and concentration of copper and mercury in water and biota of the Patos Lagoon estuary, Brazil. *Atlântica, vol. 5*, p. 35-42.

Tavares, T. (1982) Caracterização ecológica da baía de Todos os Santos (Bahia-Brasil) sob impacto de poluição por metais pesados. *Atlântica, vol. 5*, No. 2, p. 116. Abstract in Portuguese.

MERCURY IN FRESH WATER SYSTEMS – A SYNOPSIS

J.R. Ikingura
University of Dar es Salaam, Faculty of Science, Tanzania

Abstract

The knowledge of mercury speciation, partition, and accumulation in fresh water systems has improved significantly within the last decade. The advent of highly sensitive and accurate techniques for the detection and analysis of mercury in a wide range of environmental matrices and development of ultra-clean sample handling and analysis protocols have greatly enhanced the accuracy of Hg analyses. This in some cases has led to revision of previously published data on background levels of mercury, especially in water.

Primary mercury species of environmental significance in the cycling of mercury in surface environment include Hg^0, Hg_2^{2+}, and Hg^{2+}. From these species a number of inorganic and organic compounds and complex ions are formed in aquatic systems. Various research efforts have been directed towards understanding processes and roles of environmental factors that are involved in the transformation of inorganic mercury species into more toxic and highly bio-accumulative methylmercury in the mercury cycle. Environmental factors such as temperature, pH, redox potential (Eh), organic matter content, nutrient supply and microbial activity are known to influence mercury methylation and its uptake by fish and other organisms in the aquatic environment. However, the roles and importance of the individual factors may differ in different environments and hence making it difficult to extend or apply data from one area or geographical location to another. More challenging is the complexity of evaluating the interplay of the different factors in a single unified field or laboratory study.

In spite of the challenges in understanding the mercury cycle in fresh water systems, significant steps have been made for example in determining net methylation rates of mercury at ambient mercury levels using high specific activity ^{203}Hg radiotracer. The mechanism for bioaccumulation of mercury, particularly methylmercury, in fish is being re-examined in light of new data suggesting effective transfer of methylmercury at low trophic levels as the main cause for the bioaccumulation rather than the lipophilic character of methylmercury. Environmental factors affecting the oxidation, mobility and bioavailability of mercury from gold mining operations under tropical conditions are being actively investigated. These and other issues of mercury research in freshwater systems are discussed in the paper.

Introduction

Our understanding of mercury behaviour in fresh water systems has improved considerably in recent years because of intensive efforts in mercury research by the international community. Useful literature on mercury has accumulated from the biannual conference series on "Mercury as a Global Pollutant" since 1990, when the first conference was launched in Stockholm, Sweden. Valuable information on mercury in temperate aquatic ecosystems has been derived mainly from elaborate studies conducted in the Swedish and Finnish lakes (e.g. Bishop et al, 1995; Lee et al., 1995; Verta and Matilainen, 1995), the Experimental Lakes Area (ELA) of northern Canada (Rudd et al, 1995), the Onondaga Lake in New York (Jacobs et al, 1995), and in a number of lakes in the Adirondack region of USA (Driscoll et al, 1995). Studies of mercury pollution in the Brazilian

Amazon have provided some new insights on mercury behaviour in the aquatic environment, especially under tropical conditions.

Issues that have received special attention in the last decade include mercury sources and budget in freshwater systems (e.g. lakes, reservoirs), the role of various environmental factors such as temperature, pH, redox potential, dissolved organic carbon, micro-organisms, and various ligands in the mobility, methylation, and bioaccumulation of mercury in aquatic systems, and mechanisms of methylmercury transfer to fish. Others include the dynamics of mercury in watersheds, distribution and partitioning of mercury and methylmercury between sediment, water and the aquatic food web, and the determination of important sites for mercury methylation and demethylation.

This paper gives a brief review of mercury in freshwater systems and points out areas where more research efforts are needed to better understand the fate and impacts of mercury in freshwater ecosystems. The distribution, transformation and accumulation of mercury species in three main compartments of the aquatic system namely sediment, water, and biota are discussed in the light of experimental and field data from various regions.

Mercury in freshwater sediments

Sediments are the largest depositories or sinks for various metals including mercury in aquatic environment. Weathering of terrestrial rocks and erosion of soil provide ample sources of mercury to aquatic systems. Mercury released by weathering is transported by surface water in solution or particulate matter before getting deposited in river and lake sediments. Mercury concentrations in pristine sediments depend on the mercury levels in the country rocks that are undergoing weathering. Sediments derived from country rocks with elevated mercury concentrations have high mercury content and vice versa.

Wet and dry atmospheric deposition of mercury, especially in industrialised regions, contributes to mercury load in soil, sediment, and water bodies (Hoyer et al., 1995). The discharge of mercury from point sources produce high concentrations of mercury in sediments in the affected areas. The point sources include chloro-alkali plants, paper and pulp industries, battery factories, and industries using mercury in the production of electric and electronic components. Mercury contaminated municipal waste sludge also may contribute to mercury load in sediments.

The use of mercury in gold extraction is a major source of mercury contamination of river and lake sediments in many developing countries in Latin America, Africa and Southeast Asia. Mercury concentrations as high as >20 mg/kg have been reported in river sediments contaminated by gold mining operations in the Amazon (Malm et al, 1990) and in Philippines (Appleton et al., 1999).

The most abundant mercury species in sediments is Hg^{2+}, which may occur as free ions (reactive Hg^{2+}) in pore water and colloidal particles in the sediments, or may be adsorbed on mineral grains and organic matter. Unreactive Hg^{2+} in sediments may be firmly bound or locked in mineral compounds or particles. Elemental mercury (Hg^0) is formed by the reduction of Hg^{2+} in sediments under strong reducing conditions. Areas receiving point source discharges of Hg^0 could have high concentrations of elemental mercury in sediments, as in the case of gold mining areas were metallic mercury is used in gold extraction. Organic mercury species, especially monomethylmercury (CH_3Hg^+), are formed in the sediments by the methylation of inorganic mercury. Methylmercury in sediments is associated mainly with organic matter, especially humic matter. The equilibrium level of methylmercury generally do not exceed about 1.5% of total mercury present in the sediments (Hovart, 1996).

Mercury in freshwater

Concentrations of total mercury in natural waters are generally very low, typically in the range of a few nanograms per litre. Thus accurate determination of the concentrations of mercury species in water poses serious challenges. Water mercury levels reported in old literature before the advent of ultra-clean and ultra-sensitive water mercury analysis protocals generally tend to be higher than those reported in recent years. While reliable and accurate determinations of total mercury in water are now available in numerous literature, very few analyses of other mercury species particularly methylmercury are found. Better understanding of mercury speciation in water is thus still beset with difficulties of obtaining reliable quantitative data for common mercury species (Hg^0, Hg^{2+}, CH_3Hg^+). Dimethylmercury ($(CH3)_2Hg$) has been reported in deep ocean water but its presence in fresh surface water is not ascertained (Mason et al., 1995a).

A survey of published data on total mercury (THg) and monomethylmercury (MeHg) levels in uncontaminated rivers and lakes shows the reported values to be mostly in the range of 0.1 to 5 ng/L for THg, with MeHg making approximately 1 to 10% of the THg. For example, THg and MeHg levels of 3.64 ng/L to 4.04 ng/L and 0.25 to 0.65 ng/L, respectively, are associated with drainage rivers in northern Sweden (Lee et al., 1995). In pristine Adrondack lakes in USA, the concentration of THg in drainage lakes range from less than 1ng/L to above 6 ng/L while MeHg concentrations range from 0.03 to 0.70 ng/L (Driscoll et al, 1995). Total MeHg is reported to be about 10% of THg in most Adirondack lakes. At the Experimental Lakes Area (ELA) in northern Canada, THg MeHg concentrations in streams and surface runoff that drained upland areas and wetlands were more variable. Most streams had THg concentrations between 2.2 and 14 ng/L and MeHg between 0.11 and 2.7 ng/L (Kelly et al, 1995). The MeHg levels were generally elevated in streams draining a catchment with a headwater wetland.

Research findings from whole-ecosystem lake studies have shown that there are three important sources of MeHg to aquatic systems, namely precipitation, surface runoff and streams from wetlands, and in-lake methylation (Rudd, 1995). The relative importance of these sources varies in different ecosystems.

The relationship between MeHg and THg in aquatic systems has been extensively explored in several studies to determine if THg concentration could be used as a good predictor of MeHg concentration (Kelly et al, 1995). The studies suggest that THg might be a good predictor of MeHg concentrations for lakes within individual geographic areas but not for streams or runoff. The relationship of MeHg to THg was found to be highly variable with time in streams and surface runoff whereas in the lakes the relationship was linear, with correlation coefficients in the order of 0.7 to 0.8. The slopes of the regression lines for mercury data from the lakes in different geographical locations varied by a factor of 3, suggesting that a predictive relationship between MeHg and THg in one region may not be applied in another (Kelly et al., 1995).

Mercury in fish

Mercury is probably the only metal known to accumulate in large concentrations in fish and which undergoes biomagnification up the food chain. About 60-95% of mercury accumulated in fish muscle is normally methylmercury (Hovart, 1996). However, methylmercury percentages in fish muscle could be much lower (<50%) in highly contaminated areas. Mercury levels in fish are known to increase with fish age and trophic level. Thus high trophic level predatory or piscivorous fish species generally contain higher mercury concentrations than low trophic level non-predatory species. According to the World Health Organisation (WHO, 1976), freshwater fish from non-polluted areas contain mercury levels in the range of 100-200 µg/kg. However, there are increasing data in the literature showing much lower mercury concentrations in freshwater fish (< 50 µg/kg), both from tropical and non-tropical areas (Ikingura and Akagi, 1996).

Mercury concentrations exceeding the WHO limit of 0.5 mg/kg have been found in high trophic level fish species in lakes or reservoirs in Scandinavia and north America (Schilts and Coker, 1995; Anderson et al., 1995,), and in several rivers impacted by mercury pollution from gold mining activities in the Amazon (Malm et al., 1995a, 1995b; Akagi, 1998). High mercury concentrations have been found also in fish in remote lakes and rivers without any direct influence of anthropogenic mercury input (Driscoll et al., 1994). In the latter case, the high background of mercury in the soils and country rocks or long-range atmospheric mercury transport and deposition are considered to be sources for elevated mercury concentrations in the aquatic system and the associated fish.

Severe toxicological effects of mercury in humans are largely due to exposure to methylmercury through prolonged consumption of fish containing high mercury concentrations. However, despite many years of research, processes and mechanisms leading to methylmercury production and accumulation in fish in aquatic systems are still poorly understood. We are still unable to predict which levels of total mercury or MeHg/THg ratio in water column lead to high accumulation of mercury in fish or which physical-chemical parameters favour the production of methylmercury in sediment or water and the effective transfer of methylmercury to fish. Mason et al. (1995b) have shown that most of the discrimination between inorganic and MeHg occurs during trophic transfer while the major enrichment factor is between water and phytoplankton. Hence, MeHg levels in fish are considered to be determined by water chemistry which controls MeHg speciation and uptake at the base of the food chain and not the lipophilic character of MeHg.

Studies conducted in Swedish and other lakes have demonstrated the presence of a negative correlation between total mercury concentration in fish and water pH (Anderson et al., 1995; Horwitz et al., 1995), whereby the decrease of lake pH was accompanied by the increase of mercury levels in fish. The role of pH in regulating MeHg levels in water and the uptake of MeHg by fish is, however, not well clarified. Miller and Akagi (1979) demonstrated that lowering the water pH does not necessarily increase the total amount of MeHg in sediment-water systems but instead increases the solubility or partitioning of mercury into water and hence the amount of mercury available for uptake by fish. Other studies indicate that pH changes affect the overall production and partitioning of MeHg in the aquatic systems (Mauro et al., 1999). In other aquatic biota, the concentration of total mercury and the percentage of methylmercury are variable depending on a number of parameters including the species of organism, water depth, trophic level and feeding habits.

Mercury transformation and partitioning

The major processes of mercury transformation in freshwater environment involve the inter-conversion of Hg^{2+}, CH_3Hg^+ and Hg^0 species. Redoxic reactions with or without bacterial involvement are responsible for the inter-conversion of elemental and mercuric mercury. The conversion of mercuric to methylmercury and vice versa is now widely accepted to be mediated by micro-organisms. Among the diverse bacteria known to methylate Hg in culture, sulfate reducers are considered to be important methylators in the natural environment (Kelly, 1995). While many studies on mercury transformation have focused on understanding the environmental factors controlling the production, partitioning and bioaccumulation of methylmercury in aquatic systems, very few studies have investigated the degradation of methylmercury. So far no methods have been developed to determine gross methylation or demethylation under laboratory or field conditions. Equilibrium methylmercury concentrations reflect the relative rates of mercury methylation and demethylation processes in a given environment.

Methylation of mercuric ions (Hg^{2+}) occurs predominantly in aquatic sediments and to a lesser extent in the water column (Compeau and Bartha, 1984). Several studies have demonstrated that highest net methylation rates occur in the uppermost, 1 cm layer of the sediment column, below the sediment-water interface (Ikingura et al, 2000). Also roots of floating aquatic macrophytes have been identified as important sites for Hg methylation (Mauro et al., 1999). Factors that have been found to control methylmercury synthesis and accumulation in aquatic systems include temperature, microbial activity, organic matter content, Hg^{2+} concentration, sulfide levels, pH, and redox potential. Warm temperatures favour higher levels of MeHg production as demonstrated by seasonal variations in MeHg levels in streams draining wetland catchments (Lee et al, 1995) and MeHg synthesis in aquatic macrophyte roots (Mauro et al, 1999). High organic matter content and high microbial activity intensity promote MeHg production and accumulation as indicated by the increase of MeHg concentrations in water and fish in new reservoir impoundments (Anderson et al, 1995) The increase of Hg^{2+} concentration increases methylmercury synthesis and accumulation within certain limits depending on other environmental conditions (Ikingura and Akagi, 1999). The redox effects on MeHg synthesis are thought to be linked to the types of microbial metabolism they permit (Compeau and Bartha, 1984). Results of epilimnetic and hypolimnetic methylmercury studies in several lakes have confirmed the synthesis and stability of methylmercury to be favoured by anaerobic than aerobic conditions (Matilainen, 1995). High sulfide levels in reduced sediments suppress the methylation by reduction of Hg^{2+} concentration through mercury volatilisation (Hg^0) or precipitation (HgS). Low pH (3-6) conditions stimulates methylmercury production in the water column and at the water-sediment interface (Wren and Stephenson, 1991; Mauro et al., 1999) and favours the partitioning of MeHg into the water.

Determination of net methylation rates of mercury in aquatic systems has been achieved using both isotopic and non-isotopic methods (Stordal and Gill, 1995; Guimaraes et al., 2000; Ikingura and Akagi, 1999). A few studies have used high specific activity ^{203}Hg as a tracer to study the methylation at ambient environmental mercury levels (Gilmour and Riedel, 1995). Net Hg methylation rates reported from various studies vary widely and can not be compared because of the differences in experimental and environmental conditions under which the studies were conducted. The knowledge gained from the methylation studies is, however, useful in predicting the levels of methylmercury production that would be expected under specific environmental conditions. For example, in the Experimental Lakes Area (ELA), in northern Canada, and Gulf Breeze, Florida

(USA), methylation studies in sediment-water cores spiked with ^{203}Hg indicated net methylation rates in the order of 0.25 to 50 pg/cm^2/day at 30°C (Stordal and Gill, 1995) when THg concentrations were about 1.5 to 37 ng/L in water. Net methylation rates were significantly higher in the Gulf Breeze cores than in the ELA cores with comparable initial THg concentrations in water, suggesting the importance of environmental factors other than THg concentration in regulating MeHg synthesis and accumulation in aquatic systems. These results also demonstrated the limitations of applying methylation results from one geographical area to another.

Aquatic mercury cycling

While considerable efforts have been made to develop mass balance models to simulate mercury cycling in freshwater lake ecosystems, the models have so far found limited application and acceptability. The main purpose for mercury cycling models (MCMs) is to provide an analytical framework for understanding and predicting the behaviour of mercury in specific ecosystems. Thus a model developed for lake ecosystems in one geographical area may not be applied to lakes in other geographical locations because of the differences and complexity of internal and external environmental parameters for the ecosystems. Nonetheless, lake-specific models for mercury cycling are useful in predicting the transport and fate of mercury. The Onondaga Lake (NY,USA) mercury cycling model (Henry et al, 1995) is a good example.

Conclusion

A review of experimental and whole-ecosystem studies on mercury mobilisation, transport, deposition, transformation, distribution and bio-accumulation indicates that mercury dynamics in freshwater systems are controlled by a multitude of physical-chemical processes and environmental factors which eventually determine the fate of mercury in the aquatic environment. Mercury mobilisation from terrestrial sources through weathering and erosion processes constitutes the largest source of mercury to freshwater aquatic systems. Wet and dry atmospheric mercury deposition has been identified to be another important source of mercury for aquatic systems in and around industrialised regions experiencing Hg emissions from power plants and other industrial activities. Point source Hg discharges associated with gold mining operations in developing countries produce local hot spots of elevated Hg concentrations in aquatic sediments. These hot spots are likely to be sources for long-term aquatic environmental mercury contamination and mercury-related ecosystem hazards in the affected regions.

Sediments constitute the largest sink for inorganic mercury and other metals in aquatic environment. The transformation of inorganic mercury to more toxic and bioaccumulative organic mercury species, such as monomethylmercury, occurs largely in sediments and more so at the water-sediment interface. Various studies have indicated that biomethylation is a dominant process in aquatic systems than chemical or abiotic methylation. Fair knowledge has been gained from research concerning the roles of various environmental factors in the methylation of mercury but complete understanding of the methylation mechanism is still not yet realised. The relative rates of methylation and demethylation processes determine the equilibrium of environmental methylmercury concentrations and the availability of methylmercury for biaoccumulation in fish and other organisms. Demethylation processes responsible for the reduction of methylmercury in the aquatic environment are, however, poorly understood.

For future work, more research efforts are needed to clarify the important factors in the methylation and demethylation of mercury and the accumulation of methylmercury in the fish food chain so as to predict areas where mercury transformation in aquatic systems poses greater environmental and health risks. More research emphasis should be given to understanding the behaviour of mercury in tropical ecosystems for which the available database is inadequate to predict and evaluate long-term impacts of mercury pollution.

References

Akagi, H. (1998) Studies on mercury pollution in the Amzon, Brazil. *Global Environ. Research*, 2, 193-202.

Anderson, M.R., Scruton, D.A., Williams, U.P., and Payne, J.F. (1995) Mercury in fish in the Smallwood reservoir, Labrador, twenty one years after impoundment. *Water, Air, and Soil Poll.*, **80**, 927-930.

Andersson, P., Borg, H., and Karrhage, P. (1995) Mercury in fish muscle in acidified and limed lakes. *Water, Air, and Soil Poll.*, **80**, 889-892.

Appleton , J.D., Williams, T.M., Breward, N., Apostol, A., Miguel, J., and Miranda, C. (1999). Mercury contamination associated with artisanal gold mining on the island of Mindanao, the Philippines. *Sci. Tot. Environ.*, **228**, 95-109.

Bishop, K, Lee, Y.H., Pettersson, C., and Allard, B. (1995) Terrestria; sources of methylmercury in surface waters: The importance of the riparian zone on the Svartberget catchment. *Water, Air, and Soil Poll.*, **80**, 435-444.

Compeau, G. and Bartha, R. (1984) Methylation and demethylation of mercury under controlled redox, pH, and salinity conditions. *Applied and Environ. Microb.*, **48**, 1203-1207.

Driscoll, C.T., Blette, V., Yan, C., Schofield, C.L., Munson, R., and Holsapple, J. (1995). The role of dissolved organic carbon in the chemistry and bioavailability of mercury in remote Adirondack lakes. *Water, Air, and Soil Poll.*, **80**, 499-508.

Driscoll, C.T., Yan, C., Schofield, C.L., Munson, R., and Holsapple, J. (1994) The mercury cycle and fish in the Adirondack lakes. *Environ. Sci. Technol.*, **28**, 136-143 A.

Gilmour, C.C. and Riedel, G.S. (1995) Measurement of Hg methylation in sediments using high specific-activity ^{203}Hg and ambient incubation. *Water, Air, and Soil Poll.*, **80**, 747-756.

Guimaraes, J.R.D., Ikingura, J.R., and Akagi, H. (2000) Methylmercury production and distribution in river water-sediment systems investigated through radiochemical techniques. *Water, Air, and Soil Poll.*,**124**, 113-124.

Henry, E.A., Dodge-Murphy, L.J., Bighan, G.N., and Klein, S.M. (1995). Modelling the transport and fate of mercury in an urban lake (Onondaga Lake, NY). *Water, Air, and Soil Poll.*, **80**, 489-498.

Horwitz, R.J., Ruppel, B., Wisniewski, S., Kiry, P., Hermanson, M., and Gilmour, C. (1995) Mercury concentrations in freshwater fishes in New Jersey. *Water, Air, and Soil Poll.*, **80**, 885-888.

Hovart, M. (1996). Mercury analysis and speciation in environmental samples. *In* Global and regional mercury cycles: sources, fluxes and mass balances" (Baeyens, W., Ebinghaus, R., and Vasiliev, O., Eds.), pp. 1-30. Kluwer Academic Publishers, London.

Hoyer, M., Burke, J., and Keeler, G. (1995) Atmospheric sources, transport and deposition of mercury in Michigan: Two years of event precipitation. *Water, Air, and Soil Poll.*, **80**, 199-208.

Ikingura, J.R. and Akagi, H. (1996) Monitoring of fish and human exposure to mercury due to gold mining in the Lake Victoria gold fields, Tanzania. *Sci. Tot. Environ.*, **191**, 59-68.

Ikingura, J.R., Akagi, H. (1999) Methylmercury production and distribution in aquatic systems. *Sci. Tot. Environ.*, **234**, 109-118.

Ikingura, J.R., Akagi, H., and Guimaraes, J.R.D. (2000) Evaluation of mercury methylation in fresh and pre-dried sediments using ^{203}Hg radioisotope. *Jour. Environ. Sci.*, **12**, *Supplement*, 66-75.

Jacobs, L.A., Klein, S.M., and Henry, E.A. (1995) Mercury cycling in the water column of a seasonally anoxic urban lake (Onondaga Lake, NY). *Water, Air, and Soil Poll.*, **80**, 553-562.

Kelly, C.A., Rudd, J.W.M., Louis St.,V.L, and Heyes, A. (1995) Is total mercury concentration a good predictor of methylmercury concentration in aquatic systems? *Water, Air, and Soil Poll.*, **80**, 715-724.

Lee, Y.H., Bishop, K., Pettersson, C., Iverfeldt, A., and Allard, B. (1995) Subcatchment output of mercury and methylmercury at Svartberget in northern Sweden. *Water, Air, and Soil Poll.*, **80**, 455-465.

Malm, O, Pfeiffer, W.C., Souza, C.M.M., and Reuther, A. (1990) Mercury pollution due to gold mining in the Madeira River basin, Brazil. *Ambio*, **19/1**, 11-15.

Malm, O., Castro, M.B., Bastos, W.R, Branches, F.J.P., Guimaraes, J.R.D., Zuffo, C.E., and Pfeiffer, W.C. (1995a) An assessment of Hg pollution in different gold mining areas, Amazon Brazil. *Sci. Tot. Environ.*, **175**, 127-140.

Malm, O., Branches, F.J.P., Akagi, H., Castro, M.B., Pfeiffer, W.C., Harada, M., Bastos, W.R., and Kato, H. (1995b) Mercury and methylmercury in fish and human hair from the Tapajos river basin, Brazil. *Sci. Tot. Environ.*, **175**, 141-150.

Mason, R.P., Rolfhus, K.R. and Fitzgerald, W.F. (1995a). Methylated and elemental mercury cycling in surface and deep ocean waters of the North Atlantic. *Water, Air, and Soil Poll.*, **80**, 665-677.

Mason, R.P., Reinfelder, J.R., and Morel, F.M.M. (1995b) Bioaccumulation of mercury and methylmercury. *Water, Air, and Soil Poll.*, **80**, 915-921.

Matilainen, T. (1995) Involvement of bacteria in methylmercury formation in anaerobic lake waters. *Water, Air, and Soil Poll.*, **80**, 757-764.

Mauro, J.B.N., Guimaraes, J.R.D., and Melamed, R. (1999) Mercury methylation in a tropical macrophyte: Influence of abiotic parameters. *Appl. Organometal. Chem.*, **13**, 631-636.

Miller, D.R., and Akagi, H. (1979). pH affects mercury distribution, not methylation. *Ecotoxic. Environ. Safety*, **3**, 36-38.

Rudd, J.W.M. (1995) Sources of methylmercury to freshwater ecosystems: A review. *Water, Air, and Soil Poll.*, **80**, 697-713.

Schilts, W.W. and Coker, W.B. (1995) Mercury anomalies in lake water and in commerially harvested fish, Kaminak Lake area, District of Keewatin, Canada. *Water, Air, and Soil Poll.*, **80**, 881-884.

Stordal, M.C. and Gill, G.A. (1995) Determination of mercury methylation rates using a 203-Hg radiotracer technique. *Water, Air, and Soil Poll.*, **80**, 725-734.

Verta, M., and Matilainen, T. (1995) Methylmercury distribution and partitioning in stratified Finnish forest lakes. *Water, Air, and Soil Poll.*, **80**, 585-588.

WHO (1976) Environmental Health Criteria 1: Mercury.

Wren, C.D. and Stephenson, G.L. (1991) The effect of acidification on the accumulation and toxicity of metals to freshwater invertebrates. *Environ. Poll.*, **71**, 205-241.

Mercury Species Over Europe – MOE. Project Overview and highlights from atmospheric measurements

John Munthe and Ingvar Wängberg
IVL Swedish Environmental Research Institute, PO Box 47086, SE-402 58 Göteborg Sweden
john.munthe@ivl.se

Introduction

The overall objective of MOE (Mercury Over Europe) is to provide new insights into the cycling of mercury species in the European atmosphere. MOE includes emission inventories, atmospheric modelling, process studies and field measurements.

Field measurements within MOE were co-ordinated with the parallel research programme MAMCS (Mediterranean Atmospheric Mercury Cycle System, co-ordinator N. Pirrone, CNR) focussing on mercury cycling in the Mediterranean region. In the field measurements, mercury species in air were measured at 5 sites in Northwest Europe and at 5 coastal sites in the Mediterranean region during 4 seasons. Total Gaseous Mercury (TGM) was monitored either using continuous instrumental methods or 21 h sampling on Au-cartridges for manual analysis. Total Particulate Mercury (TPM) concentrations were obtained by collecting airborne particles on micro quartz fibre filters using a new developed technique allowing for high performance at a low cost. The presence of Reactive Gaseous Mercury (RGM), i.e. $HgCl_2$, was determined using mist chambers and KCl-denuder techniques. Gaseous Methyl Mercury (GMeHg), i.e. CH_3HgCl (g), was measured using a new method developed within the present project. The sampling air is extracted through a 1 dm^3 volume mist chamber containing diluted HCl/KCl solution at a flow rate of 10 - 15 $dm^3\ min^{-1}$ (Lee *et al.*, in prep.). This method allows determination of GMeHg concentrations as low as 0.5 pg m^{-3} at 6 - 8 h sampling time. Additionally, automatic RGM measurements with high time resolution were performed at the Rörvik site. The result of the MOE and MAMCS field campaigns constitute a unique set of environmental mercury data that will be utilised in modelling work within both projects.

Results

In Figure 1, a summary of average concentrations of TGM, TPM and TPM obtained in the MOE and MAMCS measurement campaigns is presented. TGM is relatively evenly distributed in the atmosphere over Europe, in comparison to the species RGM and TPM. Slightly elevated concentrations of TGM are found in the Mediterranean region in the summer campaigns, which may be an effect of re-emissions of mercury from the sea surface.

Total Particulate Mercury (TPM) concentrations are generally higher in the Mediterranean stations than at the stations in the North. This may be explained by local emission sources or a higher photochemical oxidation capacity in the marine boundary layer air in combination with generally higher aerosol levels e.g. from windblown dust and sea-salt. RGM concentrations are also significantly enhanced in the Mediterranean most likely for the same reasons as for TPM.

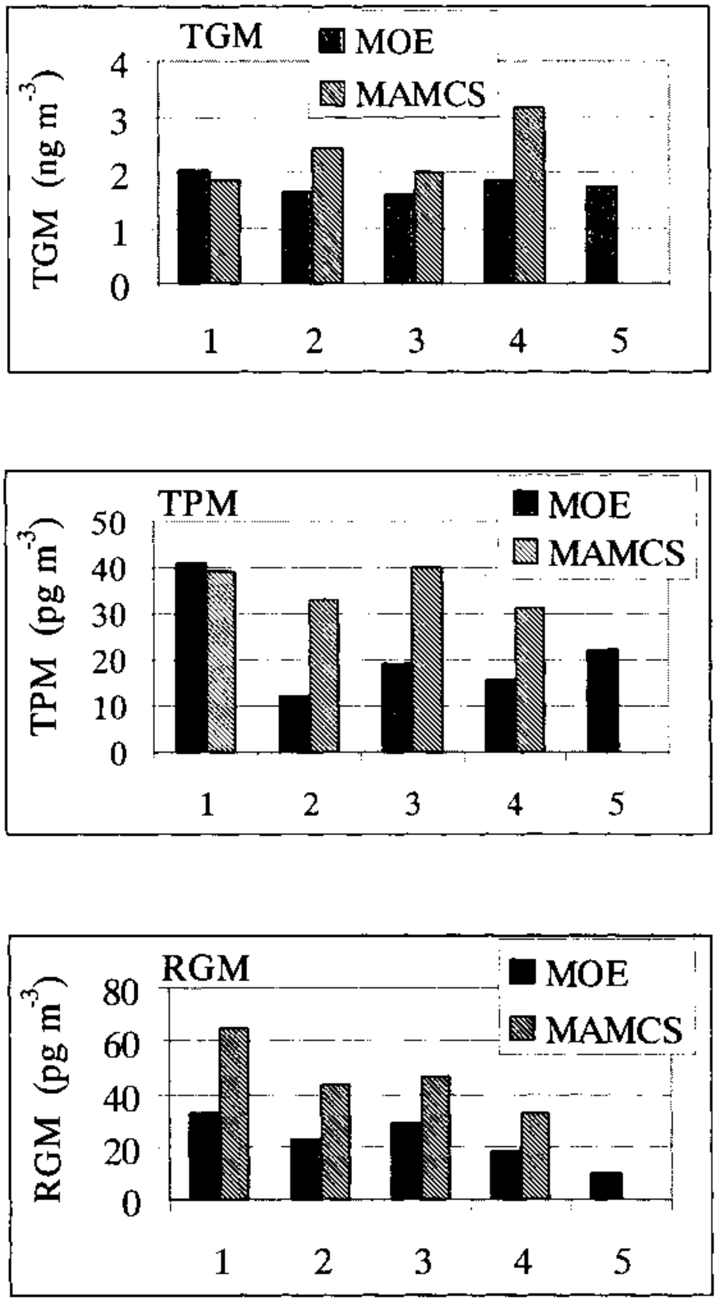


Figure 1. Average TGM, TPM and RGM values obtained in MOE and MAMCS measurement campaigns (from Wängberg *et al.*, 2001).

The TGM data obtained in MOE shows a clear south to north gradient with concentrations gradually decreasing from the German site Neuglobsow to Zingst (DE), Rörvik (SE) and Aspvreten (SE). The exception to this trend is Mace Head where the lowest concentrations were expected due to the prevailing westerly winds and absence of local sources, see Figure 2.

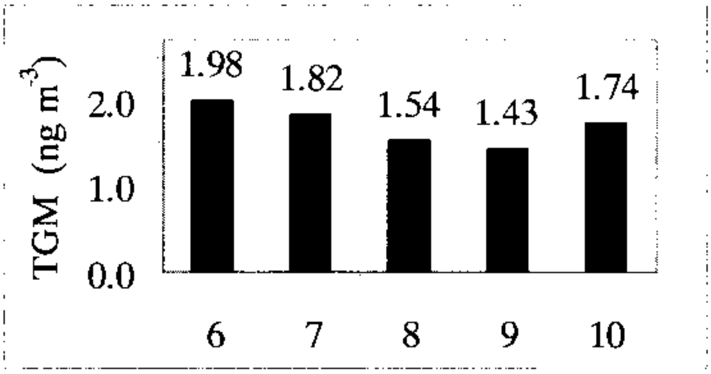


Figure 2. Average TGM from the MOE measurement sites. The averages are based on the data from all 5 MOE campaigns.

The most reasonable explanations for the elevated levels found at Mace Head is re-emissions of mercury from the sea-surface in the north Atlantic. Very high levels of dissolved gaseous mercury have been reported from this region (Mason *et al.*, 1998).

In Figure 3, results from TPM measurements obtained in the fifth MOE campaign are presented. The very clear episode with elevated concentrations at all 4 measurement sites indicate that significant transport from south to north occurred during this period.

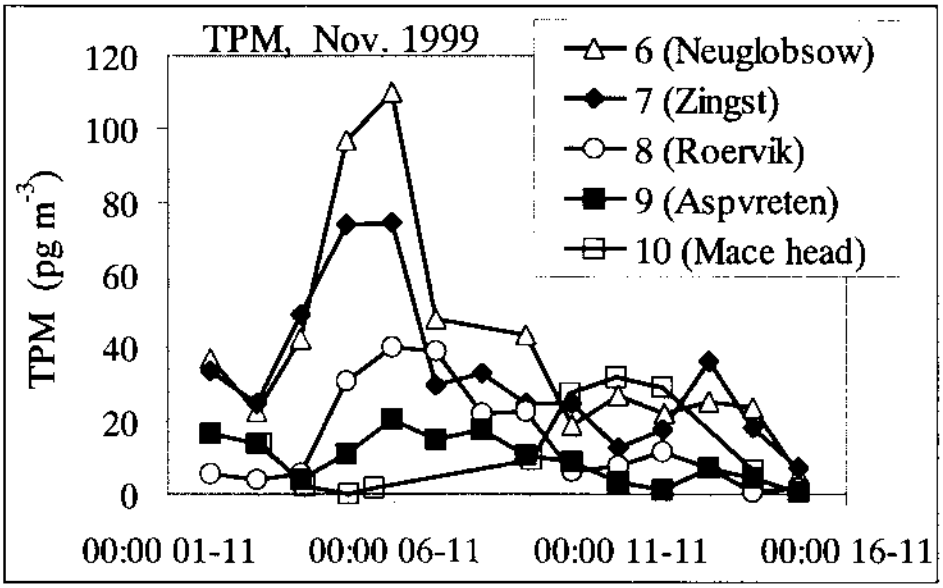


Figure 3. Time series with 21 h average TPM obtained during the fifth MOE campaign. The measurements started 12:00 and ended 09:00 the following day. TPM data are plotted against mean time.

This transport pattern can be verified by evaluating air mass trajectories for this period. In Figure 4, a calculated backward trajectory for the Rörvik site is presented (Wängberg *et al.,* 2001).

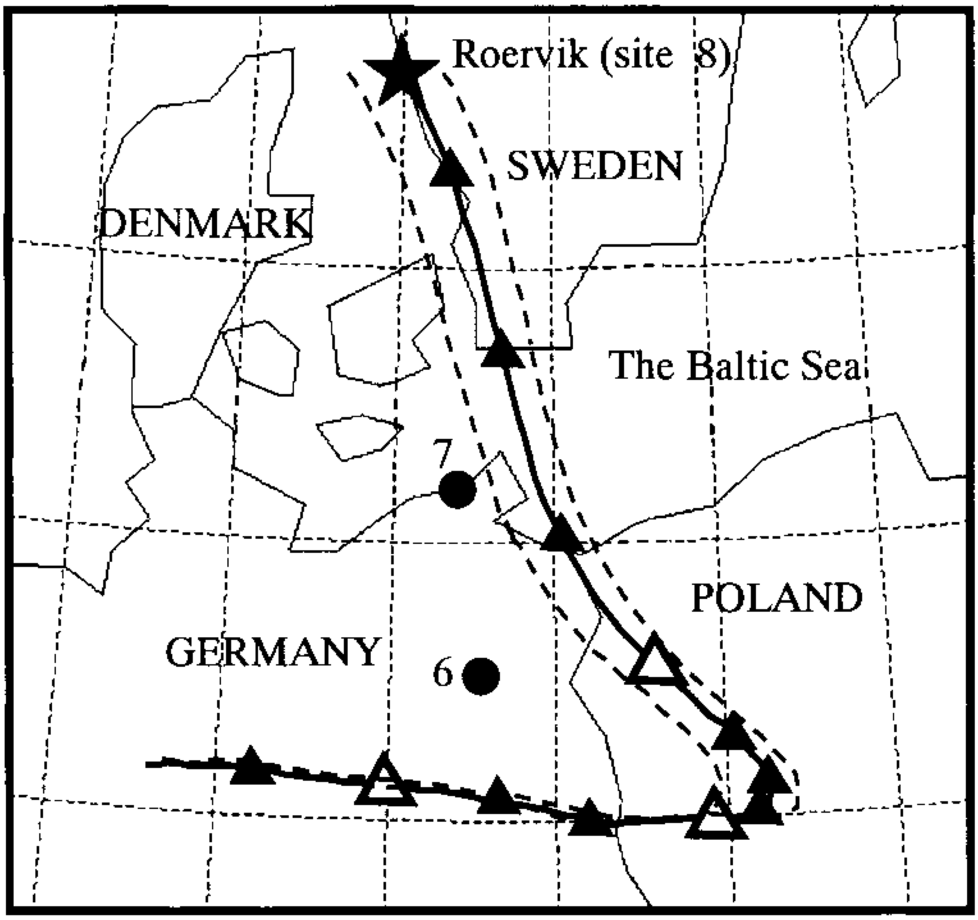


Figure 4. 82 h back trajectories starting at Rörvik 22:00, 5 November 1999. The starting height was 20 m. Filled triangles mark 6 h time intervals and the open triangles indicate 24 h intervals. Dotted lines are back trajectories calculated with starting points 37 km west and 37 km east of the Rörvik site.

Trajectories calculated for the preceding days show a significantly different transport pathway with the air masses being transported from the west and thus not passing over the main source region in central Europe.

For gaseous methylmercury (Figure 5) and RGM (Figure 6) the distribution at the MOE sites is more difficult to interpret.

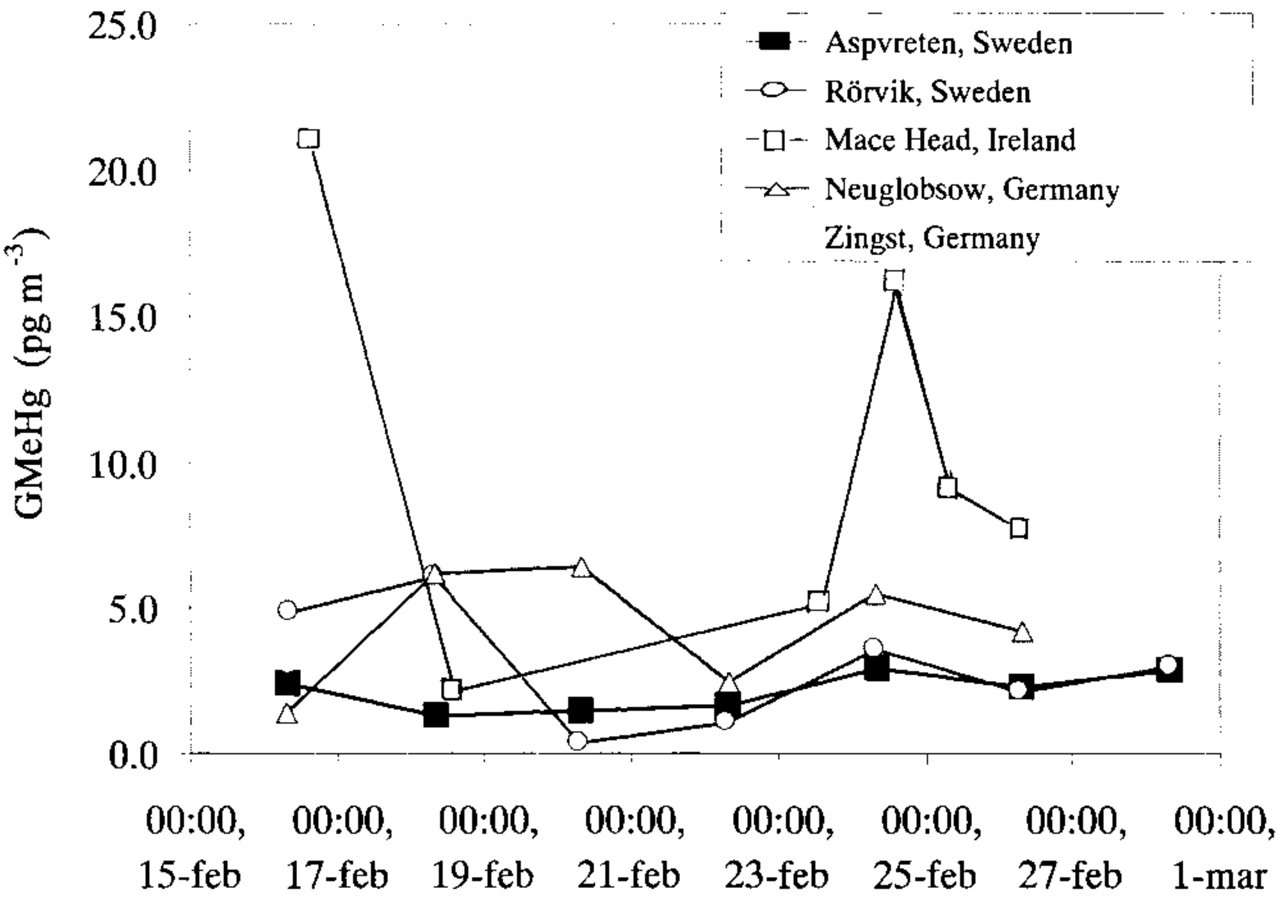


Figure 5. Gaseous methylmercury in air at MOE sites during one campaign.

The highest GMeHg concentrations are found at the Mace Head site. As for TGM, this suggests that the sea surface may be a significant source. Mason *et al* (1999) reported significant concentrations of dimethymercury in seawater. Dimethylmercury is volatile and emissions from the sea followed by chemical degradation to MeHg is a feasible explanation. The concentrations found at Neuglobsow is slightly elevated in comparison to those observed at Rörvik and Aspvreten. This may indicate some influence of anthropgenic sources, either of MeHg or other chemical precursors such as dimethylmercury or RGM.

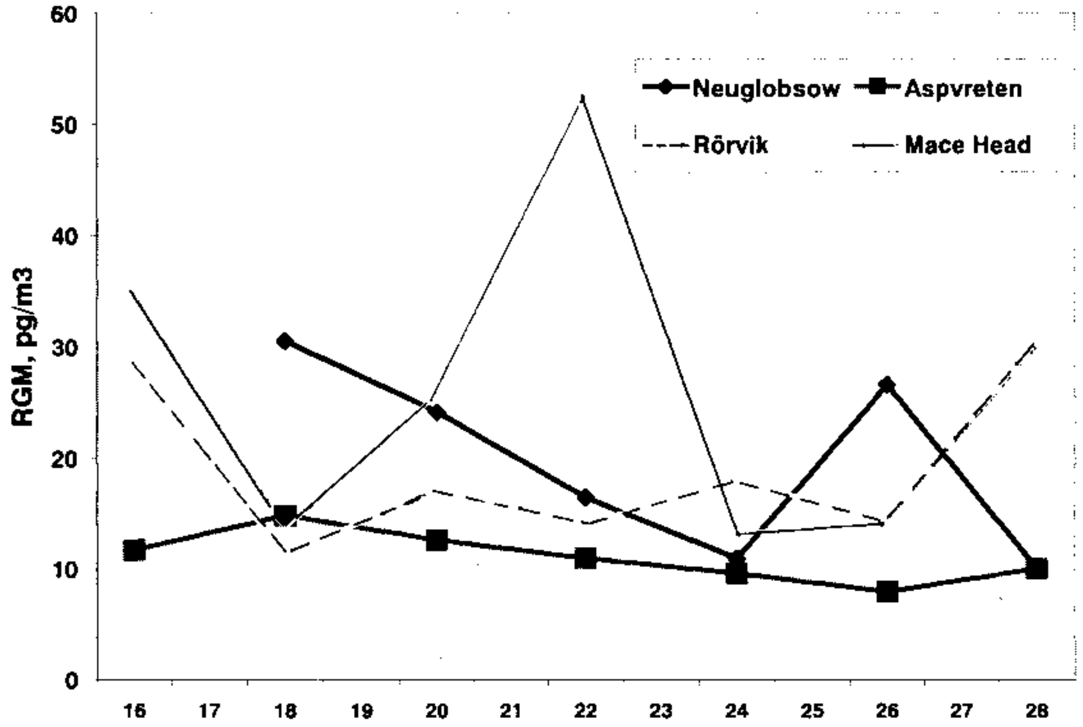


Figure 6. Reactive Gaseous Mercury at MOE sites during one campaign.

The distributions of RGM at the MOE sites is similar to that of GMeHg with elevated concentrations foud at Mace Head and concentrations at Neuglobsow slightly higher than at the Swedish stations. For RGM, a sea surface source is very unlikely since this by definition is a water-soluble form of mercury. Possibly oxidation processes occurring in the marine boundary layer can explain these findings. RGM is however, readily dry deposited and should be expected to be found in highly varying concentrations. RGM is known to be emitted from combustion sources but despite this the south to north gradient found is very weak.

References

Mason, R.P., Fitzgerald, W.F. and Morel, F.M.M. 1995. The biogeochemical cycling of elemental mercury: Anthropogenic influences. *Geochim. Cosmochim Acta* **58**, 3191 –3198.

Wängberg, I., Munthe, J., Pirrone, N., Iverfeldt, Å., Bahlman, E., Costa, P., Ebinghaus, R., Feng, X., Ferrara, R., Gårdfeldt, K., Kock, H., Lanzillotta, E., Mamane, Y., Mas, F., Melamed, E., Osnat, Y. , Prestbo E., Sommar, J., Spain, G., Sprovieri, F., Tuncel, G. 2001, Atmospheric Mercury Distribution In Northern Europe and in the Mediterranean Region. *Atmospheric Environment in press.*

Presentation at NIMD Forum- 2001 (Proceedings Ed. H. Akagi), March 2001

Recent Research on Missing Sources and Sinks in the Global Hg Cycle: The Role of the Arctic as a Sink

S. E. Lindberg[a], S. Brooks[b], C-J Lin[c], and K. Scott[d]

[a]Environmental Sciences Division, Oak Ridge National Laboratory[1]; [a]NOAA Atmospheric Turbulence and Diffusion Division; [b]Oak Ridge Associated Universities, Oak Ridge, Tennessee USA, [c]Lamar University, Texas, and [d]University of Manitoba, Canada
(email for corresponding author: SLL@ornl.gov)

ABSTRACT

In the past decade many new sources of diffuse mercury emissions have been measured. These sources significantly increase the estimates of global Hg emissions, and suggest there may be missing Hg sinks in global models. Mercury levels in Arctic wildlife are elevated above normal levels, but there are few known Arctic Hg sources, and long range transport of HgE must be considered. The discovery of mercury depletion events (MDE, similar to ozone depletion) at Alert, NWT suggests a mechanism for Hg accumulation from the global pool. One hypothesis is that HgE is transformed into a reactive gaseous mercury (RGM) species which deposits locally. This species has never been measured in the Arctic, and MDE's must be confirmed at other Arctic sites.

We have measured total gaseous Hg at Barrow, AK since September, 1998 with an automated analyzer. The initial data provide the first confirmation of MDE's at this more southerly, Arctic site. MDE events begin soon after Polar sunrise, and persist until snowmelt. During MDE Hg exhibits a strong correlation with ozone, suggesting a links with the reactions that destroy tropospheric ozone. We have developed a simple model using local meteorological data which closely simulates the patterns of depletion events, and suggest that turbulence and temperature are important factors. There are a number of potential reaction pathways with Hg and Br to explain the depletion events, which suggest that during these events HgE is oxidized to RGM. The model predicts a Hg deposition rate much higher than measured in the eastern US. To test our hypotheses, we have measured RGM prior to and following polar sunrise during winter 2000, and plan to measure Hg speciation and evasion in snowmelt during polar summer.

INTRODUCTION

Mercury levels in Arctic wildlife are known to be elevated, often significantly above normal levels (e.g. Dietz et. al. 2000), and levels have been increasing over time (e.g. Wheatly

---

[1] Research sponsored by the NOAA Arctic Program and the US EPA Office of International Programs. ORNL is managed by UT-Battelle for the U.S. Department of Energy.

and Wheatly 1988). However, there are no known Arctic sources of reactive gaseous mercury (RGM), and long range transport of elemental Hg vapor (HgE) must be considered. The recent discovery of so-called mercury depletion events (MDE, characterized by extremely low levels of HgE, well below global background concentrations) at Alert in the Canadian high Arctic (Schroeder et. al. 1998) suggests a possible mechanism for Hg accumulation from the global background pool of elemental Hg. One hypothesis for MDE is that HgE is transformed into a reactive gaseous mercury (RGM) species which then deposits locally. This reactive atmospheric species has never been measured in the Arctic, but methods we developed in our lab suggest that RGM has the capability to deposit rapidly by dry and wet deposition (Lindberg and Stratton 1998). Elevated levels of aerosol Hg have been reported during MDE periods (Schroeder and Lu, pers. comm.), but fine aerosol Hg would be less likely to deposit quickly to the local snow surface than would RGM. Hence, it is crucial to quantify RGM in the Arctic.

Prior to beginning speciation studies, MDE's must first be confirmed at other Arctic sites. This was the initial objective of the Barrow Arctic Mercury Study (BAMS), which has since expanded to include speciation and flux studies. This paper will briefly describe the project and some initial TGM measurements from the winter/spring of 1999. New measurements of atmospheric Hg speciation as RGM have been initiated as part of BAMS during September, 1999, and these data will be presented at the conference and published elsewhere.

METHODS

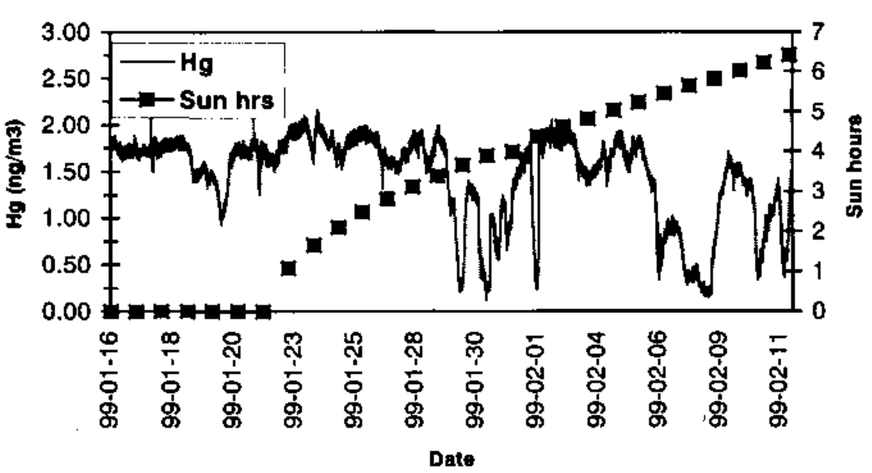
We initiated routine measurements of TGM (primarily gaseous Hg°) at Barrow, AK in September, 1998 with an automated Tekran 2537A analyzer. Descriptions of the Tekran and its operating parameters have been published (the device collects airborne TGM by gold amalgamation, then releases the trapped Hg by thermal desorption for quantification by CVAFS; e.g. see Lindberg et. al. 2000). TGM is sampled with a 5-min time resolution using a heated Teflon inlet line mounted ~5 m above the ground on a mast 3 m above the roof of the NOAA CMDL building. There are no Hg sources within the CMDL, no emission points on the roof, nor any known major Hg point sources in the town of Barrow, which is located ~10 km southwest of the CMDL. The CMDL is located near the peninsula at Point Barrow, ~2 km from the shoreline, and is surrounded primarily by water to the N, E, and W. Prevailing winds are from the NE. Barrow is geographically the northern-most point in Alaska, located at 71° 19' N, 156° 37' W, and the CMDL is ~9m above mean sea level. In latitude, Barrow is ~1600 km south of Alert. We expect to continue TGM sampling at Barrow until 2001. We began sampling RGM at Barrow using the Tekran 1130 in September, 1999, and intend to measure TGM and RGM simultaneously during the winter-spring of 2000 and 2001. RGM is being sampled with an automated KCl-coated denuder using a method not yet published in the open literature (Stevens et. al. 1999). RGM data will not be reported in this paper. Ancillary data available at the CMDL include routine meteorological data and trace gases such as ozone (e.g. Oltmans et. al. 1989).

RESULTS AND DISCUSSION

The initial year's data provide the first confirmation of MDE's at this more southerly Arctic site. This is also the first evidence that the MDE phenomenon could be a widespread characteristic of Arctic dawn, since Barrow is several thousand km from Alert. The events begin within a few days of polar sunrise (Fig. 1), and persist until snowmelt (not shown). During this period, Hg exhibits a strong correlation with ozone ($r^2 = 0.75$), suggesting a possible link with the chemical reactions which destroy tropospheric ozone. These reactions are thought to include reactive bromine, perhaps both in the aerosol and in the gaseous phase (eg. Fan and Jacob, 1992), and are a common feature at Barrow (Oltmans et. al. 1989). In comparison, there is no correlation between O_3 and Hg° in the months before polar sunrise ($r^2 < 0.1$).

Published data may provide some clues of the causes of MDEs. Field data indicate that both gaseous and aerosol Br exhibit strong seasonal peaks at Barrow, between about February and June (polar sunrise to snowmelt; Berg et. al. 1983). During this period, aerosol Br may increase by as much as 20-fold over typical concentrations during the rest of the year, and these peaks are highly reproducible from year to year. Peak aerosol Br levels may exceed 100 ng/m^3 during these events. Hypotheses for the sources of this Br include aerosol enrichment by bubble bursting from the sea-surface microlayer, by gaseous reactions resulting from organic Br emissions from marine algae (eg. bromoform is thought to be emitted by ice algae), and/or by heterogeneous gas/solution reactions at the interface of hygroscopic sea salt aerosols. Several reactive airborne Br species may result (eg. Dickerson et. al. 1999), some of which have the potential to oxidize Hg° (e.g. BrO, BrCl). Many of these compounds arising from reactive Br exhibit a strong diel pattern, indicating the importance of sunlight and photochemical reactions. Some of our early data suggest that

Fig. 1. First Confirmation of Mercury Depletion Events in the U. S. Arctic, Barrow, AK (January, 1999).



peak depletion periods tend to occur at midday.

To better understand the periodicity of MDEs, one of us (S.B.) developed a simple predictive model of these phenomena using local meteorological data. This model closely simulates the patterns of TGM depletion events during 1999, and suggest that atmospheric turbulence intensity and temperature are important factors. This simple model can be used to predict Hg deposition rates based on the assumption that the depleted Hg is accumulating locally by dry deposition. The model predicts short-term (~20 weeks) dry fluxes that are much higher than annual wet deposition rates measured in the eastern U.S. For example, wet deposition rates reported at more than 25 sites in the eastern U.S. by the NADP Mercury Deposition Network (MDN) are typically in the range of ~10-20 $\mu g\ m^{-2}\ y^{-1}$ (NADP/MDN, 1999), while the model suggests a flux of ~50-60 $\mu g\ m^{-2}$ at Barrow during February-May alone. The model is being improved with more detailed meteorological data, especially high-resolution measurements of UV-B, available during the winter of 2000. These new data support a very strong diel pattern to the MDEs (not shown).

There are a number of potential reaction pathways between Hg° and Br which could explain the occurrence of depletion events by oxidation of HgE to some HgII compound such as HgO (one possible form of RGM). Hence, it will be critical to measure RGM in the Arctic during and after these events, and to confirm the predicted levels of accumulation of Hg in surface snow at Barrow (levels as high as 80 ng/L are predicted from the first generation model). These are the primary objectives for the BAMS project during 2000-2001, which will include the first measurements of bioavailable Hg in Arctic snow measured with a bioreporter method (K. Scott and C. Kelly, in prep.).

Another interesting twist in our data is illustrated in Table 1. Our data from June 1999 suggest that some of the oxidized Hg accumulating in the snowpack may be reduced and evaded as HgE following snowmelt. This is manifested as an elevated and long-lived spike in ambient Hg° following snowmelt (persisting from ~ mid-June to mid-August). To test our various hypotheses, we plan two major campaigns in 2001: 1) to measure RGM, aerosol Hg, reactive Br, and Hg in snow prior to and following polar sunrise during winter-spring, and 2) to measure Hg speciation in snow and runoff and evasion of Hg° over melting snow during spring-summer. There is also a strong need to develop and apply a near-real-time micrometeorological method to directly quantify Hg fluxes to the surface at Barrow and other Arctic sites to confirm the mechanism of Hg accumulation in snow. Gradient methods based on modified Bowen ratio have been published for TGM (eg. Meyers et. al. 1996, Lindberg et. al. 1995), and these or related approaches such as relaxed eddy accumulation may be viable at these sites for RGM and related species.

Table 1. Statistics for TGM concentrations and related parameters at Barrow, AK

Day No.	Dates	Mean TGM (ng/m^3)	StdDev TGM	Mean Sun Hours	Mean Aerosol Br[a] (ng/m^3)	Period
292 - 322	Oct 18 - Nov 19	1.76	0.07	4.5	6	Fall
365 - 20	Jan 1 - 20	1.71	0.29	0	12	Winter
30 - 146	Jan 30 - May 25	1.37	0.75	13	35	Arctic dawn
64 - 74	Mar 4 - 13	0.71	0.56	10.5	55	a single MDE
165 - 195	Jun 13 - Jly 13	2.30	0.39	24	4	post snowmelt

---

[a] Derived (approximated) from Berg et. al. (1983)

ACKNOWLEDGMENTS

We wish to thank the sponsors of this project for their continued support (the NOAA Office of Arctic Research, and the U. S. EPA Office of International Activities), Dan Endres and Malcolm Gaylord for local support at CMDL, F. Schaedlich for extended assistance with Tekran equipment, S. Oltmans for Barrow ozone data, and R. Stevens and M. Landis for help with development and deployment of automated RGM denuder methods now in use at Barrow.

REFERENCES

Berg, W. W., P. D. Sperry, K. A. Rahn, E. S. Gladney (1983), Journal of Geophysical Research, Vol. 886719-6736.

Cheng, M. D. and W. H. Schroeder (2000), Journal of Atmospheric Chemistry, Vol. 35101-107.

Dickerson, R. R., K. P. Rhoads, T. P. Carsey, S. J. Oltmans, J. P. Burrows and P. J. Crutzen (1999), Journal of Geophysical Research, Vol. 104, No. D1721,385-21,395.

Fan, S.-M. and D. J. Jacob (1992), Nature, Vol. 359 522-524.

Lindberg, SE, A. Vette, C. Miles, and F. Schaedlich (2000), Application of an automated mercury analyzer to field speciation measurements: Results for dissolved gaseous mercury in natural waters. Biogeochemistry, 48(2), 237-259.

Lindberg, S. E. and W. J. Stratton (1998), Atmospheric mercury speciation: Concentrations and behavior of reactive gaseous mercury in ambient air. Envir. Sci. & Technol. 32:49-57.

Lindberg, S.E., K-H. Kim, T.P. Meyers, and J.G. Owens (1995), A micrometeorological gradient approach for quantifying air/surface exchange of mercury vapor: Tests over contaminated soils. Envir. Sci. Technol. 29:126-135.

Meyers, T.P., M.E. Hall, and S.E. Lindberg (1996), Use of the modified Bowen ratio technique to measure fluxes of trace gases. Atmos. Envir. 30: 3321-3329.

National Atmospheric Deposition Program (2000), NADP Data Report 2000-01. Illinois State Water Survey, Champaign, IL.

Oltmans, S. J., R. C. Schnell, P. J. Sheridan, R. E. Peterson, S.-M. Li, J. W. Winchester, P. P. Tans, W. T. Sturges, J. D. Kahl, and L. A. Barrie (1988), Atmospheric Environment, Vol. 23, No. 11 2431-2441.

Schroeder, W. H., K. G. Anlauf, L. A. Barrie, J. Y. Lu, A. Steffen, D. R. Schneeberger, and T. Berg (1998), Nature, Vol. 394, July 23 331-332.

Stevens, R.K., Schaedlich, F.H., Landis, M., Schneeberger, D., Prestbo, E., Lindberg, S. and Keeler, G. (1999), Automated instrument designed to measure Hg° and reactive gaseous mercury in near real time: Design and operational characteristics. Paper presented at the 5th International Conference on Mercury as a Global Pollutant, Rio de Janeiro, Brazil, May, 1999.

Wheatley, B. and M. A. Wheatley (1988), Arctic Medical Research, Vol. 47, Suppl. 1, pp. 163-167 163-167.

THE MERCURY BIO-GEOCHEMICAL CYCLE: SITE SPECIFICITY AND DATA INTERPRETATION

Ricardo Melamed[1], *Zuleica Castilhos*[1], *Mario G.Ribeiro Jr.*[1], *Roberto C. Villas Bôas*[1],
Jane B.N Mauro[2], *Jean R.D Guimarães*[2], *Sérgio Coelho-Souza*[2]

[1]CETEM/MCT – Center for Mineral Technology/Ministry of Science and Technology
Av. Ipê 900 – Ilha da Cidade Universitária – 21941-590 - Rio de Janeiro – Brazil
RMelamed@cetem.gov.br

[2]Laboratório de Radioisótopos, IBCCF/UFRJ
Bloco G-CCS - Ilha da Cidade Universitária 21949-900 - Rio de Janeiro - Brazil

ABSTRACT

The complexity of the cycling of mercury in the environment is reflected by the many reactive processes involving various mercury oxidation states, species, and complexes, and ultimately the various pathways leading to bioaccumulation. In addition, research studies, more recently conducted in tropical regions, have identified important differences between the mercury cycling in Northern and Southern Hemispheres.

This paper focuses on some particularities at the regional scale that illustrate the controversy on the effects of physico-chemical properties on the mercury cycling at the global scale.

Examples of these particularities are: the mobility of mercury in a given matrix is dependent not only on the mercury species involved in the reaction process but also on the complex formed; the lack of bioaccumulation in regions with high inputs of elemental mercury used in informal gold mining; temperature control on methyl mercury uptake; the effects of pH and dissolved organic carbon on bioaccumulation; the role of soils as an important reservoir and release of mercury to aquatic systems.

It is hoped that these review paper will point out some intriguing results found in the literature, allowing some discussions about the specifics of these findings and possibly raising perspectives for future research.

INTRODUCTION

The biogeochemical cycle of Hg in the Amazon is considered still poorly understood. Previous studies were concentrated in the dynamics of Hg in gold mining areas. Very few measurements were made out of these areas, to investigate the impact of gold mining at the larger regional scale. In these far regions Total-Hg inputs triple the levels of pre-industrial times. More recently, the "natural sources" of Hg became on focus because depth integrated Hg stocks are much higher than expected from anthropogenic Hg sources. Soils, with Hg levels reaching up to 212 ng/g are being considered the largest reservoir and the greatest potential source to the aquatic environment via "podzolization". High Hg levels in fish and in human hair were associated to low pH and high DOC (Forsberg et al., 1999)

In the wetlands of Brazil, in a region where Hg inputs are very high, Hg concentration in the soils is relatively low (9-45 ng/g), and the high end concentration occurs only in seasonally flooded soils. Fish survey indicated very low levels (average=77 ng/g w.w.), which was concluded to be a result of low Hg availability or biomass dilution of MeHg (Guimarães et al., 1999)

Recently, there were alarming reports about Hg contamination in hair of riverine people and fish in the Negro river basin, characterized by high DOC and low pH (4.5), an area where gold mining is non-existent. Observed Hg concentration in soils (with high desorption rates), water, rain and atmosphere are higher than expected for a non-impacted area (Table 1, Fadini and Jardim, 1999):

Table 1: Hg in different compartments of the Negro river basin, Amazon

Compartment	Hg
Surface water	6.7 ng/L
Atmosphere	4.5 ng/m^3
Rainfall	9.8 ng/L
Soils	164 ug/kg

Considering an annual precipitation in the region of 1,500 mm, it yields an accumulation rate of 14.7 ug Hg/m^2/year, which is about the triple of Hg input of pre-industrial times.

Oxisols constitute an important reservoir of Hg with 100-200 mg/m^2/m depth, occurring in association to Fe/Al oxides, thus sandification and podzolization processes are a natural export of Hg down slopes, with erosion enhancing even further the transport of fine clayey particles enriched with oxy-hydroxides and Hg.

A study conducted along 800 km of the Madeira river (Miller et al., 1999) showed that the lateritic soils exposed along the basin are developed in old alluvial sediments eroded from the Andes, suggesting that much of the elevated Hg levels in soils and sediments come from the Andes and transported by the Madeira river. Thus, in regions affected by gold mining activities in the Amazon region, the highest Hg concentrations were not found in rivers where mining activities take place but at the outlet of Andean sub-basins exploited for alluvial gold with fish concentrations reaching up to 5.2 ug/g.

In the Southern Hemisphere, where anthropogenic inputs are generally lower, different patterns of mercury cycling were identified. In lakes characterized by high rainfall, shallow (6m) and high temperature (30 °C), water column MeHg is low due to rapid uptake and biomagnification, while in lakes characterized by high rainfall, but cooler temperatures, water column MeHg is high reflecting in lake methylation and supply of MeHg from upper catchment (Apte et al., 1999).

MERCURY CYCLING IN SEDIMENTS

Anthropogenic mercury emissions into the atmosphere and atmospheric deposition have increased significantly since pre-industrial times, especially in the Northern Hemisphere. In the Southern Hemisphere, increases in mercury depositions can also be observed, which is mainly attributed to informal gold mining, the so called garimpos (Lacerda and Marins, 1997; Lacerda and Solomons, 1998) and to industrialization (Lacerda and Marins, 1997; Lacerda *et al.*, 1999; Ribeiro Jr. e Lacerda, *in press*). In general, Hg generated by these activities is deposited near the source.

Hg migration in sediments was observed in a single core (Figure 1) collected from a dam in Alta Floresta City, Mato Grosso State, constructed for electric energy generation, and used for fishing and other recreation activities. Figure 2 shows the variation with depth in organic matter, Fe and C, while Figure 3 shows the Hg profile of the sediment core.

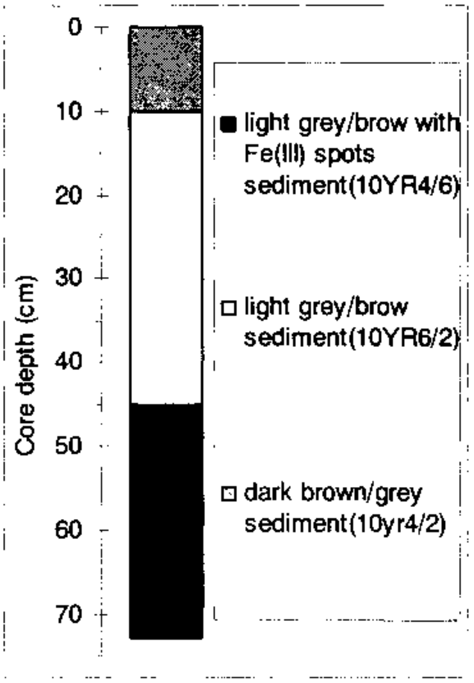
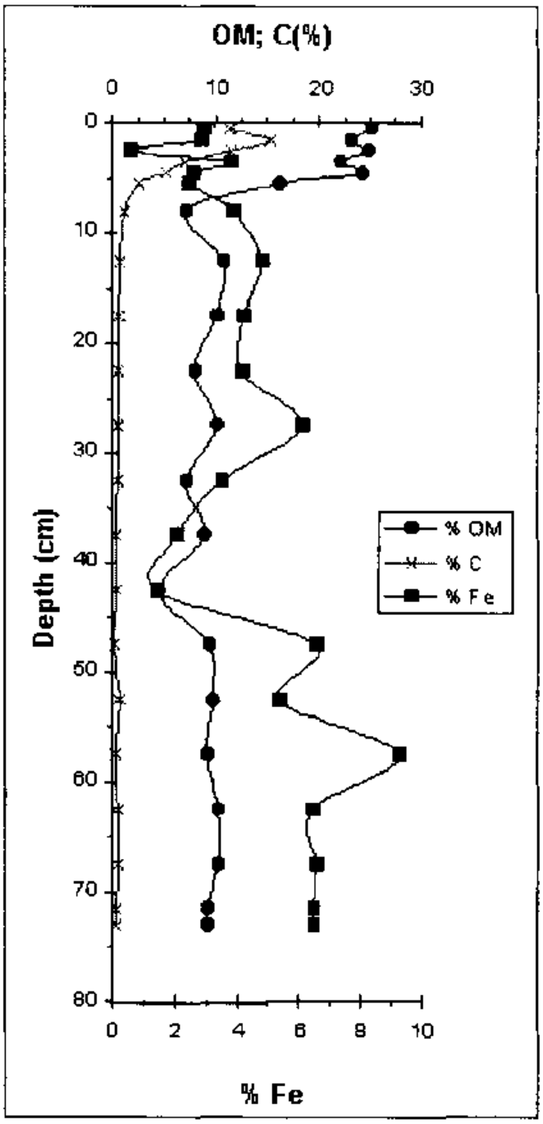


Figure 1: Sediment core description.
The code in parenthesis is the Münsel color chart classification.



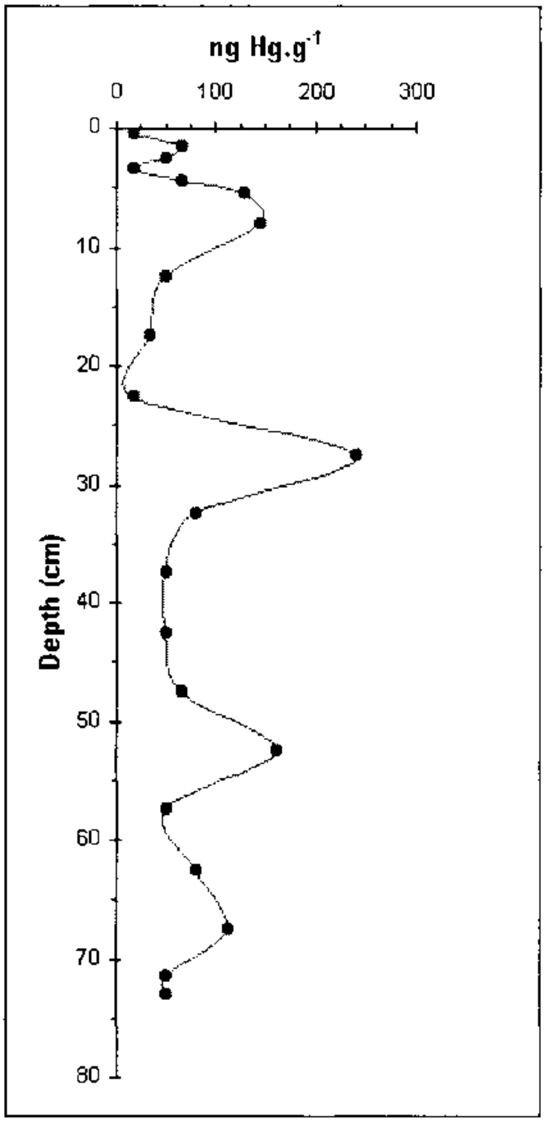


Figure 2: Variation of Fe, organic matter (OM) and C in the sediment core.
Figure 3: Variation of mercury concentration with depth.

An unexpected increase in mercury concentration occurs below 30 cm core depth, different from other anthropogenic generated heavy metals that show a decrease in concentration with core depth. The increase in the Fe content at the same core depth suggests that the iron is responsible for this Hg accumulation, as observed by Roulet and Lucotte (1995) and Rosolen *et al.* (1999). Correlation coefficients between Hg and the other parameters can be observed in Table 2.

Table 2: Correlation coefficients between Hg with Fe, OM and C

		_Correlation coefficients_*		
		Fe	MO	C
Total core	0 – 73 cm	0,2251	-0,2575	-0,2763
Depth intervals	0 – 10 cm	0,1455	-0,8587	-0,6792
	10 – 45 cm	0,5797	0,7023	0,7064
	45 – 73 cm	0,7401	0,5332	0,5988

* for a confidence interval of 99.5 %

The data in Table 2 show no significant correlation between Hg concentration with Fe, OM and C, when considering the total sediment core. However, if we consider each core section, according to Figure 1, different perspectives are found. A significant negative correlation between Hg concentration with OM and total C content is observed at the 0-10 cm interval. At the 10-45 cm layer, Hg concentration correlates well with all parameters, but the correlation of Hg was more significant for OM and C than for Fe. At the 45-73 cm layer, all parameters are also correlated with the Hg concentration, but in this layer the correlation with Fe was more significant than the correlation with OM or total C.

These results suggest that Hg and Fe(II) migrate in association with organic complexes. Observation of the sediment core reveals the presence of red Fe nodules at the lower layer suggesting that at the lower depth Fe(II) is oxidized by oxygen enriched groundwater during seasonal level fluctuations. Another plausible mechanism for Hg migration, which was not tested, is the formation of polysulphides.

METHYLATION

Sulfate-reducing bacteria are considered as the main Hg methylators (Compeau and Bartha, 1984). These microorganisms are usually found where redox potentials are low, although it has been stated that they can also be found in oxic environments (Jorgensen and Bak, 1991). The presence of inorganic Hg (at different oxygen conditions, oxic and anoxic) can result in methylmercury formation, either mediated by sulfate-reducing bacteria or other microorganisms and/or, although less likely, through abiotic processes. This is illustrated by the detectable Hg methylation observed in plankton samples obtained in surficial waters of a floodplain lake, the Araipá, located in the Tapajós river basin, Brazil. Another evidence is the methylmercury formation in two different strains of cyanobacteria originated in laboratory cultures. A cultivated complex of calcareous microalgae and bacteria was also demonstrated to have a low but detectable methylation potential. Methylmercury formation observed in incubations of the above-cited microorganisms has been similar to levels obtained by other authors in water and surface sediments. Nevertheless, higher levels (up to 44 %) are found in the roots of floating aquatic macrophytes from tropical lakes (Mauro et al, 1999, Guimarães et al, 1998, 2000) that usually present high temperatures and where the large macrophyte stands provide a hypoxic microenvironment suitable for an optimal growth of sulfate-reducing bacteria. Very low redox potentials (highly reducing conditions) may inhibit Hg methylation, probably by the formation of sulfide complexes, and the investigation of different matrices oriented to determine the redox threshold beyond which methylation is suppressed, is a relevant issue. Anyhow, the methylmercury production by plankton and/or its associated bacteria should not be underestimated due to its wide distribution and abundance in many water bodies. We suggest more investigations regarding Hg methylation by plankton in order to better estimate its importance and to verify if this process is indeed performed by plankton itself or by other associated microorganisms.

Hg CONTAMINATION IN FISH - THE DOSE-RESPONSE APPROACH

It has been demonstrated that from 75% to 95% of all Hg accumulated in tissues of fish is usually MeHg, with inorganic Hg as the source (Jernelöv and Lann, 1971; Huckabee, 1979). In fish, the rate of absorption of MeHg is faster than absorption of Hg, and the clearance rate is slower, with the net result being high MeHg to total Hg is generally very high in fish. Some authors suggested that this ratio could change depending on the degree of Hg contamination in aquatic systems (Anderson and Depledge, 1997; Kherig and Malm, 1999).

Hg is a xenobiotic, a non-essential metal (Eaton and Klaassen, 1996), which under natural microbial activities (Pak and Bartha, 1999) present in sediments and water column brings MeHg as a Hg detoxification product (biotoxification process). Bioaccumulation is a more direct study of mercury reaction at the environmental interface of the aquatic organisms. The direct bioaccumulation, or the bioconcentration factor (BCF) of Hg is defined as the ratio of Hg concentration in fish tissue to the Hg concentration in water. The indirect bioaccumulation or biomagnification is the accumulation of a chemical in a given species according to its trophic level in the food chain (Bruggeman, 1982).

Both dissolved, inorganic Hg and MeHg, accumulate in phytoplankton (Herring et al., 1998) by passive diffusion across the membrane (Mason et al., 1995) or by facilitated diffusion transport (Watras et al., 1998). The BCF of Hg in this trophic level is approximately 103 with 15% as MeHg (Porcela, 1994). However, in contrast to MeHg, inorganic Hg is not biomagnified as the trophic transfer from phytoplankton to zooplankton. The uptake most certainly is determined by exposure and the reduced polarity is the most likely explanation for the exceptionally high bioavailability of MeHg (Phillips and Buthler, 1978). MeHg attains its highest concentration in the tissues of fish at the top of aquatic food chain (WHO,1989). Carnivorous species in a food chain are placed at a higher trophic level than non-carnivorous species (Laws, 1981). It is generally agreed that Hg concentrations in carnivorous fish are higher than in non-carnivorous species (WHO, 1989; Cabana et al., 1994; Watras and Huckabee, 1994; Castilhos and Bidone, 2000).

The factors controlling the accumulation of Hg in fish are not yet fully understood. In general, three different approaches have been used to understand the processes involved in the contamination of Hg in fish. The first approach has been to survey streams, lakes and watersheds and to correlate contaminant body burdens in biota with geochemical, physical and biological descriptors of aquatic systems (Driscol, 1994; Watras et al., 1998). The second approach has been to examine the biological process involved in pollutant uptake by biota, especially under controlled laboratory (Freitas and Hart, 1975; Lawrence and Mason, 2000) or controlled aquatic body conditions (Watras and Bloom, 1992). The third approach has evolved as a combination of field measurements and mechanistic simulation models to quantify the influence of the various processes of the contaminant uptake rates (Poust et al., 1996).

Aquatic organisms accumulate Hg from water and food (including organic matter and sediments), but their relative importance remain controversial (Norstom et al., 1976; Rodgers and Quadri, 1982; Spry and Wiener, 1991; Post et al., 1996).

Many parameters have been considered important in the bioaccumulation and/or biomagnification of Hg in fish. Among them, the natural and/or anthropogenic loadings of mercury in sediments and water column (Lockhart et al., 1999 suggest that recent loadings of mercury to lakes could influence mercury levels in fish), the physico-chemical parameters of water, such as temperature, hardness, alkalinity, acidity, pH, dissolved organic carbon, oxi-reducing conditions (Spry and Wiener, 1991; Gilmour and Henry, 1991) and salinity (Stumm and Morgan, 1996). The organic matter (OM) is a well known carrier of Hg in natural and polluted water and the complex formed is suggested to reduce metal bioavailability and to control Hg speciation in aquatic systems (Hudson et al., 1994; Kainz et al., 1999; Lawrence and Mason, 2000) while others have correlated high levels of Hg in fish with DOC (Forsberg et al., 1999). Thus, bioproductivity (Hakanson, 1984) and primary production (quantity and kind) are important parameters besides physical dimension of aquatic system and climatic seasonality. Indeed, the "reactive" mercury levels in water column and sediment (Gonçalves, 1999), the microbiology activities to methylate mercury and their methylation rates, and the concentration of antagonist chemical agents, such as Se and Zn (WHO, 1992; Hakanson, 1980) or agonist (eg: Al) (Driscoll et al., 1994), should influence the bioaccumulation of mercury in fish. In addition, local biota's physiological-dependent factors, like size, length, age, sex and metabolic rate (Walker, 1976 Olson, 1976; Phillips, 1980; Nicoleto and Hendricks, 1988; WHO, 1989; Reinfelder et al, 1998) and also, food-chain characteristics (Cabana et al, 1994) should influence bioaccumulation as well. It has been suggested that seasonal changes in fat and protein could influence the metal levels in biota (Osbourn, 1979) and its distribution between different internal organs.

Hg transference factors in aquatic trophic chain have been studied by comparison of Hg means values among different trophic levels (phytoplankton, zooplakton, invertebrates, macrofites and fish) (Porcela, 1994; Wren and Stephenson, 1991) or by comparison of Hg tissue levels among fish species with different food habits (Castilhos, 2000). The transference factors of Hg through the food-chain involving Amazonian ichtyofauna suggested that biomagnification may occur in both the contaminated and background areas (Castilhos and Bidone, 2000).

Hg levels in fish for spatial and/or temporal comparisons have been normalized by the mean of Hg content in 1 kg of fish (Johnels et al., 1967; Häkanson, 1991), or by using only fish of one year of age (Post et al, 1996), or by using a specific length (Scruton, et al., 1994), or by using a specific weight (Watras et al., 1998). However, even when only one species of fish is utilized as bioindicator or biomonitor for Hg accumulation, the results show high variability in the levels of Hg (Jonhels et al., 1967; Kehrig, 1992; Pinho, 1998; Castilhos, 1999). Positive correlation between concentrations in muscle and both size, length and/or age for a given species has been well documented (WHO,1989; WHO, 1992, Leah et al., 1991; Simonin et al., 1994; Thornton et al., 1996 Andersen and Depledge, 1997, Castilhos et al., 1998), with higher fish having higher Hg concentrations, and this relationship could be pointed out as an important reason for high relative standard deviation of fish tissue mercury levels.

Castilhos et al (2001) suggested a field dose-response approach as a tool for environmental Hg contamination assessment in fish (DRAC). This methodology was used to assess the Hg contamination in the Tapajós River Region by gold mining activities. The results showed different daily uptake doses by Tucunaré (*Cichla* spp.) between uncontaminated and contaminated areas (~3.5 times), and this difference could be attributed to the Hg load differences between the studied areas.

The dose-response relationship has the competence to absorb inter-individual variabilities. Responses are of two kinds: quantal and quantitative. In a quantal test, an organism either shows the response under study or does not show it. Thus, a certain percentage of test organisms will show the response within some stated conditions. In a quantitative or graded test, each organism responds to a variable degree. Quantal test are designed to estimate the concentration of a test material that affects 50% of the test organisms, the median effective dose (ED 50% or ED50). One must choose the effect to be observed. Thus, this is a quantal rather than a graded response, since the specific effect is either present or absent. The ED50 for accumulation of Hg by fish indicate the time of exposure necessary to attain those tissue concentration levels by half of the exposed individuals. Some methods are used to calculate ED50. Among them, there is the "probit" method (American Public Health Association, 1985; Ross and Gilman, 1985). The potential times of exposure were inferred from estimated age and were transformed in their logarithms. The frequency of responses were transformed in "probit" units.

If we accept that exposure and response can be interrelated as: $t.C = k$, where t is the time of exposure, C is the daily uptake rate of Hg and k is a constant related to a response (adapted from Dämgen and Grünhage, 1998). Although bioaccumulation/biomagnification processes cannot be interpreted as a pharmacological/toxicological "effect", one could suggest that as higher the internal dose (bioaccumulation), higher potential aquatic risk. MeHg has been classified as clastogen substance and toxic effects such as icthyomutagenesis have been associated to MeHg exposure and intensity of dose (probabilistic risk) (Schoeny, 1997).

Castilhos and Lima (2001) have tested the applicability of the proposed methodology by using literature data. Seven fish species from 8 different aquatic ecosystems, including lentic and lotic freshwater, estuarine and marine ecosystems were chosen from scientific publications, in which Hg levels in muscles and estimated age or measured length for individual specimens were available. For the work in the literature that present the relationship between the levels of Hg in tissues and the size and/or age of fish in graphical form, the program ***"GIF Coordinates"*** was utilized for acquisition of the coordinates. The objectives of this study work were: (i) to establish and compare the dose-response relationship for Hg accumulation by several different fish species from several ecosystem and time collection (ii) to estimate and compare the daily Hg uptake rate by those several different fish; and (iii) estimate and compare the potential time of exposure necessary for Hg accumulation to reach 500 μg/kg, the concentration limit for human consumption adopted in many countries.

The results are presented in Table 3, which displays the popular name of the fish, the local and time of sampling, food habit, number of collected specimens, observed effect, daily uptake rate estimate, estimate of time of exposition to reach 500 μg/kg.

Comparing the EO values (median of Hg concentration in μg/kg) in the population sampled, for piscivorous and zooplanktivorous fishes, the following crescent order of contamination is achieved: Sepetiba Bay (corvina) < Ilha Grande Bay (corvina) = Guanabara Bay (corvina) = lagoons - Santarém (tucunaré) < Conceição Lagoon (corvina) < Tongue River- reservoir (white crapie) < Tongue River-reservoir (walleye) = Tongue River-reservoir (pike) = Tapajós River (tucunaré) < Tongue River- reservior (sauger) < Tucuruí River- reservoir (tucunaré).

However, considering the daily uptake rate estimates and or the time necessary for half of the specimens to reach 500 μg/kg, the order of contamination is altered to: Tongue River-reservoir (white crapie) < Conceição Lagoon (corvina) < Ilha Grande Bay < Guanabara Bay < Sepetiba Bay < Tongue River-reservoir (pike = sauguer= walleye) = lagoons-Santarém (tucunaré) < Tapajós River (tucunaré) < Tucuruí River-reservoir (tucunaré).

Considering data from the work Kherig, when the average of Hg levels are normalized with respect to the average weight of the fish, no alteration in the order of contamination is achieved, as compared to the order obtained through the average rate of daily uptake. The DRAC shows advantage to allow the expression of time of exposition, giving a dynamic evaluation (μg/kg/day), capable of being utilized in predictive models. In addition, it may orient the sampling, requiring only 30 individuals for each aquatic system (Castilhos et al, 2001b). For the Tucuruí system possible differences in the rate of growth of tucunaré should be investigated, because the data indicates the existence of dwarf species in reservoirs.

The DRAC is a simple and fast methodology and can be applied to any data bank including spatial and temporal contamination assessment for environmental contaminants that showed bioaccumulation, such as organochlorides.

Table 3. Popular and scientific names, food habit, locality, date and number of fish collected (N) and bibliografic references; observed effect (OE;$\mu g.Kg^{-1}$), estimated daily uptake rate (DUR; $\mu g.Kg^{-1}.dia^{-1}$), estimated time of exposure to attain $500\mu g.Kg^{-1}$ (T, years) and bibliografic reference.

Popular name	Scientific name	FH*	Locality	Date	N	OE ($\mu g.Kg^{-1}$)	DUR ($\mu g.Kg^{-1}.day^{-1}$)	T (years)
Northern Pike	*Esox lucius*	P	Tongue River- Reservoir(EUA)[1]	1978	56	300	0,2	6,8 [5]
Sauger	*Stizostedion canadense*	P	Tongue River- Reservoir(EUA)[1]	1978	31	350	0,2	6,8 [5]
Walleye	*Stizostedion vitreum*	P	Tongue River- Reservoir(EUA)[1]	1978	26	300	0,2	6,8 [5]
White crapie	*Pomoxis annularis*	Z	Tongue River- Reservoir(EUA)[1]	1978	36	200	0,05	27 [5]
Corvina	*Micropogonias furnieri*	C	Guanabara Bay (RJ, BR)[2]	1992	56	100	0,15	9,1 [6]
Corvina	*Micropogonias furnieri*	C	Ilha Grande Bay(RJ, BR)[2]	1990-1991	57	100	0,13	10,5 [6]
Corvina	*Micropogonias furnieri*	C	Sepetiba Bay (RJ, BR)[2]	1990-1991	60	80	0,10	13,7 [6]
Corvina	*Micropogonias furnieri*	C	Conceição Lagoon(SC, BR)[2]	1990-1991	42	130	0,08	17 [6]
Tucunaré	*Cichla spp*	P	Lagoons -Santarém (PA, BR)[3]	1992	28	100	0,2	6,8
Tucunaré	*Cichla spp*	P	Tapajós River (PA, BR)[3]	1992	41	300	0,8	2
Tucunaré	*Cichla spp*	P	Tucuruí River- Reservoir (PA, BR)[4]	1995	61	1000	1,65	0,83 [5]

- P = piscivorous; C= carnivorous benthic; Z = zooplanktivorous
- [1]-Phillips et al., 1980; [2]-Kherig, 1992 (data non-normalized); [3]-Castilhos et al., 2001; [4]-Porvari, 1995; [5]-Castilhos and Lima, 2001; [6]-Castilhos, 1999b

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION (1985) Part 800 Toxicity test methods for aquatic organisms, In: 16th Edition, Greenberg AE, Trussell RE, Clesceri, L. and Frason MAH (ed) Standard Methods for Examination of Water and Wastewater. Baltimore, Maryland, USA, p. 689-819

ANDERSEN, JL and DEPLEDGE, M.H. (1997) A survey of total mercury and methylmercury in edible fish and invertebrates from Azorean Waters. Marine Environmental Research, 44(3): 331-350

BRUGGEMAN WA (1982) Hidrophobic interactions in the aquatic environment. In: Hutzinger O (ed) The Handbook of environmental chemistry, vol 2. Spring-Verlag, Germany, p 205

CABANA G, TREMBLAY A, KAFF J, RASMUSSEN JB (1994) Pelagic food hain Structure in Ontario Lakes: A determinant of mercury levels in lake trout (Salvelinus namaycush). Can J Fish Aquat Sci 51:381-389

CASTILHOS, Z.C. et al (1998) Increase of the background human exposure to mercur through fish consumption due to gold mining at the Tapajos river region, Pará State, Amazon. Bull Environ Contam Toxicol 61 (2): 202-209

CASTILHOS, Z.C (1999) Gestão em poluição ambiental: Análise da contribuição dos garimpos de ouro na contaminação por mercúrio da ictiofauna e das águas fluviais na região do rio Tapajós, Estado do Pará, Brasil. PhD thesis, 200p.

CASTILHOS, Z.C (1999b) Estimativa da taxa de captação diária de Hg por Micropogonias furnieri a partir da interrelação dose-resposta em quatro estuários brasileiros. "Workshop" Efeitos de Poluentes em Organismos Marinhos, UFF/FAPERJ; 08 a 10 de novembro de 1999,p.22.

CASTILHOS, Z.C. and BIDONE, E.D. (2000) - "Mercury Biomagnification in the icthyofauna of the Tapajós River Region, Brazil". Bull Environ Contam Toxicol 64:5, 693-700.

CASTILHOS Z.C. et al (2001) Bioaccumulation of Hg by Tucunaré (Cichla ocellaris.) From Tapajós River Region, Brazilian Amazon. A Field Dose-Response Approach. BECT, in press (May).

CASTILHOS Z.C. et al (2001b) Manual de coleta de peixes para aplicação da DRAC e para a investigação de efeitos bioquímicos. Projeto CNPq/CETEM/UFF.

CASTILHOS, Z.C. and LIMA, C (2001) A field dose-response approach as a tool for spatial and temporal environmental Hg contamination assessment in fish. VI ICMGP-2001

CLARKSON TW (1994) The toxicology of mercury and its compounds. In: Watras CJ, Huckabee JW (ed) Mercury pollution: Integration and synthesis. Lewis Publishers, Boca Raton, Florida, USA, p. 631-640

COMPEAU, G. AND BARTHA, R. (1984) *Applied Environ. Microbiol.* **48** (6): 1203-1207.

DÄMGEN, U. and GRÜNHAGE, L. (1998) - "Response of a grassland ecosystem to air pollutans. V. A toxicological model for He assessment of dose-response relationship for air pollutants and ecosystems". Environ Pollut 101:375-380.

DRISCOLL, C.T.; SCHOFIELD, C.L.; MUNSON, R. K.; YAN C.; KOLSAPPLE, J.G. The mercury cycle and fish in the Adirondack lakes. Environ. Sci. Technol. 28(3): 136-143, 1994.

FADINI AND JARDIM (1999) Levels of Hg in the Negro river basin in Amazon

FORSBERG ET AL. (1999). The biogeochemistry of mercury in the Negro river basin. V International Conference on Mercury as a Global Pollutant

GILMOUR, C.C. and HENRY, E.A. Mercury methylation in aquatic systems affected by acid deposition. Environ. Pollut. 71(2/4): 131-170, 1991.

GONÇALVES, G.O (1999) Distribuição de mercúrio nas lagoas do litoral nordeste fluminense.. Dissertação de mestrado-Geoquímica, UFF, Niterói, 60p

GUIMARÃES, J.R.D., MEILI M., MALM O. AND BRITO, E. M. S. (1998) *Sci. Total Environ*. **213**: 165-175.

GUIMARÃES, J.R.D., MEILI, M., HYLANDER, L.D., SILVA, E.C., ROULET, M., MAURO, J.B.N., LEMOS, R.A. (2000) *Sci. Total Environ.* 261 : 99- 107.

HÅKANSON, L. (1980) "An Ecological Risk Index Aquatic Pollution Control. A Sedimentological Approach." Water Research. v.4, p.975-1001.

HÅKANSON, L. (1984) "On the Relationship Between Lake Trophic Level and Lake Sediments". Water Research, v.18, n.3, p303-314.

HAKANSON, L. (1991) - "Mercury in fish-geographical and temporal perspectives". Water Air Soil 55:159-177.

HERRIN, RT et al (1998) Hypolimnetic methylmercury and its uptake by plankton during fall destratification: a key entry point of mercury into lake food chains? Limnol. Oceanog. 43(7): 1476-1486

HUCKABEE JW, ELWOOD JW, HILDEBRAND SG (1979) Accumulation of mercury in freshwater biota. In: Nriagu JO (ed). The biogeochemistry of mercury in the environment, Elsevier, North Holland, Amsterdam, p. 277-302

HUDSON, R.J.; GHERINI, S.A.; WATRAS, C.J.; PORCELLA, D.B. Modeling the biogeochemical cycle of mercury in lakes: The mercury cycling model and its application to the MTL study lakes. In: In: Watras C.J. and Huckabee J.W. editores. Mercury pollution: Integration and synthesis. Lewis Publishers, Boca Raton, Florida, USA, 1994. p. 473-526.

JOHNELS, A.G.T.; WESTEMARK, T.; BERG, W.; PERSSON, P.I. and SJONSTRAND (1967) - "Pike (Esox lucius L) and some other aquatic organisms in Sweden as indicators of mercury contamination in the environment". Oikos 18:232-33.

JORGENSEN, B.B. AND BAK, F. (1991) *Appl. Environ. Microbiol.* **57** (3): 847-856.

KAINZ, M et al (1999) Relationship of methylmercury between zooplankton and natural lake sediments. VICMGP, p. 229.

KHERIG, H. (1992) - "Estudo comparativo dos níveis de concentração de mercúrio total em corvinas (Micropogonias furnieri) de quatro estuários brasileiros". Dissertação de mestrado - Rio de Janeiro – PUC

LACERDA, L. D. AND MARINS, R. V. 1997. Anthropogenic Mercury Emissions to the Atmosphere in Brazil: The impact of Gold Mining. Journal of Geochemical Exploration. 58.p: 223 - 229.

LACERDA, L. D. AND SOLOMONS, W. 1998. Mercury from Gold and Silver Mining: A Chemical Time Bomb?. Springer-Verlag, Berlin.

LACERDA, L. D.; RIBEIRO, M. G.; CORDEIRO, R. C. SIFEDDINE, A. AND TURCQ, B. Mercury Atmospheric Deposition Over Brazil During the Past 30,000 Years. Ciencia e Cultura. v.51 (5/6). p.363-371.

LAWS, E. Aquatic Pollution; an introductory text. Willey-Interscience Published, 1981. 200p.

LAWRENCE, AL and MASON, RP (2000) Factors controlling the bioaccumulation of mercury and methylmercury by the estuarine amphipod Leptocheirus plumulosus. Environmental Pollution, January, 2000.

LOCKHART, WL et al.(1999) Mercury in fish from North Canadian lakes. V ICMGP, p. 230.

MASON, RP, REINFELDER JR, MOREL F M M (1995) Bioaccumulation of mercury and methylmercury. Wat Air Soil Pollut 80:915-921

MAURO, J.B.N., GUIMARÃES, J.R.D. AND MELAMED, R. (1999). *Appl. Organomet. Chem*., **13**: 631-636.

NICOLETO, PF and HENDRICKS, AC (1988) Sexual differences in accumulation of Hg in four species of centrachid fishes. Can. J. Zool. 66: 944-949

OSBOURN, D (1979) Seasonal changes in fat, protein and metal content of the liver of starling Sturnus vulgaris. Environ. Pollut. 19: 145-155, 1979.

OLSSON, M. (1976) Mercury level as a function of size and age in Northern pike, one and five years after the mercury ban in Sweden. AMBIO, 5(2): 73-76

PARK, R and BARTHA, R (1999) Mercury methylation by interspecies hydrogen and acetate tranfer between sufidogens and methanogens . Apl. Environ. Microbiol., 64(6): 1990-1998

PINHO, AP. (1998) Mercúrio total em elasmobrânquios e teleósteos da costa leste do Brasil. Dissertação de mestrado, Departamento de Biofísica, UFRJ, Rio de Janeiro, 95p.

PHILLIPS, GR and BUTHLER, DR. Trans. Amer. Fish. Soc., 107: 853-861

PHILLIPS, D.J.H. (1980) - "The effects of age (size, weight) on trace metals in aquatic biota. In: Mellanby K (ed) Quantitative aquatic biological indicators". Applied Science Publishers, Essex, England.

PHILLIPS, G.R..; LENHART, T.E.; GREGORY, R.W. (1980) - "Relation between trophic position and mercury accumulation among fishes from the Tongue river reservior, Montana. Environmental Research, 22: 73-80.

PORCELA, DB (1994) Mercury in the environment: Biogeochemistry . In: Watras CJ, Huckabee JW (ed) Mercury pollution: Integration and synthesis. Lewis Publishers, Boca Raton, Florida, USA, p. 3-19

PORVARI, P. (1995) - "Mercury levels of fish in Tucuruí hydroeletric reservior and in river Mojú in Amazonia, in the state of Pará, Brazil". The science of the total Environment 175: 109-117.

POST, J.R.; VANDENBOS, R. and MCQUEEN, J. (1996) - "Uptake rates of food-chain and waterborne mercury by fish: field measurements, a mechanistic model, and an assessment of uncertainties". Can J Fish Aquat Sci 53: 395-407.

REIFELDER, J. R. et al (1998) Trace element trophic transfer in aquatic organisms: a critique review of the kinetic model approach. The science of the total Environment 219: 117-135.

RODGERS, D.W. and QUADRI, S.U. Growth and mercury accumulation in yearling yellow perch, Perca flavenscens , in the Ottawa River, Ontario. Environ. Biol. Fishes, 7:377-383, 1982.

ROSOLEN, V.; LAMOTTE, M.; ROUER, O.; BOULET, R.; TRINCHET, J. AND MELFI, A. J. 1999. Evidências de Formação de Horizontes de Argila Manchada por Processos de Deferruginização. Anais do V Congresso de Geoquímica dos Países de Língua Portuguesa & VII Congresso Brasileiro de Geoquímica, Porto Seguro, Bahia, Brasil. p: 413 – 415.

ROULET, M. AND LUCOTTE, M. 1995. Geochemistry of Mercury in pristine and Flooded Ferralitic Soils of a Tropical Rain Forest in a French Guyana, South America. Water, Air and Soil Pollution. 80.p: 1079 - 1088.

SCHOENY, R. Use of genetic toxicology data in U.S.EPA risk assessment: the mercury study report as an example. Environ Health Perspect. 104 (Suppl. 3): 663-673, 1996

SCRUTON, D.A.; PETTICREW, E.L.; LEDREW, L.J.; ANDERSON, M.R.; WILLIAMS, U.P.; BENNET, B.A. and HILL, E.L. (1994) -"Methylmercury levels in fish tissue from three reservoir systems in Insular Newfoundland, Canadá". In: Watras CJ, Huckabee JW Mercury Pollution: Integration and Synthesis. Lewis Publishers, USA, 727p.

SIMONIN, H.A et al (1994) Mercury in yellow perch from Andirodac Lakes (New York, US). In: Watras CJ, Huckabee JW (ed) Mercury pollution: Integration and synthesis. Lewis Publishers, Boca Raton, Florida, USA, p. 457-469

SPRY, D.J. and WIERNE, J.G. Metal bioavailability and toxicity to fish in low-alkalinity lakes: a critical review. Environ. Pollut. 71: 243- 304, 1991.

STUMM, W and MORGAN, JJ (1996) Aquatic chemistry, 3rded.

THORNTON, K.W.; PRICE, A.; ARMSTRONG, M.; NIX, J.; GIESE, J.; TURMAN,D. (1996) Factors affecting fish mercury concentration in Arkansas lakes and streams, USA. Mercury as a Global Pollutant-4th International Conference, Agust 04-08, Hamburg, p. 185.

WALKER, TI (1976) Effects of species, sex, length, and locality on mercury content at school shark Mustelus antarticus- Guenther from South-eastern Australian waters. Australian J. Marine and Freshwater Research, 27: 603-608.

Watras CJ, Huckabee JW (1994) Mercury pollution: Integration and synthesis. Lewis Publishers, USA, 727p

WATRAS, C.J.; BACK, R.C.; HALVORSEN, S.; HUDSON, R.J.M.; MORRISON, K.A. and WENTE, S.P. (1998) - "Bioaccumulation of mercury in pelagic freshwater food webs". Sci Tot Environ 19:183-208.

WHO (1990) Environmental Health Criteria 101: Methylmercury. Geneva, World Health Organization

WHO (1989) Environmental Health Criteria 101: Methylmercury. Geneva, World Health Organization

Mercury Contaminated Sites: A Review of Remedial Solutions

Jennifer Hinton, jhinton50@hotmail.com
Marcello Veiga, veiga@mining.ubc.ca
Dept. of Mining and Mineral Processing Engineering
University of British Columbia, Vancouver, BC, V6T 1Z4, Canada

The physio-chemical characteristics of mercury have made it a favourable component for many industrial and agricultural applications. Significant anthropogenic Hg sources include fossil fuel combustion, the chloralkali industry and pharmaceuticals. Modifications to industrial practices and stricter regulations have led to emission reductions in many countries; however, this has been countered by the increased use of Hg for gold amalgamation, particularly in tropical, developing countries. Under any of these circumstances, varying quantities and forms of Hg have been inevitably released to environments where it can pose a risk to human and ecological health. Given the unique behaviour of Hg, remediation of Hg contaminated sites can be complicated and costly. This paper reviews commonly employed and emerging techniques to mitigate Hg pollution and describes key design considerations and concerns associated with each method. Well-established *ex-situ* (external) techniques, such as physical separation and thermal treatment of excavated materials, are discussed in theory and practice. Potential *in-situ* (*i.e.* in place) Hg recovery methods, such as soil vapour extraction combined with soil heating and the use of leaching agents, are also explored. Finally, containment strategies are described for sites where more conclusive measures cannot be employed.

Keywords: mercury sources, mercury pollution, remediation, mitigation.

CHARACTERIZATION OF CONTAMINATED SITES

Industrial activities use Hg for various purposes and can therefore introduce Hg into the environment in many different forms. As the toxicological impacts of Hg are largely dependent on speciation, understanding its transformations and the impacts of various chemical forms is vital to the prevention of harmful human and environmental health effects. Ultimately, the Hg species present in a given environment depends on the initial released form, the thermodynamic stability of this compound and the transformation rate of the released form to a more stable one (Baeyens *et al*, 1979). These issues must be well understood to effectively design and evaluate appropriate remedial solutions in Hg impacted areas.

Depending on the mercury source and how it was released into the environment, mercury may be present in concentrated "hot spots" or dispersed over extensive areas, as is often the case in agricultural applications or atmospheric deposition adjacent to power generating facilities. Often, contamination is initially suspected on the basis of historical land use (*e.g.* in the production of primary batteries). Once an initial investigation of land use has been conducted, soil and groundwater should be sampled to determine the presence of contaminants. A detailed site will determine the extent and distribution of contamination and characterize geologic units significant to contaminant mobility. This typically involves subsurface drilling and/or shallow trench sampling, generally using a backhoe, and may be accompanied by the installation of piezometers to sample and monitor groundwater and collection of surface water samples. Primarily due to the volatile nature of many forms of Hg, the health of those carrying out work at the site must be protected throughout the investigation.

The recent metallic Hg spill in Choropampa, Peru, brought to light the importance to consider the form of Hg present when conducting sampling programs. During the clean-up that followed this accident, an analytical procedure was employed to detect metallic Hg "hot spots" along the roadsides. Although the procedure was accurately conducted, the size of the sample analyzed (1 gram) was too small to sufficiently represent the content of Hg in soils from a given location.

When released into the environment, metallic Hg often produces a "nugget effect", i.e. individual droplets increase the analyzed concentration in discrete locations of the sample. Consequently, larger samples are always needed to avoid sampling errors. It has been established that the most effective procedure for detection of metallic Hg "hot spots" is a semi-quantitative method involving pyrolysis of 30g soil samples (CETEM, 1989). In the screening stage, analytical precision of small, discrete samples is essentially irrelevant to the practical identification of hot spots. For comparison, the appropriateness of sample sizes for gold sampling is shown in Table 1. If the same approach applies to sites contaminated with metallic Hg, to sample a soil with 4 ppm Hg as drops of 0.25mm, 1 kg of material is needed to be representative. Dry pulverization of the entire kilogram sample is required to reduce the size of the mercury droplets and, in this case, smaller sub-samples can be used for chemical analysis. It has been established that visible identification by panning is a reasonable and inexpensive method to identify metallic Hg hotspots until levels of 3 ppm (CETEM, 1989).

Size of Gold Particle (mm)	kg of sample required	
	4 ppm Au	1 ppm Au
2.0	400	1000
1.0	50	200
0.5	8	30
0.25	1	4
0.125	0.1	0.5
0.062	0.02	0.05
0.031	0.002	0.006
0.015	0.0002	0.002
0.008	0.00002	0.0001

Table 1. - Required Sample Sizes to Avoid Nugget Effect (adapted from Clifton et al., 1969)

Sampling of biota present at the site and/or the implementation of bioavailability studies should be performed at this point in the site assessment. Bioaccumulation evidence must be obtained or predicted in order to evaluate appropriate courses of action. If impacts to biota are not proven or probable, containment and long-term management is more appropriate than other aggressive measures. This, of course, is based of on the acceptability of this to regulators. If bioaccumulation is occurring or likely to occur, then remediation must be implemented. The decision now is to excavate and treat or isolate impacted materials *or* address the contamination using *in-situ* methods. Currently, excavation and removal is widely practiced, as it is frequently the most cost-effective option for Hg impacted sites. However, alternative *in-situ* technologies will likely become more prevalent as they become more established and less costly.

In some cases, the decision to remove contaminated soils or sediments is based exclusively on Hg concentrations in excess of numerical criteria. In Japan, for example, the decision to dredge sediments from Minamata Bay with Hg concentration above 25 ppm was based on many site-specific factors such as tidal range, sediment-to-water transfer rate and a safety factor of 100 in fishing zones (Kudo and Turner, 1999). In British Columbia, Canada, after the construction of a Convention Center on an old contaminated site, the Government established guidelines for Hg concentration in soils. The BC Ministry of Environment (1989) determines that soils or sediments with Hg concentration between 2 and 10 ppm require remediation to levels below 2 ppm if the land is to be used for residential and recreation purposes. For sites with concentrations above 10 ppm Hg, all uses of land are restricted pending the application of appropriate remedial measures that reduce contaminant concentrations to less than 10 ppm.

It is clear that the simple soil or sediment analysis does not provide enough evidence to support remediation actions. Sites with 25,000 ppm of Hg in British Columbia have been analyzed. This tailing from a chlor-alkali operation must definitely be properly contained. It is evident that a risk based approach as warranted in many situations.

REMEDIATION TECHNOLOGIES

Many factors are used to assess the suitability of specific remedial measures for a given site. The distribution and properties of stratigraphic units (individual soil and rock types) and a comprehensive understanding of the site hydrogeology and hydrology, are used in conjunction with physio-chemical properties of the contaminant to predict contaminant mobility and distribution and subsequently develop means for mitigation. Once determined, these elements can contribute to a preliminary assessment of risk to ecological and human health in the impacted area and provide a basis for evaluation and subsequent design of measures for mitigation.

The determination of clean-up goals and prescribed responses are becoming increasingly dependent on identified risks to human and ecological health. Although numerical criteria are valuable indicators of the occurrence and extent of contamination, these levels are not economically or technically attainable in some situations. As shown in Figures 1 and 2, if Hg is detected in biota (*i.e.* it is bioavailable) or the risk of its subsequent incorporation into organisms is appreciable, then more aggressive and often more costly measures are warranted. In the case of dispersed mercury contamination, remedial measures are typically not feasible (Figure 1). If bioaccumulation is identified, then exposure pathways must be addressed. Possible responses include the implementation of consumption advisories to reduce intake of contaminated food, or educational programs and technology modifications to lessen exposure to Hg vapour. In James Bay, Canada, where fish from La Grande hydroelectric dam are contaminated, a very useful brochure informs the public about Hg sources, biotransformation, and dietary recommendations. The booklet also includes a list of recipes in which fish is diluted with vegetables reducing the amount of Me-Hg ingested. If Hg is distributed in a localized area (Figure 2) and bioaccumulation is evident, then remedial actions are warranted. If the potential for assimilation into the food chain is low, then containment methods are sufficient. In either situation, long term management is required until adequate protection of ecological and human health is ensured. Of course, any measure employed must be acceptable to regulators.

Figure 1: Appropriate Response to Dispersed Mercury

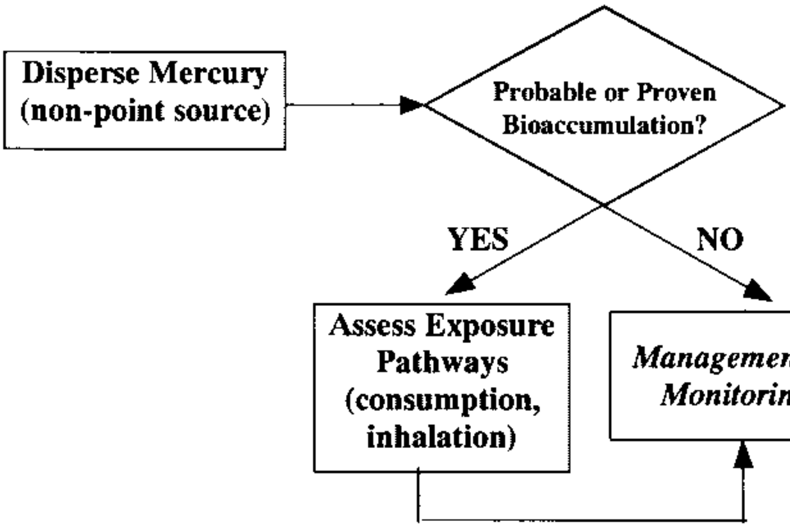
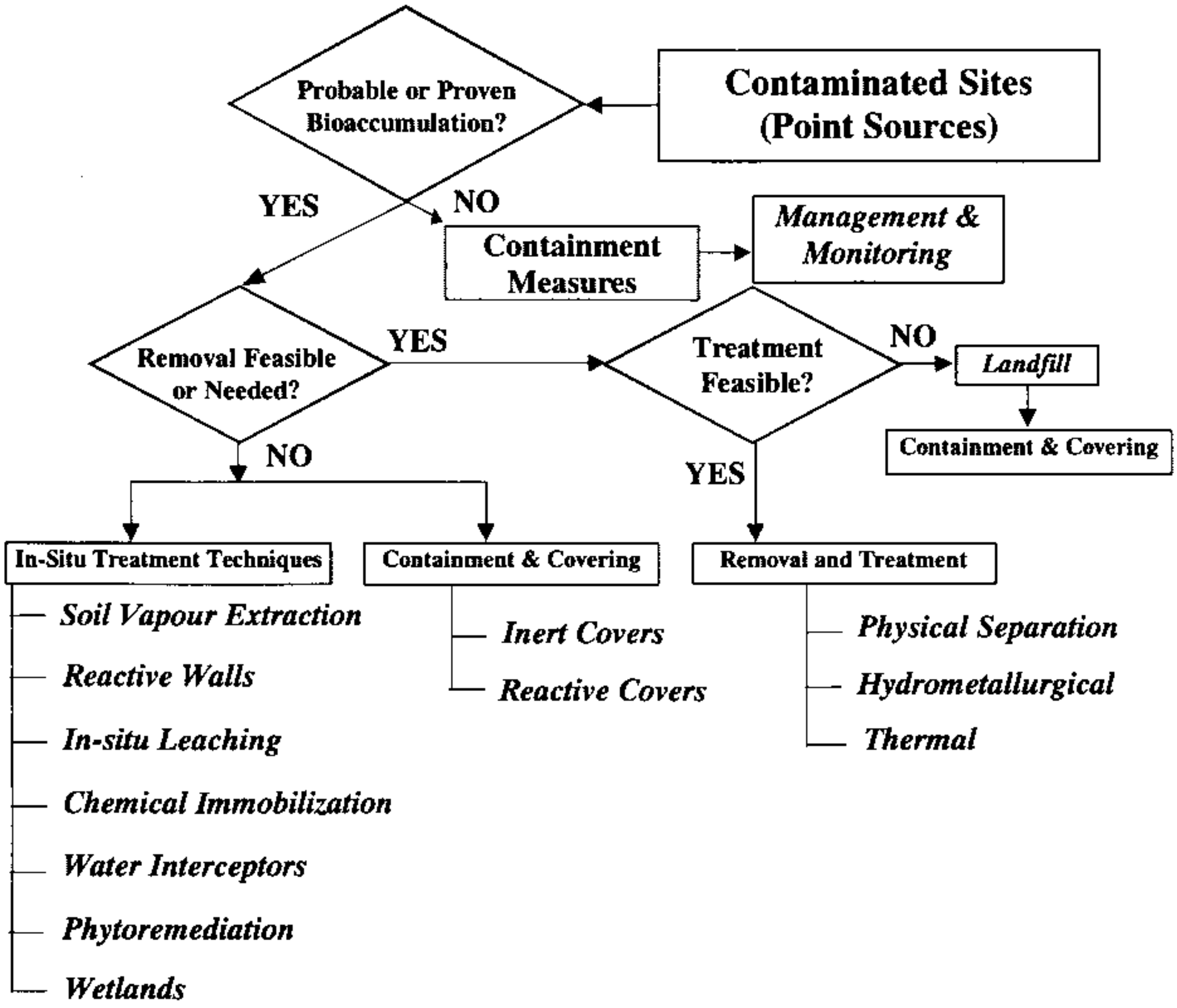


Figure 2 - Appropriate Responses to Point Source Mercury Contamination



Removal and Treatment

Excavation and *ex-situ* (*i.e.* off-site or aboveground) treatment of Hg-contaminated soils is the most frequently employed practice for Hg recovery. Although excavation can be complicated if it extends below the water table or costly if the contamination is distributed over a large area, it is essentially a well-understood practice. Mercury is subsequently liberated from excavated soil by one of the three processes described below.

Physical Separation

Classification on the basis of size fraction simple process is based on the premise that most Hg compounds have a strong affinity for the fine fraction of soils. In wet screening, metallic Hg droplets behave as a separate liquid phase being concentrated into the fines. If there is an intention to analyze Hg grain size distribution, dry sieving must be adopted. Fines, rich in clayminerals, hydrous ferric oxides and/or organics are separated generally using hydrocyclones. In this case, if metallic Hg is predominant, the separation is not effective, unless the handling process (scrubbing) causes Hg flouring (lack of coalescence) and Hg drops become really small. Then, the Hg-enriched sludge or fine fraction is dewatered and subsequently isolated or treated further (for example, via thermal methods). This classification procedure is most effective for soils dominated by coarse materials (*i.e.* sand and gravel) with some (< 20%) fines.

Veiga (1997) reviewed the techniques to concentrate metallic mercury from Hg-contaminated gold mining tailings using classical beneficiation processes, such as gravity concentration with centrifuge as well as flotation. From 60 to 80% of Hg is removed and the presence of superfine mercury or mercury adsorbed onto other minerals, such as hydrous ferric oxides, can be reasons for low recoveries. The most promising and simplest technique to remove metallic mercury from contaminated sediments is a sluice box assembled with special-amalgamating plates manufactured by Rio-Sul Ambiental Ltd. These plates, with a thin coating of mercury and silver electrolytically deposited onto a copper alloy plate, have been successfully tested in Brazil and Colombia to remove over 90% Hg from contaminated gold tailings (Veiga et al., 1995). Due to the toxicity of Hg, the occupational health of adjacent workers naturally must be considered during all kind of processing.

Hydrometallurgical Treatments

Chemical extraction of Hg from excavated soils can be induced through four primary mechanisms: 1) desorption of adsorbed species; 2) oxidation of metallic Hg; 3) use of strong complexing agents; and 4) through dissolution of precipitated Hg (*after* Hempel and Thoeming, 1999). Efficiency of any mechanism employed may decrease over time due to recomplexation and readsorption and removal of the most soluble compounds at early time. Leaching agents commonly applied to removed materials include halide compounds, such as hypochlorite or hydrobromic acid, iodine in the form of potassium iodine, and a mixture of nitric acid and NaCl (Hempel and Thoeming, 1999). Sodium hypochlorite and sodium pyrophosphate are believed to be particularly effective at liberating metals from organics, but hydrogen peroxide releases more metals from oxides and sulfides (Papp *et al.*, 1991). An *electrolytic process* to leach mercury from gold mining tailings has been developed by CETEM - Center of Mineral Technology, Rio de Janeiro and tested in two pilot plants. Hypochlorous acid is produced through eletrolysis of sodium chloride mixed with a pulp of Hg-contaminated material (Thoming *et al*, 1999).

Following Hg leaching and subsequent electrolytic recovery of the metal, the solution could be regenerated and recirculated through the system.

Thermal Treatment

As the volatility of Hg and its compounds increase with temperature, thermal heating of excavated soil is a potentially effective means for Hg recovery from contaminated soils. Hempel and Thoeming (1999) determined that all Hg compounds should volatilize at temperatures below 600 °C and, in the absence of HgO, 250 °C should be sufficient. Larry and Jose (1990) recommended heating temperatures between 600-900 °C. Matsuyama *et al* (1999) determined that low-temperature thermal treatments could adequately recover Hg from soils, were less costly and less likely to negatively alter soil properties. Matsuyama *et al* found that at temperatures of 200-210°C, even stable compounds like HgS begin to volatilize. In the presence of a catalyst, specifically iron chloride, Hg (as HgS) removal increased from 50% to 99.5% in a one-hour period at 300 °C.

Organic contaminated soils are commonly treated using thermal processes. Hempel and Thoeming (1999) described the method for organic pollutants, as it is principally the same for Hg recovery from soils. Soil is processed in batches as follows: first it is dried at a temperature of 100 °C., then transferred to a heating drum where temperatures of 600°C are maintained. Gas from these stages passes through an afterburner where, at temperatures near 800-900°C, conversion of organic contaminants to CO_2 is anticipated. This is followed by gas cooling (150°C), flow through a dust filter and a spray tower to remove dust and SO_2. If this typical configuration were applied to Hg, it would be recovered from the gas phase using a gas washing system (Hempel and Thoeming, 1999), charcoal filter (Renner, 1995), iodine impregnated scrubber or through condensation.

In-Situ Treatment Techniques

When removal of contaminated sources is not economically or technically feasible, *in-situ* treatment is the option. Methods for *in-situ* recovery of Hg are far less established than *ex-situ* techniques. As well, due to subsurface heterogeneity, more uncertainly generally exists concerning the effectiveness of *in-situ* processes, and clean-up times tend to be longer than *ex-situ* treatments. In addition, permanent monitoring is usually needed. Despite these factors, many *in-situ* technologies are very promising mainly due to the fact that contaminated soil and groundwater remain in the subsurface. In some cases, when Hg is reaching the water table, extraction wells must be installed and operate, sometimes, in perpetuity. As extracted, water must be treated at the surface, well placement and pumping rate should be chosen to ensure capture of contaminated groundwater and limit recovery of clean water.

Soil Vapour Extraction coupled with Soil Heating

Soil Vapour Extraction (SVE) uses a vacuum to force air through the unsaturated zone. Volatile and semi-volatile compounds easily partition into the vapour phase and are subsequently removed by the vacuum. The ground surface is covered with a tarp or other cover system to minimize vacuum extraction of "clean" air from the surface and ensure lateral airflow through the impacted area. The effectiveness of these systems is primarily dictated by contaminant volatility and availability to air channels, which is mainly governed by contaminant solubility and tendency to sorb to solid surfaces and soil properties.

It is well demonstrated that Hg fluxes into the vapour phase increase significantly with airflow and temperature (Ebinghaus *et al*, 1999, Sexauer Gustin *et al*., 1997). Thus, soil vapour extraction may be an effective technology for *in-situ* remediation of Hg if performed in conjunction with soil heating. Currently, soil heating can be costly over expansive areas and difficult to homogeneously heat a soil volume. As well, the effects of soil heating on physical, chemical and biological properties of soil are not well known. Despite this, soil heating combined with soil vapour extraction may in the future become an effective means of Hg removal in the vadose zone.

Permeable Reactive Walls

Permeable Reactive Walls, wherein dissolved compounds react with walls constituents to precipitate contaminants, have successfully been employed at many organic and metal impacted sites. Permeable reactive walls are engineered structures installed below the ground surface perpendicular to the flow of contaminant-laden groundwater. Walls are geochemically engineered to transform contaminants to relatively benign and/or immobile forms and ideally can operate passively for extended periods with little or no maintenance (Domenico and Schwartz, 1998). Two main criteria that must be addressed in effective design include wall permeability (ideally slightly greater than the parent material) and the level of reactivity with wall constituents (Waybrandt *et al*, 1998). Depending upon the contaminant of concern, wall constituents have ranged from organic-rich composted sewage sludge or wood chips combined with neutralizing materials (*e.g.* limestone) for removal of metals from acidic mine drainage to zero-valent iron (ZVI) for various organic and inorganic contaminants. Other materials proposed for metals studied include hydroxyapatite (Xu and Schwartz, 1994), zeolites (Li, in Press), hydrous ferric oxides and bone char phosphate (USEPA, 1997).

In-situ Leaching and Extraction

In-situ Leaching and Extraction involves the injection of chemicals to enhance Hg solubility in groundwater, thereby reducing clean-up time and improving recovery rates from groundwater. Pump-and-treat is a frequently practiced, cost-effective remedial alternative employed either for removal contaminants from the subsurface and/or hydraulic containment of a contaminant plume (Domenico and Schwartz, 1998). This technology is generally limited to treatment of contaminants impacting groundwater in a dissolved form (*e.g.* $HgCl^-$, HgS^o) or as a non-aqueous phase liquid (NAPLs, *e.g.* metallic Hg). In many natural systems, Hg is strongly adsorbed to soils and/or is present as a precipitate – in either situation, Hg may dissolve or desorb over time and thereby provide a long-term source to groundwater. To reduce clean-up time and increase the efficacy of the clean-up system, solubility-enhancing chemicals may be injected up-gradient from the zone of contamination. Two issues limit the applicability of this method of *in-situ* Hg extraction. First, it is not well demonstrated and second, the injection of leaching agents into the subsurface for the purpose of enhancing contaminant mobility is often unacceptable to regulators.

Electro-kinetic separation is an *in-situ* leaching process that involves the generation of an electric field through application of a low-voltage direct current (DC) in a soil matrix (USAEC, 2000). Heavy metals, such as Hg, migrate towards electrodes placed in the soil where they accumulate and can subsequently be removed at a lower cost than excavating the entire impacted area. In fine-grained soils (*e.g.* clays), migration of charged and uncharged species may occur by *electroosmosis* (Hempel and Thoeming, 1999). The electrokinetic separation process occurs in three stages (Sobolev *et al*, 1996). First, the metal must be transformed into a soluble form, with or without the injection of solutions. Second, the electric current mobilizes the solubilized metal towards an electrode. Finally, accumulated metals are collected at the electrode, typically through excavation.

Chemical Immobilization of Hg

Immobilization and stabilization techniques involve the *ex-situ* or *in-situ* mixing of impacted soils with additives that intend to reduce the mobility or leachability of contaminants (Domenico and Scwartz, 1998). *Stabilization* specifically attempts to bind contaminants to the solid and is often accompanied by reductions in soil permeabilities. *Solidification* techniques will improve the physical characteristics of materials, such as sediments or sludges, so they can be excavated and transported more easily.

Many suppliers propose various concoctions of stabilizing agents (*e.g.* organic polymers), although cement, calcium carbonate spiked with binders (furnace slag silicates or fly ash), asphalt or bitumen are most commonly used. Augers or adapted digging tools can mix additives with soil that has been excavated or directly in the subsurface. Subsurface mixing is less established than aboveground techniques, primarily because specialized injection and mixing equipment must be used, homogeneous treatment is difficult to ensure, and it is still a fairly costly procedure. Despite these issues, *in-situ* stabilization may in the future become an effective solution for deep and difficult to access zones of contamination.

Interceptor Systems

Interceptor Systems, such as trenches and drains, are extremely simple and effective at recovering Hg as "free product" (essentially as metallic Hg), however this treatment is limited by topograghy and stratigraphy and does not address Hg held in residual saturation.

Phytoremediation

Phytoremediation is a promising albeit unproven technology, wherein plants assimilate and concentrate metals from soils. Metal recovery typically occurs through subsequent harvesting and combustion of plants (Anderson *et al*, 1999). Plants that accumulate substantial quantities of metals from soils, known as *hyperaccumulators*, may be species specially developed for extraction of certain metals, or native species possessing a resistance to toxic effects of specific metals. This technique holds much promise for the cost effect remediation of shallow soils over a fairly widespread area, but issues such as limited access to vegetation by wildlife and time required for clean-up must be addressed.

Passive Remediation - Wetlands

The use of wetlands for Hg immobilization is a somewhat controversial issue as wetland-type environments are intrinsically amenable to the conversion of Hg to its highly toxic form, methylmercury (MeHg). Despite this potential, the US Department of Energy (Anon, 2000) has had notable success in significantly limiting the amount of MeHg generated (<1 ng/L) in an artificial wetland, mainly by controlling the amount and form of sulfur present. Mercury bound to decomposed organic matter descends to the bottom of anaerobic cells where it reacts with gypsum to form relatively insoluble sulfide. Ultimately, the wetland will treat up to 1 million gallons of water daily.

Containment and Covering

Clean-up of many contaminated sites is often not feasible, generally due to financial or technical reasons. There are several geotechnically-engineered approaches to prevent infiltration of solutions that may react with the contaminated material (*e.g.* organic acids react with metallic mercury) and/or inhibit off-site migration of contaminants. Each system has limitations with respect to emplacement depth and uncertainty concerning permeability and barriers may intend to surround the contaminated zone entirely or remove the potential for groundwater flow through the source. Two different approaches are applied to classify covering materials, based on the reaction potential with Hg: a) inerts b) reactives (Veiga and Meech, 1995).

Inert Covers

This technique employs low permeability materials, such as compacted clays, sand, natural soils mixed with stabilizers or bentonite, or geosynthetic membranes. Design considerations include grading of surfaces to direct drainage and the long-term integrity of the seals. Elements such as weather effects (frost, desiccation of clays), damage from burrowing animals or vegetation or degradation of synthetics by sunlight can all be significant. Aquarium studies show that 6 cm of sand or gravel applied over sediments spiked with $HgCl_2$ (250 ppb Hg) can prevent release of methylmercury from sediments and subsequent accumulation by fish (guppies). Thinner layers of sand, appeared to be unsatisfactory (Bongers and Kattak, 1972).

Slurry walls are low permeability barriers generally composed of excavated material mixed with bentonite or a mixture of cement and bentonite. Walls can be constructed as a trench, which are typically 0.5 to 2m wide and installed to a maximum depth of 50m, or using specialized equipment (Domenico and Schwartz, 1998). Notable installation systems include augers that mix bentonite with native soil (limited to 60m depths) and a procedure wherein parallel steel plates are forced into the ground using vibration and filled with bentonite slurry as the steel is retracted. The latter method is more susceptible to leakage, as it must be constructed in sections and is considerably thinner than the other methods. *Grout walls* or *grout curtains* are similar to slurry walls constructed through the pressurized injection of gelling or solidifying liquids into the subsurface (Domenico and Schwartz, 1998). *Sheet pile walls* are installed using driving or vibratory forces. In the past, joints between adjacent piles have tended to leak until voids are filled with fines. Recently, however, a technique proposed by the University of Waterloo in Canada in which joints are sealed during construction has been demonstrated in field tests to be extremely effective (Domenico and Schwartz, 1998).

Dredging of contaminated aquatic sediments is often difficult to achieve without re-suspension of sediments and thus contaminants, then removal of material is not always practical. *Sub-aqueous cap* of clean and ideally isolating material over contaminated sediments is a technique that should satisfy three basic purposes: physical isolation of sediments from benthic organisms, prevention of sediment re-suspension and transport, and reduction of the contaminant migration into the water column (Palermo, 1998). The U.S. Army Corp of Engineers has developed detailed specifications for design, construction and monitoring of these systems (Palermo, 1998).

Reactive Covers

This technique usually consists of mixing materials that react with mercury compounds with materials with sealing properties (inert covers). The reactive components of the covers are adsorbents able to bind Hg (II) compounds or methylmercury fixing them in a non-bioavailable form. Veiga and Meech (1995) reviewed several Hg adsorbent materials, such as activated charcoal, organic materials (*e.g.* wool, chicken feather, hair, onion skin, nylon, flour, sugar cane bagasse, rubber, peat), waste wool/polyester blend fibers, rubber (*e.g.* ground automotive tires), sulphides (*e.g.* sodium sulphide, pyrite) and hydrous ferric and manganese oxides (HFMO). Due to the obvious matter of aesthetic and environmental aggression, there are many materials that cannot be applied to large polluted areas. HFMO, by its abundance in tropical latosols, is an indicated material to be tested in remediation procedures of sites contaminated with mercury from artisanal gold mining activities.

CONCLUSIONS

Due to historic and recent industrial practices, varying quantities and forms of Hg have been released to environments where it can pose a risk to human and ecological health. In response to this, several techniques exist or are currently being developed for the remediation of mercury and other contaminants. The decision of applying remediation procedures should be focused on the evidence or possibility of occurring mercury bioaccumulation. The simple chemical analysis of contaminated soil/sediment samples is not sufficient to justify remedial actions. The remedial procedures must also consider the Hg species and the form in which mercury is distributed in the sites. Measures to treat point-sources of mercury must evaluate the feasibility or need to remove the entire contamination source. Techniques such as excavation and *ex-situ* treatment of impacted soils and containment measures are frequently practiced and well understood. Despite this, these methods are often not cost effective or technically feasible. Emerging *in-situ* procedures, such as permeable reactive walls and leaching, may ultimately become more appropriate for many Hg-impacted sites. In some situations, acceptable clean-up goals are simply not economically or technically possible, thus long term management through containment and monitoring must be employed. Ultimately, any measure employed must consider the risk to ecological or human health and have the acceptance of regulators.

REFERENCES

Anonymous, 2000. Constructing a Solution to a Mercurial Problem. *Environ. Sci. Technol., Technology Solutions*, p. 251A, June 1, 2000.

B.C. Ministry of Environment, 1989. British Columbia Standards for Managing Contamination at the Pacific Place Site. Pacific Place Guidelines, Waste Management Program, appendix N, p. 77-82, April 5, 1989.

Biester, H., Schuhmacher, P., and Muller, G., 2000. Effectiveness of Mossy Tin Filters to Remove Mercury from Aqueous Solutions by Hg(II) Reduction and Hg° Amalgamation. *Wat. Res.*, v.34, n.7, p. 2031-2036.

Bongers, L.H. and Khattak, M.N., 1972. Sand and Gravel Overlay for Control of Mercury in Sediments. Environmental Protection Agency, EPA Project 16080 HVA, U.S. Dept. Interior, Washington, D.C. 46p.

CETEM - Centro de Tecnologia Mineral, 1989. Poconé Project. Annual Report, Rio de Janeiro, Brazil, 210p.- in Portuguese.

Clifton *et al.*, 1969 quoted in Bacon, W.G.; Hawthorn, G.W. and Poling, G.W., 1989. Gold Analyses - Myths, Frauds and Truths. *CIM Bulletin*, v. 82, n. 931, p.29-36.

Cox, C.D., Shoesmith, M.A., and Ghosh, M.M., 1996. Electrokinetic Remediation of Mercury-Contaminated Soils Using Iodine/Iodide Lixiviant. *Environ Sci Tech*., v.30, n.6, p. 1933-1938.

Domenico, P.A. and Schwartz, F.W., 1998. *Physical and Chemical Hydrogeology*. John Wiley & Sons, Inc., New York, 506p.

Ebinghaus, R. Tripathi, R.M., Wallschlager, D., and Lindberg, S.E., 1999. Natural and Anthorpogenic Mercury Sources and Their Impacts on the Air-Surface Exchange of Mercury on Regional and Global Scales. In: *Mercury Contaminated Sites, Characterization, Risk Assessment and Remediation*. R. Ebinghaus, R.R. Turner, L.D. Lacerda, O. Vasiliev and W. Salomons (Eds.), Springer Publishing, New York, p. 1-50.

Hempel, M., and Thoeming, J., 1999. Remediation Techniques for Hg-Contaminated Sites. In: *Mercury Contaminated Sites, Characterization, Risk Assessment and Remediation*. R. Ebinghaus, R.R. Turner, L.D. Lacerda, O. Vasiliev and W. Salomons (Eds.), Springer Publishing, New York, p. 113-130.

Johnson, P.C., Kemblowski, M.W., and Colthart, J.D., 1990. Quantitative Analysis for the Cleanup of Hydrocarbon Contaminated Soils by In-Situ Soil Venting. *Groundwater Monitoring Review*, Spring edition., p. 413-428.

Kudo, A. and Turner, R.R., 1999. Mercury Contamination of Minamata Bay: Historical Overview and Progress towards Recovery. In: *Mercury Contaminated Sites, Characterization, Risk Assessment and Remediation*. R. Ebinghaus, R.R. Turner, L.D. Lacerda, O. Vasiliev and W. Salomons (Eds.), Springer Publishing, New York, p. 143-158.

Larry, R., and Jose, L., 1990. *Final Best Demonstrated Available Technology (BDAT) Background Document for Mercury Containing Wastes*. D009, K106, P065, P092, and U151. U.S. Environmental Protection Agency, Washington, p. 1-5.

Matsuyama, A., Iwasaki, H., Kigaki, K., Yabuta, H., Sano, T., and Akagi, H., 1999. Study on the Remediation Technology of Mercury-Compound Contaminated Soil by Low Temperature Thermal Treatment. In: *Mercury Contaminated Sites, Characterization, Risk Assessment and Remediation*. R. Ebinghaus, R.R. Turner, L.D. Lacerda, O. Vasiliev and W. Salomons (Eds.), Springer Publishing, New York, p. 421-440.

Palermo, M.R., 1998. *Design Considerations for In-Situ Capping of Contaminated Sediments*, Wat. Sci. Tech., v.. 37, n. 6-7, p. 315-321

Papp, C.S.E., Filipek, L.H., Smith, K.S., 1991. Selectivity and Effectiveness of Extractants Used to Release Metals Associated with Organic Matter. *Applied Geochemistry*, v. 6, p. 349-353.

Rodrigues-Filho, S. and Maddock, J.E.L., 1998. Assessment of the Heavy Metal Pollution in a Gold "Garimpo", In: *Environmental Geochemistry in the Tropics*. J.C. Waseerman, ed., London, Springer, p. 193-210.

Sexauer Gustin, M., Taylor, G.E., and Maxey, R.A., 1997. Efect of Temperature and Air Movement on the Flux of Elemental Mercury from Substrate to the Atmosphere. *J.Geophysical Research*, v. 102, n. D3, p. 3891-3898.

Sobolev, I.A., Barinov, A.S., Prozorov, L.B., and Kuptsov, V.M., 1996. Remediation of Hg Contaminated Soil. *Environmental Geotechnics*, Kamon (ed.), Ballerna, Rotterdam, p. 1083-1087.

Thoming, J., Sobral, L., and Wilken, R.D., 1999. Electroleaching: A Mobile Clean-up Process for Mercury Contaminated Materials. In: *Mercury Contaminated Sites, Characterization, Risk*

Assessment and Remediation. R. Ebinghaus, R.R. Turner, L.D. Lacerda, O. Vasiliev and W. Salomons (Eds.), Springer Publishing, New York, p. 441-456.

Unger, A.J.A., Sudicky, E.A., and Forsyth, P.A., 1995. Mechanisms Controlling Vacuum Extraction Coupled with Air Sparging for remediation of Heterogeneous Formations Contaminated by Dense Non-Aqueous Phase Liquids. *Water Resources Research*, v. 31, n. 8, August 1995, pp. 1913-1925.

U.S. Army Engineering Corp., 2000. In-Situ Electrokinetic Remediation for Metal Contaminated Soils. USEAC Environmental Technology Website, http://aec-www.apgea.army.mil:8080/prod/usaec/et/restor/insitu.htm.

U.S. Environmental Protection Agency, 2001. Remediation Technology Database, Website: www.epareachit.org.

Veiga,M.M.. 1997. *Introducing New Technologies for Abatement of Global Mercury Pollution in Latin America*. Ed. UNIDO/UBC/CETEM. Rio de Janeiro, 94p. ISBN: 85-7227-100-7.

Veiga,M.M. and Meech,J.A., 1995. Gold Mining Activities in the Amazon: Clean-up Techniques and Remedial Procedures for Hg Pollution. *Ambio*, v. 24, n. 6, p. 371-375.

Veiga, M.M.; Veiga,A.T.; Franco, L.L.; Bonagamba, M.; Meech, J.A., 1995. An Integrated Approach to Mercury-Contaminated Sites. Proc. Eco Urbs' 95, p.51-53. Rio de Janeiro, Jun. 19-23, 1995.

Waybrandt, K.R., Blowes, D.W., and Ptacek, C.J., 1998. Selection of Reactive Mixtures for Use in Permeable Reactive Walls for Treatment of Mine Drainage. *Environ. Sci. Technol.*, v. 32, p. 1972-1979.

Xu, Y., and Schwartz, F.W., 1994. Lead Immobilization by Hydroxyapatite in Aqueous Solutions. *J. Contam. Hydrol.*, v. 15., no. 3, p. 187-206.

Health Effect－epidemiology of chronic low-dose exposure

MAKOTO FUTATSUKA

Department of Public Health, Kumamto University School of Medicine

A number of epidemiological studies have been carried out on populations prenatal exposed chronically to low doses of methylmercury. Tabel 1 summarizes some key methodological aspects of those studies. I would like to try to make reviews of those articles.

Mc Keown-Eyssen et al. (1983) studied 234 12-to 30-month-old Cree children for whom prenatal methylmercury exposure was estimated on the basis of maternal hair samples. The subjects lived in four communities in northern Quebec. For 28% of the mothers, hair samples were collected during pregnancy. The mean maternal hair mercury concentration was approximately 6ppm, with 6% of samples exceeding 20ppm. One of four pediatric neurologists blinded to individual mercury exposure status, measured physical status and conducted a neurological examination. The only neurological findings significantly associated with prenatal methylmercury exposure were abnormalities of muscle tone or reflexes in boys. The authors noted about the one significant adverse association identified: the abnormalities of muscle tone and reflexes in boys were isolated, mild, and of doubtful clinical importance; the absence of a coherent dose-response relationship; the absence of consistency across sex; and the possibility that the finding reflect chance, lack of normality in the distribution of the exposure index, or residual confounding.

Infant's status on neurological examination was also evaluated as an end point in a study of 131 children in Mancora, Peru (Marsh et al. 1995). Maternal hair was used as the index of exposure (geometric mean 7.05ppm). No end point was significantly associated with maternal hair mercury concentration. Cordier and Garel (1999) reported on the association between methylmercury exposure and neurological status in 248 9-month old to 6-year old children living in gold mining regions of French Guiana. The median maternal hair mercury concentration was 6.6ppm. An association was observed in boys between increased reflexes and higher maternal hair mercury concentrations in a cohort of 9 month old to 6 year old children. But these findings were different by the different examiner, therefore, the authors advised caution in interpreting those data.

The three major prospective long term studies,. the New Zealand, the Seychelles and the Faroes projects are worthy of notice.

Kjellströn et al.(1986) studied a cohort of New-Zealand children for whom prenatal methylmercury exposure was estimated on the basis of maternal hair samples as well as dietary questionnaires collected during the period when the child was in utero. Although exposure information was collected on nearly 11,000 women, the authors focused on 935 women who reported eating fish more than three times per week during pregnancy. Efforts were made to match each child in the high mercury group with a reference child on the basis of maternal ethnicity, hospital of birth, maternal age, and child age. In the follow-up evaluations completed when children were 4 years old, 38 exposed and 36 reference children were tested. On the Denver Development Screening Test (DDST), 52% of the children in the high mercury group had an abnormal or questionable result compared with 17% of the children in the control group ($P<0.05$). Those subjects were followed up at 6 year of age. Three controls were matched to each high mercury child on the basis of confounding factors. Fifty seven fully matched groups of four children each participated in a follow up evaluation of neurodevelopmental status at 6 years age (Kjellstrom et al. 1989). In the high mercury group, the mean maternal hair mercury concentration was 8.3ppm. A battery of 26 psychological and scholastic tests was administered. On the results treated statistically, scores on the Test of Language Development-spoken language quotient (TOLD-SL)and Mc carthy Scales of Children Abilities perceptual-performance scale(MCPP) were inversely associated with maternal hair mercury concentration ($P<0.05$).

Seychelles Child Development Study (SCDS) consists of a cross-sectional pilot study and following cohort study. As to the pilot study, all women attending an antenatal clinic were asked to provide hair samples during and after pregnancy for 2 years before the start of study. A total of 804 infants were subsequently enrolled in the study, and tested during three visits over 2 months in 1987-88 (Meyers et al. 1995). They were evaluated between the age of 5 and 109 weeks by one blinded pediatric neurologist. Mean maternal hair merucury concentration was 6.1ppm. The statistical analyses focused on three end points; overall neurological examination, increased muscle tone, and deep tendon reflexes in the extremities. No association was evident between maternal hair mercury concentration and abnormal findings.

The main cohort of the SCDS consisted of 740 mother-infant pairs. When the infants were 6.5 months old, one blinded pediatric neurologist administered the same neurological examination that had been used in the pilot study. The frequency of abnormal findings was not significantly associated with maternal hair mercury concentrations (Meyers et al.1995). The ages at which a child was able to walk without support and to say words were determined by an interview with a child's primary caregiver conducted at the 19-month evaluation (Meyers et al. 1997, Axtell et al. 1998).

Those date were available for 738 children. The major finding of the analyses was that the association between age at walking and talking, and maternal hair mercury was not significant. The Bayley Scales of Infant Development (BSID)was administered by blinded examiners to children in the SCDS cohort at ages 19 and 29 months (738 at 19 months and 736 at 29 months)(Davidson et al. 1995). In addition, at 29 months, six items of the Infant Behavior Record (IBR) were completed by the examiner. Those scores at 19 and 29 months were not significantly associated with maternal hair mercury concentration during pregnancy. In the main SCDS, 711 children from the original cohort were evaluated at 66 months of age using a battery of standardized neurodevelopmental tests (Davidson et al. 1998). The pattern of scores of the six primary end points did not suggest an adverse effect of either prenatal or postnatal mercury exposure. The associations that were found were consistent with enhanced performance among children with increased exposure to methylmercury.

The faroe project is a long-term cohorts study from 1986 to the present. Steuerwald et al. (2000) recruited a cohort of 182 singletone, full-term infants born in the Faroe Islands and evaluated the associations between neurological function at 2 weeks of age and various dietary contaminants and nutrients. The primary outcome variable was the neurological optimality score (NOS), which reflects an infant's functional abilities, reflexes, responsiveness, and stability of state. The exposure biomarkers measured were mercury concentration in maternal hair, cord whole blood, and cord serum. Measurements were also taken of 18 pesticides (or metabolites), and 28 polychlorinated biphenyl (PCB) congeners in maternal serum and breast milk, selenium in cord whole blood, and fatty acid in cord serum. There was a significant inverse relationship between NOS scores and cord whole blood mercury concentrations. The mean concentration was 20.4 μ g/L. Ages at milestone achievement of motor development were investigated in a 21-month birth cohort (1,022 infants, 1986-87) of children (Grandjean et al. 1995). Milestone data were obtained from maternal interviews and the observations of district health nurses who visited the homes on several occasions during the children's first year of life. Three miletones were selected for analysis; sits without support, creeps, and gets up into standing position with support. The age at achievement was significant inverse association with children's hair mercury concentration at 12 months. Comprehensive evaluations were conducted at approximately 7 years of age on 917 of the surviving members of a 1986-87 birth cohort of 1,022 singleton births (Grandjean et al. 1997). In multiple regression analyses, increased cord blood mercury concentration was significantly associated with worse scores on finger tapping, continuous performance test, WISC-R digit span, Boston Naming Test, and the California Verbal Learning Test-children, and the pattern of brainstem auditory evoked potentional (BAEP). Grandjean and colleagues assembled an additional study cohort of 351 children 7 to 12 years old from four riverine communities in Amazonian Brazil (Rio Tapajos) with increased exposure to methylmercury due to the consumption of fish contaminated by upstream gold-mining activities (Grandjean et al. 1999). Among children, the mean hair mercury concentration ranged from a geometric mean of 11.9 ppm to 25.4 ppm for the hightest; 80% of the children exceeded 10ppm. The battery of neurobehavioral tests administered to the children focused on motor function, attention, visual-spatial function, and short-term memory. Children's hair mercury concentrations were significantly associated with their scores on finger tapping, Santa Ana dextry test, WISC-Ⅲ digit span, Stanford-Binet copying and recall, and Stanford-Binet bread memory. In a cross-sectional study of 149 6 to 7 year old children living in a fishing village on Madeira, many of the same such as the Faroe study neurophysiological tests were administered(Murata et al. 1999). Because patterns of fish consumption were considered to be stable, current maternal hair mercury concentration was used as a measurement of a child's prenatal mercury exposure; mean 9.6 ppm. With respect to brainstem auditory evoked potential, maternal hair mercury was significantly associated with Ⅰ-Ⅲ and Ⅰ-Ⅳ interpeak latencies at both 20 and 40HZ, as well as with total latencies for peaks Ⅲ and Ⅴ. Those results are similar to the findings in the Faroe Island study.

As above mentioned, some study results appear to be conflicting. Several studies have detected significant methylmerucury associated increases in the frequency of abnormal and questionable findings on standardized neurological examinations, although the function importance of the apparent effects is uncertain. Recent epidemiological studies provide little evidence that the ages at which children achieve major language and motor milestone are affected appreciably by low-dose prenatal methylmerucury exposure. Some studies using the Dever Development Screening Test reported an association of low-dose methylmercury exposure on early childhood development. Of the three major prospective long-term studies, the Faroes study reported associations between low-dose prenatal methylmercury exposure and children's performance on standardized neurobehavioral tests, particularly in the domains of attention, fine-motor function, confrontational naming, visual-spatial abilities, and verbal memory, but the Seychelles study did not report such association. The smaller New Zealand study also observed associations, as did a large pilot study conducted in the Seychelles. Research is needed to determine the long-term implications of the neuropsychological and neurophysiological effects of low-level prenatal methylmercury exposure detected in children, specifically whether they are associated with an increased risk for later neurological impairments.

Table 1-1 Summary of Developmental Neurotoxicity Studies in Humans

Study Site	Size of Cohort Identified and Enrolled	Exposure Biomarker	MeHg or Total Hg Concectration	Age at Assessment	Number of Children assessed	End Points	Reference
Nothern Quebec	247	Maternal hair	Mean, 6ppm; 6%, >20ppm	12-30 mon	234	Neurological exam	McKeown-Eyssen et al. 1983
Mancora, Peru	369	Maternal hair	Geometric mean, 7.1ppm; geometric SD, 2.1; range, 0.9-28.5	?	194 (131 with both exposure and outcome data)	Neurological exam Developmental milestones	Marsh et al 1995
French Guiana	Approx.400	Matenal hair	Median, 6.6ppm range, 2.6-17.8 35%>10ppm	9month-12years	248 (neuro exam)	neurological exam fingertapping, Stanford - Binet: block design, copying, bead memory	Cordier and Garel 1999
					206(psychological exam)	MSCA: numerical memory, leg coordination	
New zealand	10,930 mothers screened. 935"high"fish consumers identified, 73"high"Hg mothers identified	Maternal hair	"High"Hg defined as>6ppm; mean, 8.3ppm in "high" Hg group; range, 6-86ppm; only 16 values>10ppm	4yr	74; 38 "high" Hg, 36 "low" Hg, including 30 matched pairs	DDST, vision, functional neurological exam	Kjellstrom et al. 1986
				6yr	237; 57 complete sets of 1 "high" Hg child, 3matched controls, and 4 incomplete sets	WISC-R, TOLD, MSCA, CDS, BWRT, KMDAT, PPVT, EBRS	Kjellstrom et al. 1989

Table 1-2 Summary of Developmental Neurotoxicity Studies in Humans

Study Site	Size of Cohort Identified and Enrolled	Exposure Biomarker	MeHg or Total Hg Concectration	Age at Assessment	Number of Children assessed	End Points	Reference
Seychelles Islands	804	Maternal hair	Median, 6.6ppm; range, 0.6-36.4	5-109wk	789	Neurological exam, DDST-R	Myers et al. 1995
(pilot)			ppm, interquartile range: 6.1	66 mon	217	MSCA, PLS, WJTA: LWI, WJTA: AP	Myers et al. 1995
Seychelles Islands	779	Maternal hair	Median, 5.9ppm; interquartile range,	6.5mon	712-737	Neurological exam, DDST-R, FTII, visual attention	Myers et al. 1995
(main)			6.0ppm; all values, <30 ppm	19 mon	738	Developmental milestones	Myers et al. 1997; Axtell et al.1998
						BSID	Davidson et al.1995
				29 mon	736	BSID	Davidson et al.1995
				66 mon	711	MSCA, PLS, B-G, WJTA: LWI, WJTA: AP, CBCL	Davidson et al.1998

Table 1-3 Summary of Developmental Neurotoxicity Studies in Humans

Study Site	Size of Cohort Identified and Enrolled	Exposure Biomarker	MeHg or Total Hg Concectration	Age at Assessment	Number of Children assessed	End Points	Reference
Faroe Islands	182	Maternal hair	Geometric mean, 4.1ppm; range, 2.5-7.4ppm	2wk, adjusted for gestational age	182	Neurological exam	Steuerwald et al.2000
		Umbilical cord blood	Geometric mean, 20.4μg/L; range, 11.8-40.0 g/L				
		Umbilical cord serum	Geometric mean, 2.5μg/L; range, 1.7-3.7 g/L				
	1,023	Maternal hair	Geometric mean, 4.3ppm; interquatrile range, 2.6-7.7ppm	Maternal interview "during the first year"	583	Developmental milestones	Grandjean et al.1995
		Maternal hair	the surviving members of the aboved cohort group	Children at 7 years of age	917	Comprehensive evaluation	Grandjean et al.1997
Amazon	351	Child hair	Mean, 11.0ppm; 80%>10ppm	7-12yeas	354	Fingertapping,WISC-Ⅲ: digit span forward,Santa Ana dexterity test, Stanford-Binet:copying, bead memory	Granjean et al.1999
Madeira	149	Maternal hair	Geometric Mean, 9.6ppm Range, 1.1-54.4 52%>10ppm	6-7years	146-149	BAEP, VP NES: fingertapping, hand eye coordination, continuous performance test WISC-R: digit apan, block design Stanford-Binet: bead memory	Murata et al 1999

The effects of aging on the neurological findings of Minamata disease patients and inhabitants of methylmercury polluted and non-polluted areas

Kimiyoshi Arimura[1], MD, Junji Wakamiya[2], MD, Masanori Nakagawa[1], MD, Mitsuhiro Osame[1], MD, Suminoro Akiba[3], MD, Makoto Futatsuka[4] ,MD,

1) The Third department of Internal Medicine, Kagoshima University
2) National Institute for Minamata Disease
3) The department of Public Health, Kagoshima University
4) The department of Public Health, Kumamoto University

Abstract

It has been over 30 years since methylmercury poisoning has been identified as the cause of Minamata disease. Just like in any other neurological disease, aging can have an effect on the manifestations of Minamata disease. Whether or not this is true remains to be determined. A population-based study was embarked on comparing inhabitants of a polluted area in the vicinity of Minamata to inhabitants of a distant non-polluted area. In the non-polluted area, a discrepant increase in the number of neurological abnormalities as affected by age was higher in females compared to males. Comparing the two populations, the differences in the slope of regression between neurological findings and age were few. Comparing the neurological findings among patients with Minamata disease and those in methylmercury polluted areas, we have found that (a) except for an impairment in tandem gait and Romberg's sign, the prevalence of neurological manifestations in Minamata disease remains high; furthermore, they tend to improve over time, (b) residents of methylmercury polluted areas do not have Minamata disease even in the subclinical level, (c) in elderly patients, aging effects resemble findings seen in the non-polluted area and (d) aging has no special effect on the core symptoms of Minamata disease.

Key Words

Minamata disease, neurological findings, aging, methylmercury-polluted area, non-polluted area, follow up study

Introduction

It has been over 30 years now since Minamata disease patients have been identified and confirmed following exposure to methylmercury. Just like in any other neurologic disease, aging can have an effect on the manifestations of Minamata disease. It is not established whether the neurological manifestations of Minamata disease will change over time with age. In the same token, residents in methylmercury-polluted areas may manifest subclinical neurological manifestations of Minamata disease undetected during the time of pollution. This study therefore aims (a) to assess the neurological findings among residents of methylmercury polluted areas matched with residents in a non-polluted area and (b) to assess the neurological manifestations of Minamata disease patients 20 years after confirmation of diagnosis.

Materials and Methods

(1) Neurological findings among residents of methylmercury polluted areas matched with residents in a non-polluted area

Board certified neurologists conducted a detailed neurologic examination on non-polluted area and polluted area whose inhabitants were more than 55 years of age. There were a total of 531 subjects in the non-polluted area and 281 subjects in the polluted area who were examined. Excluded were inhabitants who manifested other neurological disorders (e.g. stroke, Parkinson's disease, HTLV-1 Associated M yelopathy, among others). The presence or absence of neurological deficits was graded as either 1 or 0, respectively. Separate grades were made for Mini-mental status examination (MMSE), vibration sense and hand-grip. In order to evaluate the effects of aging, we performed statistical analysis to determine the correlation between prevalence of abnormal neurological findings and age using a logistic model or regression analysis. Since aging may have its effect on gender, a separate analysis was performed in males and females.

(2) Neurological manifestations of Minamata disease patients 20 years after confirmation of diagnosis

Detailed neurological assessment were conducted in three populations namely (a) diagnostically confirmed Minamata disease patients (Total:39 patients), (b) residents of a methylmercury polluted area called T-town (Total:324 residents) and (c) residents of a non-polluted area called K-town (Total: 954 residents). For a finder's diagnosis of Minamata disease in the methylmercury polluted area, discriminant analysis 1) 2) was applied.

Results

(1) Neurological findings among residents of methylmercury polluted areas matched with residents in a non-polluted area

In the non-polluted area, there were 348 males and 183 females examined, with a mean age of 71.9 years (7.3 SD). In the polluted area there were 159 males and 122 females studied with a mean age of 68.8 years (5.9 SD). As shown in Tables 1 and 2, aging had an effect on various neurological findings. In the non-polluted area, a discrepant increase in the number of neurologic abnormalities as affected by age was higher in females compared to males. The neurological findings concordant to both males and females were visual field defects, hearing abnormalities, hyperactive biceps reflex, difficulty standing on one foot, tandem gait impairment, and scores in the mini-mental status examination, hand-grip and lower extremity vibration sense. This also included difficulty in climbing stairs with activities of daily living (ADL).

For the inhabitants of the polluted area, females likewise had an increase in the number of neurologic abnormalities compared to males. Concordant neurologic findings in both males and females were pollakiuria and urinary incontinence, diminished Achilles tendon reflex, impaired tandem gait, abnormal Mann's test, abnormal hand-grip and decreased score of vibration sense. (Table 3 and 4)

Comparing the two towns, the common neurologic findings affected by aging among females were pollakiuria, agraphesthesia, difficulty in standing from a squatting position, difficulty standing on one foot, impaired tandem gait, and MMSE, hand-grip and lower extremity vibration sense scores. Among males in the two towns, hearing disturbance, neck movement limitation, impaired tandem gait, abnormal hand- grip, and decreased vibration sense in the lower extremity were common. The differences in the slope of regression between neurological findings and age were few between the two towns. (Table 5.) Furthermore, the exaggerated biceps tendon reflex was the only common finding in both sexes. Whereas aging may have an effect on the various neurologic findings of Minamata disease, only the dysmetria appeared to be significantly different. (Table 5.)

(2) Neurological manifestations of Minamata disease patients 20 years after confirmation of diagnosis

(a) Minamata disease patients

There were 27 males and 12 females diagnostically confirmed to have Minamata disease from 1972 to 1989. The mean age at the time of diagnosis was 45.7 years whereas the mean age at the time of the present examination is 65.6 years. The mean interval from the time of diagnosis to the present examination was 19.8 years. The frequency of abnormal neurological findings among Minamata disease patients tended to decrease, save for the presence of tandem gait and Romberg's sign. (Table 6.) However, the core signs and symptoms of Minamata disease remain high among these patients. Individual analysis of change of symptomatology in Minamata disease patients also tended to improve except for the worsening noted in tandem gait and Romberg's sign (Table 7).

(b) Residents of the methylmercury polluted area and the non-polluted area
Based on the neurological findings, only one out of 324 residents was highly suspect for Minamata disease following discriminant analysis. Comparing residents of the methylmercury polluted area and the non-polluted area as regards the core symptoms, no significant difference was found (Table 8).

Discussion

In a previous study of a non-polluted area, we found that aging affects various neurological findings but in a non-specific way. However, aging was found to have a more confounding effect on functions related to the lower extremities, (i.e. tandem gait, standing from a squatting position), hand-grip strength and MMS 3), 4), 5). In this present report we were able to confirm the results of the previous study despite using a different statistical approach. However, on further analysis on the effect of gender on the various neurological findings, females appeared to have been more affected than males. Comparing the polluted and non-polluted areas, the aging effects on lower extremity function are common to both areas. This result corroborates a published report 6) in which lower extremity function was highly correlated with the aging process.

It has been proposed that following exposure to methylmercury, neurologic abnormalities may manifest over time vis a vis the aging process 7). This may be explained by the aging effects on the already abnormal neuronal cell population in methylmercury exposure. In consideration of the fact that aging has a confounding effect on the neurological findings whether in the polluted or non-polluted areas, it can be inferred that the extrapolated neurological findings in the polluted area can be attributed to methylmercury exposure. In this context, it can further be inferred that these extrapolated neurological findings may have been subclinical to start with, and only manifested over time with age. The finger-to-nose test in particular was the extrapolated neurological finding among the main neurological manifestations of

Minamata disease.

This present study is able to establish that although the prevalence of neurological manifestations in Minamata disease was remains high, they tend to improve over time 8) 9) 10). The fact that the presence of disturbances in tandem gait and Romberg's sign worsened indicate that these are the manifestations that should be carefully assessed considering that these could be age-related. It is likewise notable that even the glove and stocking sensory disturbance found among Minamata disease patients improved as well. As of this present study, since there was only one newly found patient suspect for Minamata disease based on discriminant analysis, we can infer that the residents of methylmercury polluted area, by and large, do not have Minamata disease even in the subclinical level.

From the present study, it can still be generally concluded that the main neurological manifestations of Minamata disease may not change over time..

References

1. Hamada R, Igata A, Yanai H. Quantitative analysis of neurological findings in Minamata Disease. Saishin Igaku 1978, 33, 98. (in Japanese).
2. Igata A, Hmada R, Yanai H. Multivariant analysis of Minamata Disease. Shinkei Kenkyu no Shinpo (prograss of neurologica research) 1974, 18, 78-88. (in Japanses)
3. Arimura K, Izumo S, Nakagawa M, Osame M, Igata I, Hamada R. Neurological examinations in the aged inhabitants in non-polluted area with methylmercury. Annual report for the Research for Minatamata Disease. 1992, 147-179. (in Japanese).
4. Arimura K, Nkagawa M, Osame M, Igata A. The effects of aging on neurological findings in the aged inhabitants in polluted area. Annual report for the Research for Minatamata Disease. 1993, 38-45. (in Japanese).
5. Arimura K, Nkagawa M, Arimura H, Osame M, Akiba S. The effects of aging on neurological findings in the aged inhabitants in polluted area. Annual report for the Research for Minatamata Disease.1994, 67-71. (in Japanese)
6. Odenheimer G, Funkenstein HH, Beckett L, et al. Comparison of neurologic changes in 'successfully aging' persons vs the total aging population. Arch Neurol 1994, 51, 573-580.
7. Takeuchi T, Eto K. Pathological findings in Minamata disease patients. In: Minamata Disease, Arima S. ed, Seirinsha Productions, Tokyo, 1979, pp. 505-574.
8. Arimura K, MD, MD, Nakagawa M, MD, Osame M, et al. Neurological follow up study in patients with Minamata Disease. Annual report for the health effect of

heavy metals: Research for the health effect of organic mercury. 1997, 67-70. (in Japanese)

9. Arimura K, Wakamiya J, Nakagawa M, Akiba S, Osame M. Neurological follow up study in patients with Minamata Disease. Effects of aging on neurological symptoms. Annual report for the health effect of heavy metals: Research for the health effect of organic mercury. 1998, 63-66. (in Japanese)
10. Tokuomi H, Ideta T, Teramoto J, Imanishi K, Ueno H, Ymanaga H. Twenty year folloe up of Minamata disease. Shinkeinaika (Neurologica Medicine) 1980, 12, 254-260. (in Japanese)

Table 1. Effects of aging on neurological findings in inhabitants of non-polluted area (1)

	regression rate	SE	p value
female			
visual disturbance	0.06387	0.01718	0.0002
visual field constriction	0.08537	0.03195	0.0071
disturbance of ocular movement	0.07613	0.03173	0.0161
hearing disturbance	0.1167	0.01917	<0.0001
laterality of hearing disturbance	0.05862	0.02037	0.0038
upper extremity weakness	0.0758	0.03559	0.0332
dysdiadochokinesia	0.1075	0.03269	0.0007
dysmetria	0.1973	0.08794	0.0126
pollakuria	0.04355	0.02059	0.0346
fecal incontinence	0.3507	0.2184	0.0325
Lasegue sign	0.05757	0.02839	0.0059
palmomental reflex	0.05757	0.02161	0.0075
Lasegue sign	0.07759	0.02839	0.0059
hyperactive biceps reflex	0.1083	0.02946	0.0001
hyperactive patellar reflex	0.04677	0.0234	0.0462
hyperactive Achilles tendon reflex	0.04315	0.01852	0.0196
agraphesthesia of lower extremities	0.0665	0.03157	0.0354
difficulty standing form squating posi	0.0856	0.02069	<0.0001
difficulty standing on one foot	0.1166	0.02344	<0.0001
difficulty standing on toes	0.1118	0.02563	<0.0001
tandem gait impaiement	0.07984	0.02323	0.0005
disturbance of Mann's test	0.08983	0.02063	<0.0001
gait disturbance	0.07657	0.02961	0.0094
difficulty climbing stairs	0.08336	0.0203	<0.0001
male			
visual field constriction	0.09863	0.04189	0.0181
hearing disturbance	0.05231	0.02571	0.0412
limitation of neck movement	0.07575	0.02681	0.0041
constipation	0.07148	0.03296	0.0302
hyperactive biceps reflex	-0.1064	0.04346	0.0081
hyperactive brachioradial reflex	-0.07711	0.03902	0.0369
difficulty standing on one foot	0.08885	0.02814	0.0013
disturbance of tandem gait	0.08779	0.0307	0.0038
difficulty climbing stairs	0.08187	0.03898	0.0361

SE: standard error

Table 2. Effects of aging on neurological findings in inhabitants of non-polluted area (2)

	regression rate	SE	p value
female			
Mini Mental Status Examination	-0.2128	2.45E-02	<0.0001
right hand grip strength	-0.3785	3.53E-02	<0.0001
left hand grip strength	-0.332	3.29E-02	<0.0001
vibration sense of right lower extremi	-6.65E-02	2.07E-02	0.0014
vibration sense of left lower extremit	-5.38E-02	2.09E-02	0.0106
male			
Mini Mental Status Examination	-0.1233	3.72E-02	0.0011
right hand grip strength	-0.4688	8.33E-02	<0.0001
left hand grip strength	-0.5026	8.32E-02	<0.0001
vibration sense of right upper extremi	-7.79E-02	3.82E-02	0.0427
vibration sense of right lower extremi	-8.84E-02	3.12E-02	0.0051
vibration sense of left lower extremit	-7.78E-02	3.50E-02	0.0276

SE: standard error

Table 3. Effects of aging on neurological findings in inhabitants of polluted area (1)

	regressoion rat	SE	p value
female			
dysosmia	0.1183	0.04838	0.0138
dysmetria	0.368	0.1825	0.0082
abnormal muscle tone in lower extremiti	0.1983	0.1037	0.0445
pollakuria	0.1474	0.04729	0.0013
urinary incontinence	0.1634	0.07218	0.0203
hyperactive orbicularis oris reflex	0.1682	0.06753	0.0104
diminished Achilles tendon reflex	0.08671	0.03552	0.0139
abnormal joint position sense	0.1402	0.06593	0.0318
agraphesthesia of lower extremities	0.1433	0.04421	0.0008
difficulty standing form squating position	0.157	0.04077	<0.0001
difficulty standing on one foot	0.18	0.05357	0.0004
tandem gait impairment	0.1471	0.04972	0.0024
abnormal Mann's test	0.108	0.04087	0.0093
difficulty climbing stairs	0.1119	0.05545	0.044
male			
hearing disturbance	0.07203	0.03644	0.0456
limitation of neck movement	0.1261	0.04246	0.0019
neck pain during movement	0.09566	0.04422	0.0275
pollakuria	0.1254	0.05356	0.0151
urinary incontinence	0.363	0.1912	0.0114
Babinski sign	0.167	0.0745	0.0162
hyperactive patellar reflex	0.08088	0.03886	0.0349
diminished Achilles tendon reflex	0.09466	0.03941	0.0141
lower extremity disturbance of light touc	0.07463	0.03699	0.0412
tandem gait impairment	0.2649	0.1111	0.0041
abnormal Mann's test	0.1168	0.0551	0.0291

SE: standard error

Table 4. Effects of aging on neurological findings in inhabitants of polluted area

	regressoi	SE	p value
female			
Mini Mental Status Examination	-0.1925	3.81E-02	<0.0001
right hand-grip strength	-0.4574	7.71E-02	<0.0001
left hand-grip strength	-0.3933	7.88E-02	<0.0001
vibration sense of right lower extremity	-0.1024	4.80E-02	0.0343
vibration sense of left lower extremity	-0.1029	5.05E-02	0.0433
male			
right hand-grip strength	-0.6688	1.21E-01	<0.0001
left hand-grip strength	-0.5724	1.19E-01	<0.0001
vibration sense of right lower extremity	-0.1643	5.36E-02	0.0027
vibration sense of left lower extremity	-0.1793	5.42E-02	0.0012

SE: standard error

Table 5. Differences between the effects of aging on neurological findings in non-polluted and polluted area

	non-polluted area		**polluted area**		
	regressoion rate	SE	regressoion rat	SE	p value
female					
dysosmia	-2.38E-04	0.0269	0.1183	0.04838	0.0307
dysmetria	-0.007674	0.07892	0.368	0.1825	0.0215
pollakuria	0.04355	0.02059	0.1474	0.04729	0.0396
hyperactive orbicularis oris reflex	0.001621	0.02562	0.1682	0.06753	0.0178
hyperactive biceps reflex	0.1083	0.02946	0.001121	0.03516	0.0176
hypoactive patellar reflex	-0.01163	0.01684	0.0893	0.04641	0.0418
hypoactive Achilles tendon reflex	-0.04054	0.02228	0.08671	0.03552	0.0022
male					
Babinski sign	-0.14	0.07905	0.167	0.0745	0.0021
hyperactive biceps reflex	-0.1064	0.04346	0.01637	0.0445	0.0464
lower extremity disturbance of pain sensatio	-0.05659	0.05021	0.06806	0.03632	0.0374
segmental sensory disturbance	-0.09217	0.05932	0.07822	0.0437	0.0153

SE: standard error

Table 6. Frequency of abnormal neurological findings in Minamata disease patients

Neurological findings	first time	second tim	*
cranial nerve			
constriction of visual field	89	43	○
disturbance of ocular movement	58	40	○
dysosmia	56	51	○
perioral sensory disturbance	51	26	○
hearing disturbance	90	56	○
dysarthria	56	31	○
upper limbs			
involuntary movement	49	38	○
weakness	50	44	○
difficulty performing fine motor tasks	71	56	○
dysdiadochokinesia	82	56	○
dysmetria	56	49	○
lower limbs			
weakness	66	56	○
incoordination	61	41	○
sensory disturbance			
superficial sensation	100	90	○
joint position sense	63	50	○
vibration sense	84	87	—
gait and standing			
tandem gait	50	71	×
Romberg's sign	21	42	×
gait disturbance	50	42	○

○ improved, × worsened, — no change (%)

Table 7. Changes in neurological findings of individual patinets with Minamata disease

Neurological findings	Improved (%)	Worsened (%)	*
cranial nerve			
constriction of visual field	56	9	○
disturbance of ocular movement	24	5	○
dysosmia	26	21	○
perioral sensory disturbance	44	18	○
hearing disturbance	38	5	○
dysarthria	31	5	○
upper limbs			
involuntary movement	26	15	○
weakness	24	18	○
difficulty performing fine motor tasks	26	11	○
dysdiadochokinesia	28	3	○
dysmetria	26	18	○
lower limbs			
weakness	21	11	○
incoordination	34	13	○
sensory disturbance	11	0	○
superficial sensation	22	13	○
joint position sense	8	11	—
vibration sense			
gait and standing			
tandem gait	11	32	×
Romberg's sign	11	26	×
gait disturbance	29	26	—

○ improved, × worsened, — no change

Table 8. Comparison of the frequency of the core symptoms in Minamata disease between non-polluted and polluted areas.

	p value	OR	*(%)
constriction of visual field	0.775		2.3
hearing disturbance	0.827		16.4
dysmetria	0.286		2.3
dysdiadochokinesia	0.067		4.7
incoordination of lower limbs	0.034	0.37	1.3
glove & stocking type sensory disturban	<0.001	4.98	19.8
stocking type sensory disturbance	0.307		4.7
gait disturbance	0.027	0.51	4.0

OR: Odds ratio, * frequncy in polluted area

Effects of Long-Term and Low-Dose Methylmercury Exposure in the Experimental Mice

H. Satoh, K. Nakai, S. Kameo, C.-Y. Kim, and Z.-M. Liu

Environmental Health Sciences, Tohoku University Graduate School of Medicine,
2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, JAPAN

Abstract

To examine the neurobehavioral effects of low-dose long-term methylmercury exposures, female C57BL/6Cr mice at 8 weeks old were fed with one of the diets containing methylmercury at the doses of 0, 1 or 5 ppm (as Hg) for 4 weeks before mating. The mice were continuously exposed to methylmercury during pregnancy and lactation, and the offspring obtained were also exposed to the same diet. Neurobehavioral examinations including open field test and Morris water-maze test were given when the offspring were at 12 or 56 weeks old. In open field test at 12 weeks old, methylmercury exposure groups significantly increased the number of urination and defecation, and decreased the locomotion activity in female mice. At 56 weeks old, however, methylmercury exposure did not cause any significant effect. In Morris water-maze test, methylmercury exposure increased the time to reach to the hidden platform both at 12 and 56 weeks old. These results suggest that low-dose long-term methylmercury exposure caused adverse effects on emotional behaviors and spatial learning ability. Aging masked the effects on the emotional parameters.

Key words

methylmercury, low-dose exposure, long-term exposure, open field test, water-maze test, mouse

Introduction

Methylmercury has been shown to be a neurobehavioral toxicant to the developing nervous system of human fetuses[1]. Fetal poisoning cases were found in Minamata [2] and Iraqi [3, 4] disasters. In animal experiments, prenatal exposure to methylmercury also has been shown to cause neurobehavioral changes in reflexive development, locomotor activity, emotionality and cognitive function [1]. Recently, cognitive deficits were found in children of the Faeroe Islands who had prenatal methylmercury exposure through maternal consumption of whale meat and blubber [5]. The maternal hair mercury concentrations were 10 ppm or less. This study emphasizes the neurotoxic effects of prenatal methylmercury exposure at low dose. Furthermore, since such seafood-consuming populations are continuously exposed to low dose methylmercury through their lifetime, an important question arises: whether the neurobehavioral dysfunction becomes worse under a continuous exposure to low dose methylmercury until the adult or senescent age. Therefore, we are investigating the effects of long-term low-dose methylmercury exposure for the lifetime in experimental mice.

Materials and Methods

Animals

C57BL/6Cr female mice at 8 weeks old were purchased. They were given diet containing methylmercury either 1 or 5 ppm (as Hg) or 0 ppm as the control for 4 weeks before mating. The female mice were mated with non-exposed male mice of the same strain. The pregnant mice were allowed to litter. After parturition, the number of pups was culled to 6-8 per litter. During their gestation and preweaning periods, the maternal mice were continuously fed with the same diets.

The offspring mice were weaned at 3 weeks old. Then they were housed with 2 or 3 littermates of the same sex in a plastic cage. The same diet that was given to their mothers was continued.

Behavioral examinations

Open-field test

The apparatus was a white square 50 cm x 50 cm with a wall around [6]. The open-fields was illuminated by light bulbs located above each corner. A CCD video camera was hung 2 m above the open-field. A mouse was left in the open-field for 2 minutes and the behavior was captured by the video camera for analysis and recording.

Morris water-maze test

The water-maze apparatus was a shallow tub with its diameter of 1 m [6]. It was filled with water (20-21°C) and sufficient amount of milk was poured to make water opaque. A platform (10 cm in diameter) was placed 1 cm beneath the water surface. Because of opacity of the water, the platform was invisible to the mouse. The opacity is also effective to obtain better contrast of a mouse and water surface for clearer video recording. A mouse was forced to swim form a predetermined position and time to reach the platform was measured. The swimming behavior was captured by a video camera hung above the maze for analysis.

Home cage activity

Activity in a home cage for 24 hours was observed by using an infrared light sensor [6]. A mouse was housed in a plastic cage of the same type of its home cage. The light-dark cycle was controlled for 0800-2000 light period and 2000-0800 dark period.

Schedule of the behavioral tests

Open-field test and Morris water-maze test were given at the age of 12 weeks or 56 weeks, respectively. Home cage activity was observed at 12 weeks.

Determination of mercury

The mice were sacrificed for determination of mercury in tissues after each behavioral test above. The tissues were digested with a mixture of nitrate/sulfate/perchloric acid (1:4:1, v/v) by heating [6]. Then mercury content was determined by the cold vapor atomic absorption method.

Results

Physical development and body weight changes

The number of offspring born to the mothers given different levels of mercury did not differ. Body weight change between 4 weeks and 12 weeks is shown in Fig. 1.

Differences between the group given 5 ppm diet and other two groups (1 or 0 ppm diet) became remarkable as weeks increased. There were differences between male and female mice of the same diet group (Table 1).

At 56 weeks, the difference between males and females diminished but the differences due to different diets were apparent.

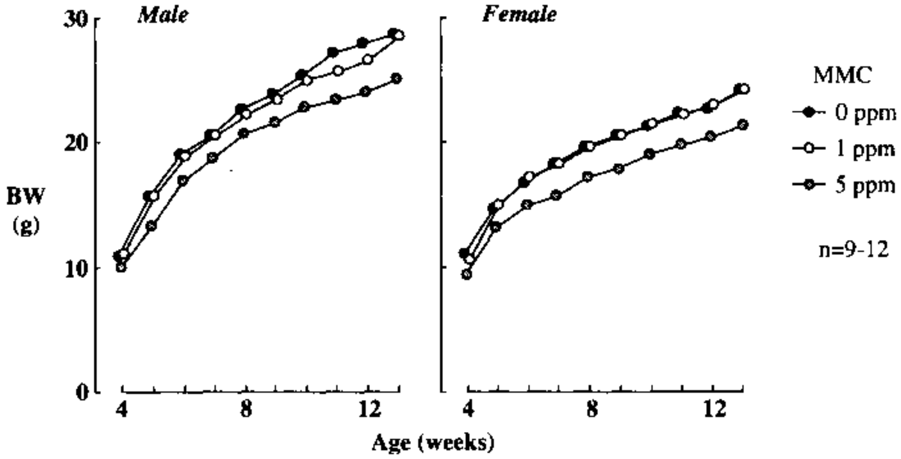


Fig. 1. Changes in body weight from 4 weeks old to 13 weeks old

Table 1. Body weight at 12 weeks old and 56 weeks old

MMC	Sex	12 weeks old		56 weeks old	
		N	Body weight (g)	N	Body weight (g)
0 ppm	Male	11	28.5 ± 0.4	10	48.4 ± 0.7
	Female	9	24.0 ± 0.5	13	48.8 ± 1.1
1 ppm	Male	11	28.3 ± 0.3	12	46.6 ± 0.9
	Female	10	24.0 ± 0.5	11	47.9 ± 1.0
5 ppm	Male	9	24.9 ± 0.4	11	45.6 ± 1.4
	Female	12	21.1 ± 0.6	12	45.5 ± 0.9

Open field test at age of 12 weeks

In the open field test, locomotion was determined in terms of moving distance for 2 minutes. Fig 2A shows locomotion. The male mice did not show significant difference whereas female mice showed decreasing trend according to different levels of mercury in diet. Numbers of urination and defecation (Figs 2B and C) showed clear increasing tendency as mercury content in diet increased, although the absolute levels were different between male and female mice.

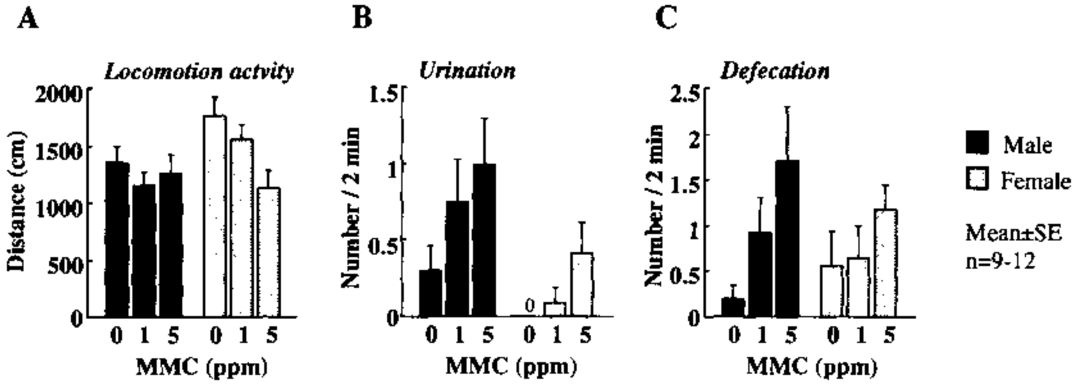


Fig. 2. Open Field test, 12 weeks old

Morris water-maze test at age of 12 weeks

As shown Figs 3A and B the groups fed with methylmercury containing diet showed the increased time to reach the platform. This is more distinctly dose-dependent in male mice. Swimming speed did not show significant differences among the different diet groups.

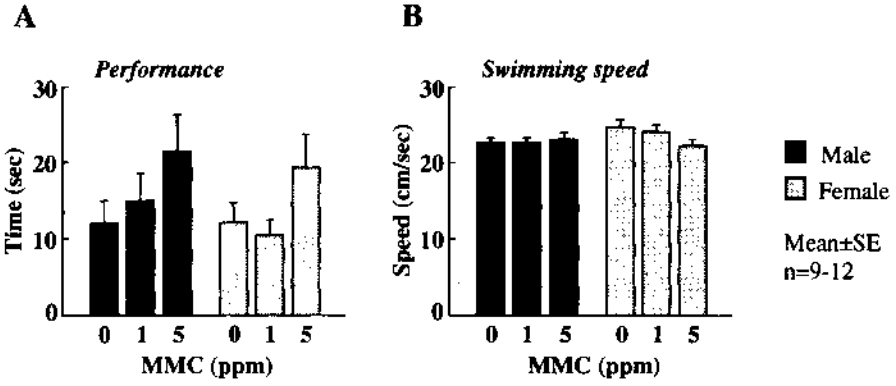


Fig. 3. Water Maze test, 12 weeks old

Open field test at age of 56 weeks

At age of 56 weeks, mice were less active than young ones in the open field-test as shown in Fig 4A. Numbers of urination and defecation (Figs 4B and C) were less than those at 12

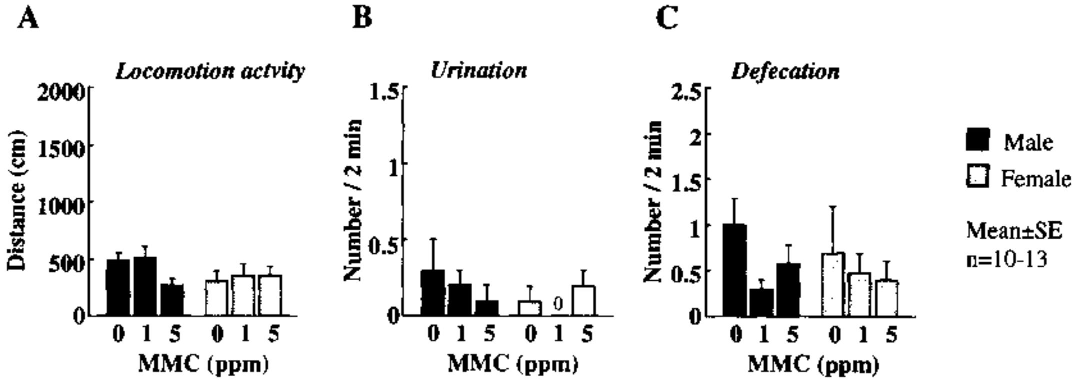


Fig. 4. Open Field test, 56 weeks old

weeks. Increasing tendency as mercury content in diet increased that was observed at 12 weeks was not obvious.

Morris water-maze test at age of 56 weeks

The results of Morris water-maze test at 56 weeks resemble to that of 12 weeks, though the increasing tendency is less remarkable in females (Fig. 5A). Swimming speeds are surprisingly similar among groups at different ages (Fig. 5B and Fig. 3B).

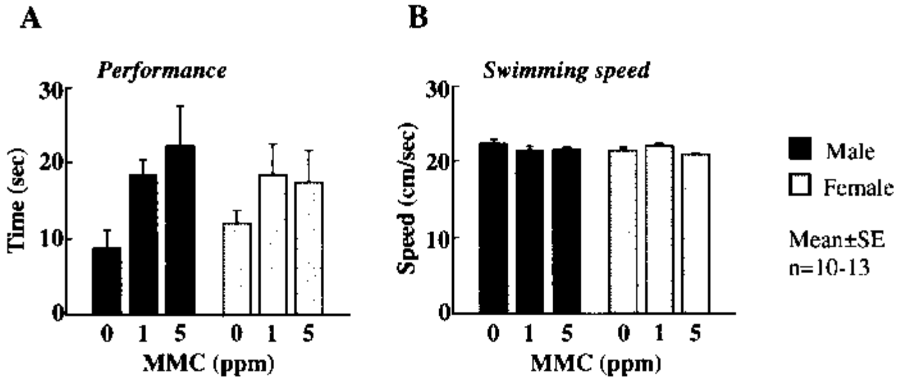


Fig. 5. Water Maze test, 56 weeks old

Home cage activity

Spontaneous activity in a home cage was shown for 24 hours being divided into 4 periods. Mercury treated mice were less active in the first light period (0800-1400).

Mercury concentrations in the brain

Total mercury concentrations in the brain at 12 weeks and 56 weeks are shown in Table 2. The concentrations increased with the elevated levels of diet mercury concentration and also the age of mice. There were distinct sex differences in the brain mercury concentrations.

Table 2. Brain total mercury at 12 weeks old and 56 weeks old

MMC	Sex	12 weeks old		56 weeks old	
		N	Brain Hg (ng/g)	N	Brain Hg (ng/g)
0 ppm	Male	6	3 ± 5	3	23 ± 5
	Female	9	0 ± 2	3	8 ± 1
1 ppm	Male	8	622 ± 36	7	1640 ± 21
	Female	7	1031 ± 49	2	2840, 3100
5 ppm	Male	5	3775 ± 278	5	7220 ± 720
	Female	8	5448 ± 125	7	9430 ± 990

Discussion

It is considered the open field test reveals the emotionality of mice, since the filed is a wide-open space with being brightly illuminated. Numbers of urination and defecation increased at 12 weeks in mice exposed to methylmercury. In our laboratory, open field test has been repeated on mice prenatally exposed to methylmercury: The exposure procedures usually employed were that mother mice were given methylmercury once in mid-gestation or three times during gestational days 12-14. The offspring of these mice did not show the increases of urination and defecation. Therefore, the increases observed in the present study may be associated with continuous exposure after birth and weaning.

The results of water maze were surprisingly similar at different ages. Since swimming speed did not change regardless of age, sex and exposure, it is plausible the differences of the time to reach the platform are due to ability of spatial cognition or learning, or both.

It can be concluded that continuous methylmercury exposure through the life span produced neurobehavioral effects different from the effects derived from the exposure during prenatal period alone. Moreover, continuous exposure did not produce the same effects at different ages as seen in the open field tests. Aging can change emotionality and it is possible that the same exposure may produce different effects at different ages. On the contrary, water maze results did not change at different ages. The test involves higher function of central nervous system and it reflects another domain of function different from the domain(s) related to open field behavior or emotionality.

It is necessary for this type of experiments investigating neurobehavioral effects of low-dose exposure to employ the behavioral examinations involving higher central nervous system functions such as cognitive and learning functions. More examinations like operant behaviors are needed.

Acknowledgment

This study is partly supported by a grant from Japan Association for Public Health.

The authors greatly appreciate technical assistance of C. Inomata, Y. Shimahara, S. Yashima, R. Ito and F. Miyoshi during experimentation. The authors also thank N. Suzuki for secretarial work.

References

1) Watanabe, C. and Satoh, H. (1996) Evolution of our understanding of methylmercury as a health threat. *Environmental Health Perspectives*, **104**, 367-379.

2) Harada, M. (1978) Congenital Minamata disease: intrauterine methylmercury poisoning. *Teratology*, **18**, 285-288.

3) Bakir, F., Damluji, S.F., Amin-Zaki, L., et al. (1973) Methylmercury poisoning in Iraq. *Science*, **181**, 230-241.

4) Amin-Zaki, L., Elhassani, S., Majeed, M.A., Clarkson, T.W., Doherty, R.A. and Greenwood, M. (1974) Intra-uterine methylmercury poisoning in Iraq. *Pediatrics*, **54**, 587-595.

5) Grandjean, P., Weihe, P., White, R.F., et al. (1997) Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicology and Teratology*, **19**, 417-428.

6) Kim, C-Y., Nakai, K., Kasanuma, Y. and Satoh, H. (2000) Comparison of neurobehavioral changes in three inbred strains of mice prenatally exposed to methylmercury. *Neurotoxicology and Teratology*, **22**, 397-403.

Research to Improve Mercury Risk Assessments

Ronald E. Wyzga, Sc. D.
Janice W. Yager, Ph.D., M.P.H.
EPRI
Palo Alto, CA 94303 USA

I. Introduction

Risk assessment has become an important policy tool. It can be used to evaluate alternative risk management options. Although risk assessments yield hard quantitative numbers, it should be kept in mind that there are considerable uncertainties associated with these numbers. In many cases the numbers produced are an upper bound for the actual risks associated with an endpoint. In fact, the product of the risk assessments for non-cancer events is not a risk per se, but a Reference Dose, RfD, defined to be an estimate (with uncertainty spanning perhaps an order of magnitude) of daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime. (US EPA, 1995)

There are several subjective judgments made in risk assessments; this is particularly true for neurotoxicants. Hard quantitative numbers are the desired output, yet there are many obstacles that must be faced before such numbers can be produced. This is particularly difficult for neurotoxicants where adverse effects are not as "clear cut" as they are for a disease with more discrete definitions like cancer. An individual is defined to have or not have cancer by pre-established clinical criteria. The percentage of individuals with this disease define the response. For neurotoxicants, current epidemiological (and clinical) practice examines a continuum of responses by severity, from very subtle responses to very frank adverse outcomes For this continuum the percentage of respondents varies; hence the response of neurotoxicants to stimuli, including environmental toxicants, can be characterized as a dose-response surface with one axis representing a continuum of possible responses, and the second axis indicating the percent of individuals responding at that level. (See Fig. 1.) It can get even more complicated because there are several domains and test batteries of neurotoxicological response. For example, motor co-ordination is a different domain from memory. For a given toxicant each of these domains has a different dose-response surface, and there is no reason why one would expect these surfaces to be parallel. In risk assessments, dose-response surfaces are rarely estimated; instead one slices the surface for a given level of severity and estimates the resulting two-dimensional dose-response curve. Hence in undertaking risk assessments for these agents, we are faced with many choices, the definition of the endpoints and dose-response curves to estimate and the methods applied for the actual estimation procedure. This paper reviews these some of these choices and suggests some research opportunities that could help to limit some of the subjective choices inherent in the current risk assessment process. Subjective decisions will, however, likely remain.

II. Issues related to Health Endpoint and Studies Considered in Methylmercury Risk Assessment

Developmental neurotoxicity has been chosen as the most sensitive, well-documented health endpoint to date for development of a health risk assessment for exposure to methylmercury *in utero*. Investigations of adverse health effects in developing organisms that are attributable to neurotoxicant environmental exposure, however, require measures that are sensitive across a wide range of exposure levels. The endpoints associated with neurotoxicity occur across a continuous range as previously discussed; further, although the brain may suffer insults from a number of sources *via* a number of different mechanistic pathways, the measured neurobehavioral endpoints are not necessarily discrete. Test methods, most often developed for individual clinical diagnosis, have nevertheless been applied to assess the human health population risks of neurotoxic agents. It is possible that the characterization of neuropsychological population effects may currently lie outside the bounds of conventional tests. Consider, for example, that the the population characterization problem is currently posed as questions about performance rather than as questions about diagnosis for which the majority of these tests were designed.

Most studies of developmental neurotoxicity employ measures known or assumed to measure developmental deviations in some reference population that may even include children with developmental abnormalities resembling those presumably caused by exposure to the neurotoxicant under study. Performance decrements on these tests in an exposed population are assumed to result from the neurotoxicant in question and the test, in turn, is assumed to be sensitive to that specific neurotoxicant.

However, parameters relating the sensitivity, specificity, and predictive value of these tests to known environmental neurotoxicant exposures have yet to be fully developed; research currently underway may serve to more clearly elucidate these exposure-effect relationships. In work now in progress, six developmental domains are being assessed in approximately 300 children using a battery of tests that include neuropsychological tests, eletrophysiological and behavioral tests of sensory and motor function and adaptations of performance tasks used previously only in animals. The objective of the study is to determine how well this test battery differentiates children with known mild neurodevelopmental deficits from those who are developmentally normal. This basic information is necessary in order to understand the classical specificity and sensitivity of these tests in terms of pre-defined functional adverse outcomes rather than relying almost solely on statistically significant differences. Choice of neurodevelopmental tests in children covers a very broad wide-ranging spectrum of individual tests – too numerous to list here. Research groups have chosen from among two major classes of tests – those that test general performance (e.g., the IQ test), and those thought to test more specific neurological developmental domains (e.g., cognitive, auditory, etc.) sometimes combining these two types of tests into one test battery.

In terms of application of children's neurological test methods to methylmercury health risk assessment, after extensive review of currently available population studies, the US National Academy of Science (NAS), in its recent report, chose the Faroes Island study upon to which to base a health risk assessment in children, the most sensitive subpopulation, since this study has reported decrements in neuropsychological test performance related to *in utero* exposure to methylmercury (NAS, 2000; Grandjean et al., 1997). Neuropsychological tests administered to 917 children at age seven were chosen by the investigators to include tasks deemed most likely to be affected by neuropathological abnormalities seen in methylmercury frank poisoning (White et al., 1994; Grandjean et al., 1997). Specific tests administered were chosen from among those showing advantageous psychometric properties in earlier field studies. One test battery was administered for each of the following five chosen domains of brain function: motor speed, attention, visual-spatial performance, language, and short-term memory. The Neurobehavioral Evaluation System (NES2) Continuous Performance Test (CPT) is performed on a computer. The CPT assesses attention and vigilance by measuring average reaction time during the last three of the four minutes of testing in a finger-tapping test and did show the most significant decremental effect of exposure. However, since data were available on CPT for only about half of the cohort, it was decided by NAS not to use this test result in a subsequent risk assessment. Instead, results from the Boston Naming Test (BNT) as the next most sensitive test were used to calculate a benchmark dose (BMD). The BNT is a test of language (word retrieval and formulation abilities) and consists of line drawings of 60 common objects (e.g., a cat) that a child has to name under time pressure (20 seconds). If the child cannot retrieve the correct word spontaneously, semantic and then phonemic cues are provided. The score is the total number of correct responses, including cues. BNT scores from the Faroes study are shown plotted in Fig. 2 (NAS, 2000, p.296). Test-retest correlation on the BNT has been described to yield a correlation of approximately 0.85 over a 12-month period in normal children six to nine years of age (USEPA, 2000a, p. 23).

Current risk assessment methodology undoubtedly was designed for the more usual situation in which one or two experimental animal studies are available with perhaps three dose points each. In this instance, one chooses one of the studies and then the first critical adverse effect that occurs at the lowest dose (binary outcome) and conducts a risk assessment with current practice tending toward use of the BMD approach. In the instance of methylmercury, however, there are a relatively large number of human observational epidemiology studies available with large numbers of individuals in the cohort in which multiple neurological endpoints, many of them with continuous outcomes, have been measured. In this regard, then, there is a question about the reliance on results from just one test chosen from among a relatively large battery of administered tests as the sole indicator of overall neurological health risk. As a possible alternative approach, the NAS report illustrated the possibility of conducting an analysis integrating test results across several acceptable studies (NAS, p. 284). This is one possible approach to adapt the "data rich" situation for methylmercury to a more realistic risk assessment paradigm. Further, it has been suggested by a USEPA scientific advisory panel that a within study integration of test scores rather than a choice of a single test measuring a limited neuropsychological endpoint may be more appropriate to a weight-of-evidence approach (USEPA, 2000a, p. 24).

In summary, currently a study or endpoint for methylmercury neurological effect in human observational studies is chosen because it was the most sensitive from a statistical standpoint. Another option that has been put forth is that one can choose an integrative measure of neurological response within or between studies. Finally, a test or test battery can be chosen because it has clear functional interpretation. Research can be designed around given tests to relate their outcomes to specific functional domains as described above. This requires that tests used be examined thoroughly and related to functional endpoints through independent studies in normal and compromised populations as is occurring in a study currently in progress.

III. Determination of Dose-Response

The traditional way to estimate the dose-response function is to assume it is a discontinuous function (e.g., a step function) and to define the point at which the risk increases above a background level. This involves using experimental data to define the No Observed Adverse Effects Level (NOAEL) or Lowest Observed Adverse Effects Level (LOAEL) and to divide that number by a series of uncertainty factors. (See below.) (US EPA, 1995). The LOAEL is defined from experimental data as the lowest level of exposure (dose) associated with a statistically significant increase in adverse responses in an experiment. When no adverse effects are observed, the NOAEL is defined as the maximum exposure level (dose) where no adverse responses were observed. The use of the LOAEL or NOAEL has several problems. It is highly dependent upon the design of the experiment from which it is estimated. For example, an experiment with many observations/individuals per exposure group can more easily detect statistically significant changes. Such experiments have greater statistical power to detect significant results; hence on the average, studies with larger numbers of individuals per dose group would have smaller NOAELS than identical experiments with larger numbers of individuals per dosing group. Secondly the dosing regime will heavily influence the NOAEL or LOAEL. For example, consider an experiment where no adverse effects were statistically defined. If this experiment had two exposure groups, one of which is the control, then the level of exposure for the other group would define the NOAEL. A similar experiment with another level of exposure would define an alternative NOAEL. Similarly the LOAEL can be influenced by experimental design. Finally in the derivation of the LOAEL or NOAEL no attention is given to responses at alternative exposure/dose groups. If, for example, the responses were related to exposure levels in a given way, it would be useful to take that into account in the risk assessment. For example, if the existing data at higher dose levels suggested an exponential *vs.* a linear dose-response relationship, this would be particularly important to take into account in extrapolating downwards to lower doses. Evidence is stronger (i.e., there is less uncertainty) if responses were to increase with increasing dose. The NOAEL/LOAEL method does not allow for consideration of such patterns.

In order to compensate for these deficiencies, an alternative method based more heavily on statistics was introduced, the benchmark dose (BMD) method. Essentially this method assumes a continuous dose-response function. Existing dose-response data are used to estimate the dose-response curve, which is extrapolated down to a specified response level, benchmark level (BMDL). This level is assumed to be such that there is little physiological/biological significance from this response to background response. A confidence interval is then applied to that level. The corresponding dose/exposure number is then treated as a LOAEL in the traditional method; i.e., the same uncertainty factors that would be applied to the LOAEL are applied to this number, the benchmark dose.

There are several issues associated with the use of the benchmark dose. First of all, it is necessary to decide the type of data to include in this assessment. As indicated above, experimental outcomes can be described with continuous data, quantal or dichotomous data in which the fraction of individuals responding at a given dose level is presented, or categorical data in which a finite number of responses are possible. For a given study there may be an option about the nature of data to include in benchmark dose calculations. In general if the categorical data are a reduction of the continuous data, one would be better served using the continuous data. No information would be thrown away, and more alternative benchmark models could be considered. Moreover, Crump (2000) has shown that converting continuous data into binary form before calculating the BMD will generally result in a smaller (more conservative) BMDL. The choice between the other two data representations may depend upon whether these is any consensus about the appropriate benchmark level, the response level to which extrapolation is made. Alternatively one could consider both measures and see whether there is coherence in estimates of the BMDs.

The choice of benchmark level defers to a consensus, which may be difficult to attain. There may be default guidelines; for example, an earlier version of the US EPA Guidelines referred to a 10% response level as being appropriate since that level is near or at the limit of sensitivity for most bioassays. (US EPA, 1995)Yet in its risk assessment for methyl mercury the NAS reverted to a 5% response level. For continuous data, there has to be some agreement about the biological significance of response. Default values, such as one standard deviation from the mean response of an unexposed group, have been proposed. Values, such as this, are dependent upon the size and other characteristics of a study. Yet the resolution of this issue is critical to the success of risk assessments. With an issue, such as mercury, where a myriad of tests have been used to assess effects, there may be insufficient experience with a given test, to allow any consensus about significance above background. The best solution appears to be efforts to make sure that the implications of various study tests and results are understood. Research is needed to link study results to functional changes in individuals. Consensus can certainly be more readily achieved for deciding the significance of functional changes.

A (mathematical) functional form must be chosen to relate the doses/exposures to responses. Often there are not enough data points to provide much guidance here, and even if there are a large number of dose-response data at higher doses, it is not clear how to extrapolate from these data points to low exposures of interest. There are several considerations here. First of all it is desirable to have some data near the benchmark level. This would provide some idea of how well alternative dose-response functions fit the data at the point of concern, the benchmark level. If no such data are available, the situation becomes difficult, if not impossible. So many alternative functions could be considered, even if the set were restricted to those that are biologically plausible. Figure 2 from the NAS report on mercury illustrates the problem well for a study with a relatively large amount of data and with some data points near the BMDL. (NAS, 2000, p.296). Three dose-response functions were fit to the data; depending upon which dose-response function were chosen, alternative benchmark levels would be chosen. The committee's report states, "Although standard statistical assessment of model adequacy could not distinguish between model, the corresponding BMDs and BMDLs differed fairly dramatically." (NAS, 2000, p. 293). In this case biological criteria were used to select the most plausible model; however, biological arguments could be made for some of the other models as well.

Recent research by Crump (Crump, 2000) has tried to facilitate this issue for continuous benchmark-dose functions. He demonstrates the considerable difference in a BMDL that model choice can have for continuous data. If one attempts to consider a range of models and consider all of them in a subsequent risk assessment, the resulting BMDLs will have "such a wide range as to make the procedure virtually uninformative". Crump advocates placing constraints upon the models used and argues from a biological perspective that alternative models should be linear or sublinear at low doses. This would include models with a threshold dose response as a special case of sublinear models. He also presents statistical arguments for ruling out supralinear curves as they are the ones that greatly increase the uncertainty in response due to model choice. A flexible model that can be fit to existing data and which is never supralinear is the K-power model (Crump, 1995;Budtz-Jorgensen et al, 2000). This model is endorsed by Crump because it is compatible with most known sublinear curves, it can fit the data well, and it is relatively simple with known statistical properties. This model was also used by the NAS its risk assessment for methylmercury. (NRC, 2000). What is needed is the application of this model to several data sets to determine whether it gives reasonable results. Monte Carlo simulations could also be used to generate data from known dose-response functions; the K-power model could then be evaluated through its application of these data.

After extrapolation has occurred to the point of interest, the confidence level about that point need be calculated; hence the level of confidence must be chosen. These limits are designed to express the uncertainty associated with a parameter estimate due to sampling and/or experimental error. The method used to obtain the confidence level is tied to the estimation method for the BMD. (See Crump and Howe, 1985.) This will depend upon the nature of the data used to estimate the BMD and the model used. Once these have been chosen, good statistical methods exist for the calculation of the confidence level. (See, for example, Kimmel and Gaylor, 1988; Chen and Kodell, 1989; and Crump (1995).) In general a 95% confidence level is chosen to ensure that with probability 0.95or higher (given several statistical assumptions) that the benchmark response value (BMR) is not exceeded. Arguments could be made, however, to choose another confidence level. The choice is a subjective one.

IV. Uncertainty Factors

An uncertainty factor (UF) is defined as one of several, generally 10-fold factors, used in operationally deriving the RfD from experimental data (as contrasted with observational data). UFs are intended to account for (1) the variation in sensitivity among members of the human population, i.e., interhuman or intraspecies variability; (2) the uncertainty in extrapolating animal data to humans, i.e., interspecies variability; (3) the uncertainty in extrapolating from data obtained in a study with less-than-lifetime exposure to lifetime exposure, i.e., extrapolating from subchronic to chronic exposure animal studies; (4) the uncertainty in extrapolating from a LOAEL rather than from a NOAEL; and (5) the uncertainty associated with extrapolation from animal data when the data base is incomplete (USEPA, 2000b). For the methylmercury database under consideration, reliance on experimental animal neurotoxicological studies is not necessary, however, since a number of large human population observational studies are available in which the most sensitive subpopulation (the human fetus) is the subject. Of the five factors listed above, the first and fifth were considered by NAS and USEPA in development of the current RfD of 0.1 μg/kg/day. As discussed at length in Dourson & Wullenweber (2001), a number of additional uncertainties have been considered by government agencies and other organizations for methylmercury health risk assessment as derived from human epidemiology studies. The UF for variability in the human population (toxicodynamics and toxicokinetics) is well supported by data and in good agreement, for the most part, among different organizations. Application of a multi-compartment pharmacokinetic model for methylmercury that includes the fetus resulted in the most realistic estimate of the interindividual toxicokinetic uncertainty factor (Clewell et al., 1999).

In addition to the above UF, database deficiencies resulting in uncertainty are a major source of wide-ranging UFs in developing an acceptable allowable level of exposure to methylmercury for all organizations. The UFs for database deficiencies ranged from 1.5 to 5 across organizations. Among those were a UF of 1.5 used by ATSDR Agency for Toxic Substances Disease Registry) to account for the possibility that the domain-specific tests used in the Faroes study might be more sensitive to subtle effects than those tests employed in the testing at approximately 6 years of age in the Seychelles. An UF of 3 was applied by USEPA for lack of a two-generation reproductive study (presumably in animals since humans have been exposed for multiple generations) and lack of data for the effect of exposure duration on sequelae of the developmental neurotoxicity effects and on adult parathesia. NAS selected a UF of 5 because of consideration of possible low-dose sequelae and latent effects, and possible immunotoxicity and cardiovascular effects that may occur at lower exposures than those at which neurotoxicological effects are presently reported to occur. It is clear, then, that database deficiencies constitute the largest area of uncertainty in development of a RfD for methylmercury and thus have possibly the largest influence on the final RfD number.

Uncertainties related to database deficiencies can be improved by initiation of new research. Further investigation to determine the critical effect for methylmercury is important. Investigation of other potential critical effects such as immunotoxicity and cardiovascular effects will either change the basis of the RfD or reduce the need for uncertainty factors if such effects are found to occur at levels above those that elicit neurotoxicity. It is possible that sufficient data already exist with which to conduct BMD analyses on these factors; alternatively, new case-control studies of fish-eating populations may be feasible to more closely examine these endpoints in relation to exposure to methylmercury. Experimental studies to examine potential developmental neurotoxic sequelae that may occur years after low exposure occurring early in life may provide information to reduce uncertainty around this issue.

V. Conclusion

A number of issues in the current risk assessment for methylmercury ranging from the risk assessment methodology itself, and the choice and definition of an adverse outcome and the uncertainties surrounding these choices have been outlined here. In order to improve mercury risk assessment in the near future, a number of research avenues should be undertaken. In terms of health effects, clearly understood and standard definitions of adverse effect(s) remain a critical outstanding issue in children's neurodevelopmental and neurobehavioral outcomes. At least one study in progress may contribute appreciably to our knowledge in this arena. A second large remaining area for research is the reduction of database deficiencies as outlined by a number of groups in their risk assessment activities and as mentioned earlier in the discussion on UFs. Studies on the impact of exposure to methylmercury on the prevalence of hypertension and cardiovascular disease in the US population can be undertaken. Effects of exposure to methylmercury at biologically plausible levels on cardiovascular and immune system functioning need to be undertaken in both experimental and observational settings. Effects on reproductive function and potential delayed neurotoxicology effects manifested in aging after relatively low exposure early in life can also be addressed.

Research can also be undertaken to improve the choice of dose-response function for application within the risk assessment process itself. Greater understanding of test instruments used in assessing neurobehavioral and neuropsychological function could aid this effort by providing more information with which to select the most appropriate benchmark level. Experience in the use of biologically relevant models, such as the flexible K-power model, applied to several different data sets may identify a small set of models that have wide applicability. Alternatively, one could hypothesize a dose-response, generate simulated data for this dose-response, and then evaluate alternative models for the simulated data set.

The objective of conducting such studies is to inform the risk assessment process by reducing uncertainties. The goal in this entire process, of course, is to eventually be able to objectively determine the body of information that is sufficient to reduce uncertainties. This, in turn, will entail leaving behind default assumptions (such as the standard UF of 10). The ability to let fall such default assumptions would indicate an appropriately high degree of confidence in the scientific method as well as the quality of information it can bring to the issue of human health risk assessment for low level exposure to methylmercury.

REFERENCES

(Reports)

Budtz-Jorgensen, E., Keiding, N., and Grandjean, P. (2000) Benchmark dose calculation from epidemiological data. University of Copenhagen, Department of Biostatistics Report 2000-4.

U.S. Environmental Protection Agency, (1995) The use of the benchmark dose approach in health risk assessment. Office of Research and Development. Washington, DC:EPA/630/R-94/007.

USEPA, (2000a) US Environmental Protection Agency, Peer Review Workshop Report on Reference Dose (RfD) for Methylmercury, Contract No. 68-C-99-238, Task Order 32, Versar Inc. Springfield, VA, December 7, 2000, p. 23.

USEPA, (2000b) US Environmental Protection Agency. Glossary. Integrated Risk Information System (IRIS). Available online at www.epa.gov/iris.

(Periodicals)

Chen, J.J. and Kodell, R. (1989) Quantitative risk assessment for teratologic effects. J. Am. Stat. Assoc. 84:966-971.

Clewell HJ, Gearhart JM, Gentry PR, Covington TR, VanLandingham CB, Crump KS, Shipp AM. (1999) Evaluation of the uncertainty in an oral Reference Dose for methylmercury due to interindividual variability in pharmacokinetics. Risk Analysis 19: 541-552.

Crump, KS. (1995) Calculation of benchmark doses from continuous data. Risk Anal. 15:79-89.

Crump, K.S. (2000) Critical Issues in Benchmark Calculations from Continuous Data. Submitted to Critical Reviews in Toxicology, Dec. 2000.

Dourson ML and Wullenweber AE. Determining the reference dose for methylmercury – how can we further reduce uncertainties? A review. NeuroToxicology (Submitted) 2001.

Grandjean P, Weihe P, White RF, Debes F, Araki S, Murata K, Sorensen N, Dahl D, Yokoyama K, Jorgensen PJ. (1997) Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol. Teratol. 19: 417-28.

Kimmel. C.A. and Gaylor, D.W. (1988) Issues in qualitative and quantitative risk analysis for developmental toxicity. Risk Analysis 8:15-20.

(Books)

Crump, K.S, and Howe, R. Chapter 9. (1995) In *Toxicological Risk Assessment.* D.B. Clayson, D. Krewski, I. Munro, eds. Boca Raton: CRC Press, Inc.

NAS (2000). National Academy of Sciences Dose-response curves fitted to cord-blood Hg data for the linear square root and log transformations (of Boston Naming Test scores). Fig. 7-5, p. 296.

National Academy of Sciences (NAS), National Research Council (NRC), Committee on the Toxicological Effects of Methylmercury.(2000) *Toxicological Effects of Methylmercury*, National Academy Press.

White RF, Debes F, Dahl R, Grandjean P. (1994) Development and field testing of a neuropsychological test battery to assess the effects of methylmercury exposure in the Faroe Islands. Proceedings of the international symposium on assessment of environmental pollution and health effects of methylmercury, Kumamoto, Japan, pp. 127-40.

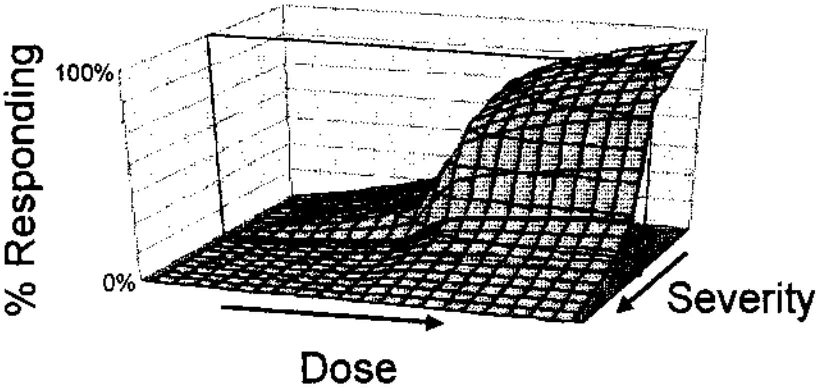


Figure 1. Hypothetical dose-response surface relating dose to response by severity

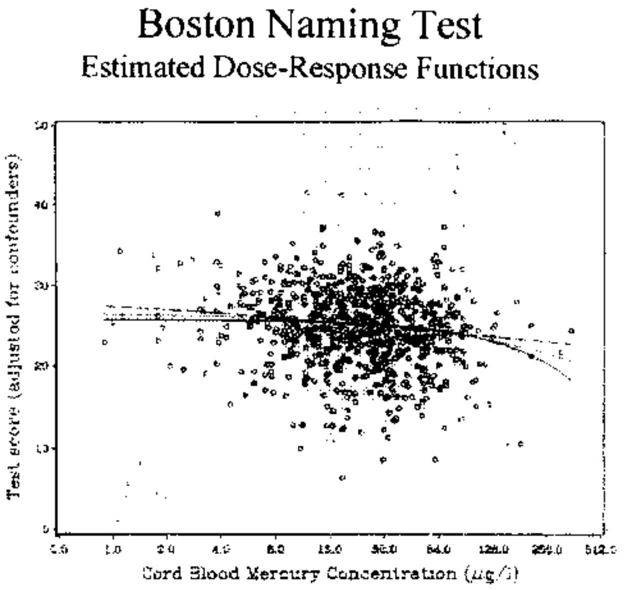


Figure 2. Dose-response curves for the Boston Naming Test test scores fitted to cord-blood mercury data for the linear, square root, and log transformations.

Use of modelling in mercury pollution studies with two case studies: Gulf of Trieste and Yatsushiro Sea

R. Rajar[1], D. Zagar[1], A. Širca[1], H.Akagi[2], S. Yano[3], T. Tomiyasu[4], K. Oki[4], M. Horvat[5]

[1]Faculty of Civil Engineering, University of Ljubljana, Slovenia; [2]National Institute for Minamata Disease, Minamata, Japan; [3]Dept. of Civil Engineering, Faculty of Engineering, Nagasaki University, Japan; [4]Dept. of Earth and Environmental Sciences, Faculty of Science, Kagoshima University, Japan; [5]Dept. of Environm. Sciences, JoZef Stefan Institute, Slovenia.

Abstract

Some basic principles of use of models in the research of pollutant dispersion processes in surface waters are described. Two case studies of modelling mercury transport are presented further on. The first is the Gulf of Trieste (Northern Adriatic), where the main source of mercury pollution is sediment from a river, arriving from a mercury mine region. By combining measurements with modelling results it was possible to describe distribution and transport of mercury and its species in the Gulf of Trieste and to calculate the mass balance for total and methyl-mercury. In the Yatsushiro Sea, some mercury has been transported from the Minamata Bay after the well-known mercury disaster. First simulation results of currents in the Yatsushiro Sea are presented, they are further on used for calculation of mass balance in the Minamata Bay, where the potential sources of Hg and their relative magnitudes are shown. Further investigations are in progress. By combining measurements with hydrodynamic and Hg cycling modelling, meaningful predictions for future behavior of Hg in both Minamata Bay and Yatsushiro Sea will be possible.

***Key words**: three-dimensional modelling; mass balance; mercury; Gulf of Trieste; Minamata Bay; Yatsushiro Sea.*

1. INTRODUCTION

During the last decades enormous effort and investment is being given to improve the quality of surface and ground waters, or at least to diminish their further pollution. One of the essential tools to attain this goal is modelling.

The basic phenomenon, which should first be simulated by a model, is the water movement, the hydrodynamic circulation. This is further on responsible for transport and dispersion of any contaminant. As often the greatest part of a contaminant is transported in the sc. *particulate form*, bound to suspended sediment, also the simulation of sediment transport should be included in the modelling. The change of contaminant concentration due to bio-chemical processes is the most difficult part of model simulation, as often the processes are not known well enough to be described reliably by equations.

There are many types of models, which can be used to this purpose. In smaller water bodies, as e.g. small lakes or bays, the pollutant concentration can be quickly equalized over the whole volume and zero-dimensional models can describe the time-development of the processes. But in large lakes or coastal seas (as e.g. the Yatsushiro Sea), the pollutant concentration is not the same at all locations of the water body, it is space and time dependent. Two or three-dimensional models should be used.

How these model tools can effectively be used in water pollution studies? They describe, simulate the phenomenon of pollutant transport and possibly some transformation processes. After calibration and verification of the model, we can be sure it can reliably simulate the phenomena. The model can give us the information on exchange of some pollutant between water bodies (e.g. between Minamata Bay and Yatsushiro Sea), it can provide the rate of sediment resuspension and transport. We can also simulate e.g. the effect of some remediation measures and find out the most effective one. Mathematical modelling is usually essentially less expensive than field measurements. One of the greatest advantages of modelling is that we can simulate some future events, which is not possible to do by measurements.

But we should emphasize, that the field measurements are absolutely necessary at any serious pollution studies. Only the combination of measurements and modelling can give good solutions. The field measurements are especially valuable for the calibration and verification of mathematical models.

Two case studies are described further on. The first is modelling of mercury cycling in the Gulf of Trieste (N. Adriatic) and the second a preliminary study of mercury transport in the Minamata Bay and Yatsushiro Sea (Kyushu, Japan).

2. MODELLING OF MERCURY IN THE GULF OF TRIESTE

2.1 Introduction

A thorough study of pollution of the Gulf of Trieste (Northern Adriatic) with mercury has been carried out. In this case mercury pollution was caused by a mercury mine, situated at a river about 100 km from the gulf. The mine was in operation for 500 years. Although it has been closed about 10 years ago, mercury contaminated sediments are still being transported from the mine region and along the river into the gulf (for more details see Horvat et al 1999).

Even 10 years after the closure of the mercury mine, Hg concentrations in river sediments and water are still very high and do not show the expected decrease in the Gulf of Trieste. Many measurements of physical, chemical and biological parameters have been carried out in recent years. Fig. 1 shows measured spatial distribution of Hg in the bottom sediments of the Gulf of Trieste. Maximum concentrations are near the river mouth, attaining maximum values of about 17 ppm.

2.2 Modelling

A two-dimensional (2D) steady-state model was first developed for the simulation of hydrodynamic (HD) circulation and mercury transport and fate. Fig. 1 presents the basic components of the model. The methylation process was also taken into account, although by simple equations, based on methylation rate and a calibration parameter. Some of the previous results were described in Rajar et al. (1997), and in Širca et al. (1999a).

As the 2D model has several significant limitations for a proper simulation of the phenomenon, an existing three-dimensional (3D) unsteady state model PCFLOW3D was later on adapted and extended with a new module for the simulation of suspended sediment transport. Many simulations have been performed first to calibrate and verify the hydrodynamic part of both models. Verification of the pollutant transport module, describing mercury transport and transformation processes was only partially possible. However the basic description of the phenomena has been successful. Fig. 2 shows a comparison of simulated and measured concentrations of total Hg in water in the gulf. The simulated concentration distribution is in agreement with measurements within the order of magnitude. For a so complex phenomena we considered this satisfactory. Some later simulations showed even better agreement.

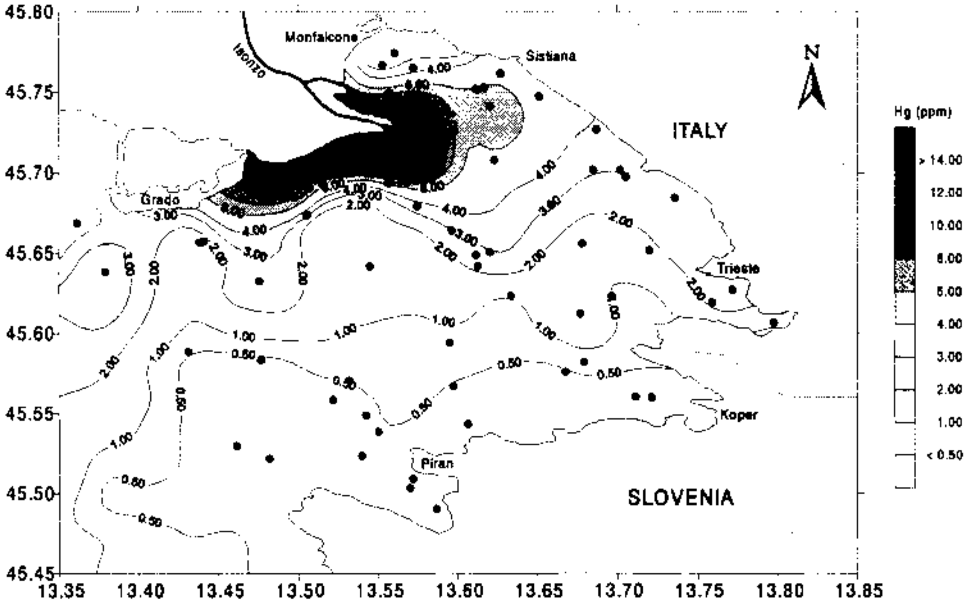


Fig. 1. Measured spatial distribution of Hg in the bottom sediments of the Gulf of Trieste (from Covelli et al., 1999).

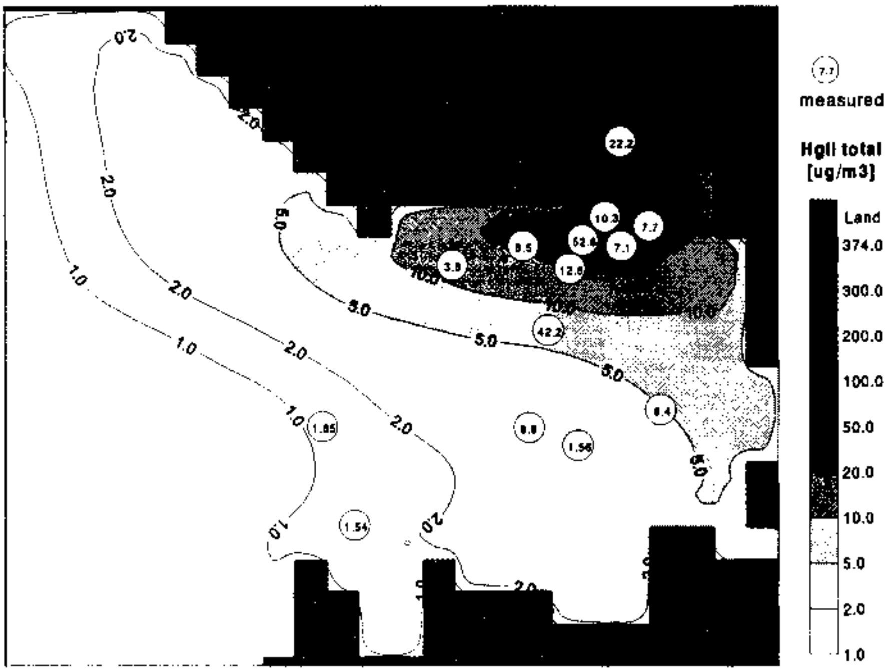


Fig. 2. Comparison of simulated and measured concentrations of total Hg in the Gulf water.

The mass balance of mercury in the gulf has been made, on the basis of modelling and measurements. It is presented in fig. 3. Such evaluation of the mass balance is very important for understanding the phenomena that are going on in a coastal sea, and for possible future predictions.

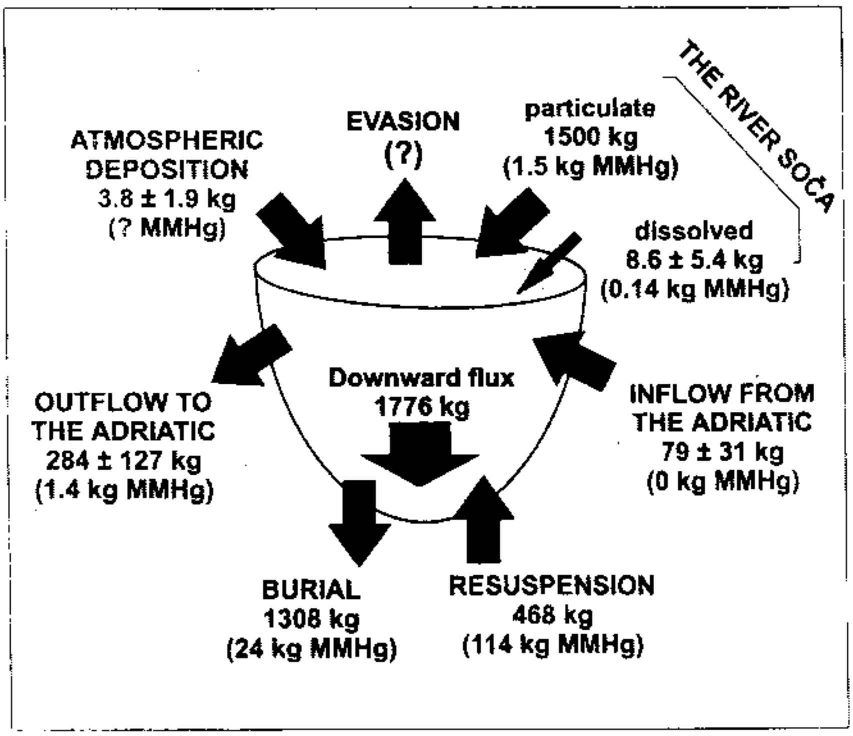


Fig. 3. Annual mass balance of total mercury in the Gulf of Trieste.

2.3 Conclusions

Although it is not possible to pretend that the very complex phenomenon of mercury transport and fate in a coastal sea has been fully resolved, the combination of modelling and measurements has contributed to a better understanding of several features of the phenomenon. We present here some conclusions, obtained on the basis of modelling and measurements, especially the ones, that can be used to understand better the phenomena of mercury cycling also in other coastal seas, as e.g. Yatsushiro Sea.

- Most Hg is transported in particulate form. Several measurements have shown that the inflow of total dissolved Hg into the Gulf is almost negligible in comparison with the inflow of Hg adsorbed to suspended particles. For example, Širca and Rajar (1997) determined that, during one year, the SoCa river brings only 3.3 kg of dissolved Hg, while the mass of particulate Hg is about 1800 kg.
- It was confirmed that the main source of inorganic Hg in the Gulf is the SoCa river, while most methyl-mercury is formed in the Gulf itself . Measurements have not shown the highest concentrations of MeHg near the river mouth but relatively far away.
- The influence of short periods of peak wind and river discharge are very important for the transport of sediments, especially because of the resuspension of the bottom sediments, which must be properly simulated.
- Wind and resuspension are the main causes of elevated concentrations of Hg along the northern shore. Once settled at the bottom near the river mouth, particulate mercury is being partly resuspended and further transported during peak wind conditions towards the west. Measurements alone could not show these phenomena clearly; modeling, however, did. The phenomenon of resuspension is very important and must be included in the model simulations.
- Benthic flux is important and needs to be simulated in the future. Measurements in one point only are so far available; it has been shown that benthic flux reaches the order of magnitude of the inflow of MeHg with the SoCa river.

- The result of modelling is an approximate mass balance: 82% of total Hg, incoming from the Soca river, settles to the bottom, 18% flows out of the Gulf with currents .
- Both 2D and 3D models have shown the correct trends of the phenomena, although a quantitative comparison with measurements was not always possible.
- The advantages of the 3D model over the 2D model are clear: it is possible to simulate the important influence of the vertical distribution of parameters. e.g. thermal and salinity stratification, and the vertical distribution of all water quality parameters.

Several points for further research can be recommended:

- Further measurements of Hg concentrations in the water column, sediment and biota are needed in different seasons for the calibration and verification of the model. Several measurements of Hg contents in fish have already been carried out.
- More detailed measurements of Hg in the Idrijca and Soca rivers, and their suspended sediments, are needed.
- As the next step, we shall concentrate our efforts on the simulation of the bio-geochemical processes of mercury in the water column, sediments and biota, revised and based on more numerous measurements for the calibration of the 3D model.

3. USE OF MODELS FOR MINAMATA BAY AND YATSUSHIRO SEA – FIRST RESULTS

3.1 Problem Description

In the Minamata Bay, the well known disaster with mercury contamination around 1956 was later very thoroughly remediated. Between 1974 and 1990 nearly 1 million m^3 of bottom sediment has been removed by dredging from the bay and safe conditions for fishing restored. But later on measurements have shown (Fig. 4), that some mercury had been transported from the Minamata Bay to the Yatsushiro Sea, mostly before the mentioned dredging.

Fig. 4 shows measured mercury concentration in the sediment of the Yatsushiro Sea (from Rifardi et al 1998). It is evident that mercury has been transported out of the Minamata bay, presumably by tidal currents. The main goals of the study (not yet finished) are the following:

(1) What is the physical phenomenon, which is causing the transport of mercury out of the Minamata Bay (and possibly back)?
(2) What physical mechanisms have caused the shown distribution of mercury in the Yatsushiro Sea, maximum concentrations being along the NE coast and W of Minamata Bay.
(3) What is the mass balance of mercury in the Minamata Bay? Description of this phenomenon will evaluate the sources and sinks of mercury in the bay, which will help understand the whole process, predict its development in the future conditions and determine if any sanitation measures are necessary.

In a collaboration project Slovenia-Japan we try to use similar model technology as in the Gulf of Trieste in order to understand the phenomenon and to give at least some partial answers to the above questions.

3.2 Short description of Yatsushiro Sea

The Yatsushiro Sea, Minamata Bay being its integral part, is almost enclosed sea, along the E side of Kyushu. Its length (in the SSE-NNW direction) is about 70 km, its width is up to 25 km in the S part and about 10 km in the N part. Its N part is shallow, the depth being of the order of 10 to 20 m, while in the S part the max. depths are 35 to 60 m. Topography of the Yatsushiro sea and Minamata

Bay was obtained from marine charts at scale 1:100.000 and 1:30.000. For the Minamata Bay the topography was obtained from marine chart at 1:7500.

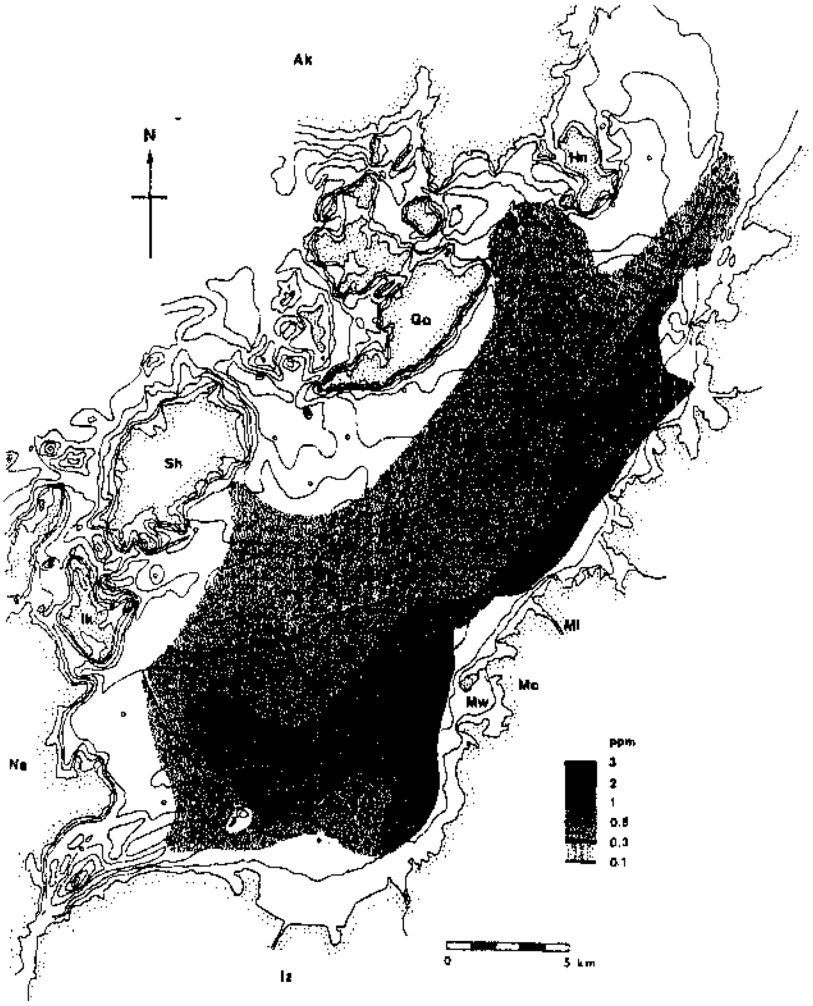


Fig. 4. Distribution of the maximum mercury content (ppm) which have been analyzed at some horizons of each core (from Rifardi et al. 1998)

There are 5 straits, connecting the Yatsushiro Sea with the neighboring seas. Table 1. gives basic dimensions and information about tidal constants in the straits, as they were used in our simulations. We should mention that the strait C (Hondo Seto) is very narrow and shallow in comparison with all the others, and it was treated as closed (no flow through it) in all our simulations.

Strait	*Width (m)*	*Depth (m)*	*Amplit. (m)*	*Time lag (deg.)*
A–Kurono Seto	400	10	1.16	224.204
B–Nagashima Kaiko	1800	61	1.28	207.834
C-Hondo Seto	<100	3 (?)		
D-Matsushima	1500	7.5	1.691	178.44
E-Misumi Ko	300	26	1.732	178.81

Table 1. *Main characteristics of the 5 straits of the Yatsushiro Sea.*

3.3 Hydrodynamic simulations

A number of hydrodynamic simulations of the circulation in the whole Yatsushiro Sea has been carried out with three purposes: (1) to calibrate and verify the model; (2) to determine the relative

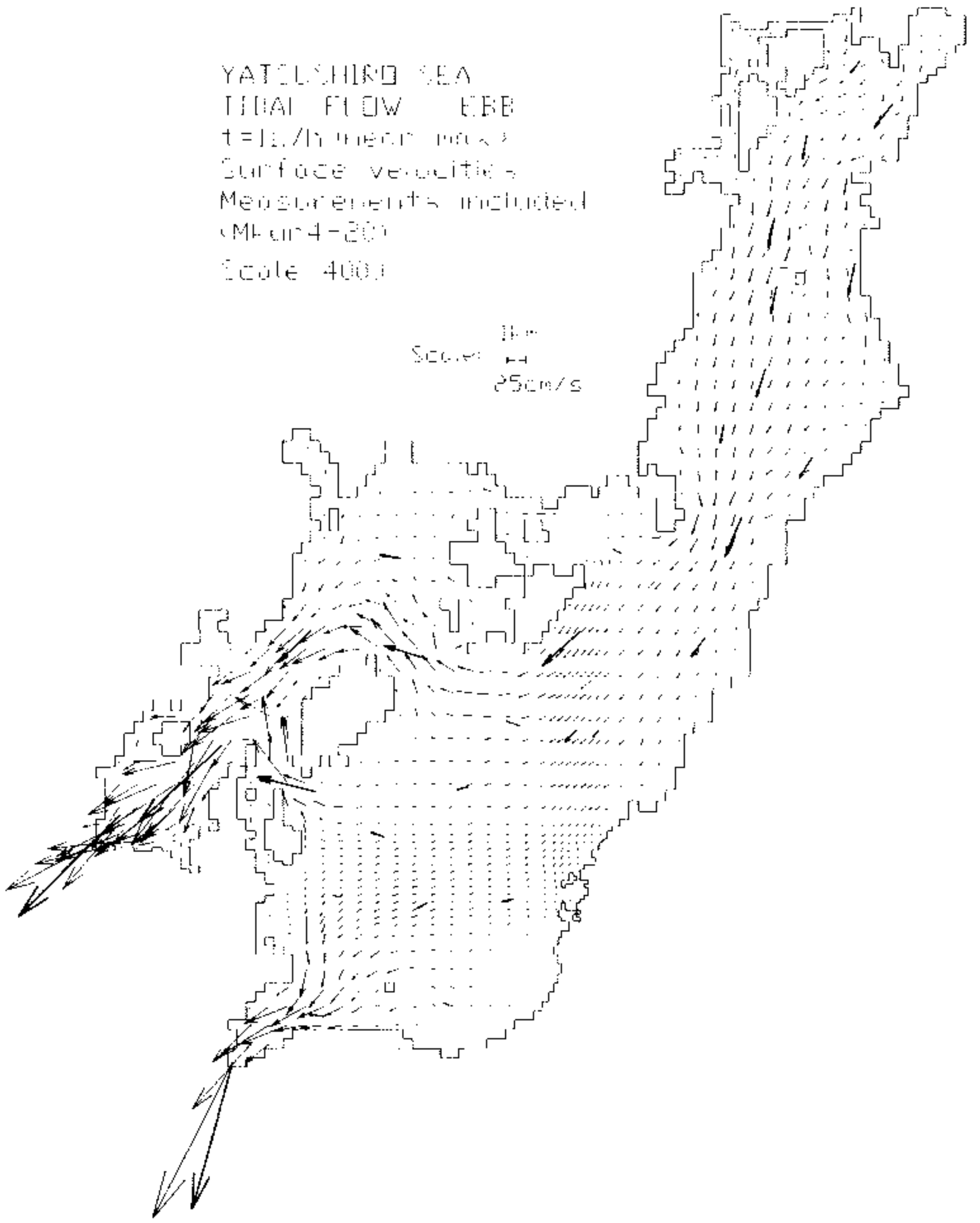


Fig. 5. Calculated and measured velocities in the Yatsushiro Sea at ebb tide. Bold vectors are measured velocities.

magnitude of the forcing factors, which influence the current circulation in the Yatsushiro Sea, and (3) to determine the value of some parameters which are used for the calculation of mass balance of Hg of the Minamata Bay, i.e. the outflow velocities or the rate of resuspension of bottom sediments.

The hydrodynamic circulation in this sea is strongly influenced by tidal currents. Measurements of currents (Hydrographic Department, Maritime Safety Agency, 1978) have shown max. velocities in the strait A to be about 4.9 knots, and at B even 6.9 knots. Therefore the first HD simulations were made with the tidal forcing. We should mention, that the amplitudes, given in table 1, were approximately evaluated for *spring* tide, more or less max amplitudes in a year. Only basic comparison of simulated and measured currents during ebb tide is shown in Fig 5. The agreement is very good in the southern part of the Y. Sea, but the simulated velocities are too small in the shallow northern part. The reason for the disagreement was found to be, that we used averaged tidal amplitudes, but measurements of currents, which were used for model calibration are valid for maximum velocities during spring tide. More detailed tuning is under way.

The second forcing factor is wind. This is strongest during winter months, blowing mainly from the north. The average winter wind was evaluated by a specific method VECTRA (Širca et al 1997) to have the magnitude of 3.35 m/s. But experience from the study of the Gulf of Trieste, as also from the study of transport of sediment from the Mururoa Lagoon, after the French nuclear experiments in the Pacific (Rajar 1998), have shown that short periods of very strong winds (typhoons) can cause 90 % or more of the annual sediment outflow. Several simulations were performed to estimate relative importance of different forcing factors. Table 2 shows the order of magnitude of current velocities caused by them. We can say, that in the straits and near them, tidal forcing is always the most important, as the max velocities are of the order of 3 - 5.0 knots (Fig. 5).
Simulations with average winter wind have shown that over most of the Yatsushiro Sea the velocities are smaller than tidal, only inside the Minamata Bay it is of the same order of magnitude. Typhoon can cause velocities, which are everywhere, except in the straits of the same order of magnitude or greater than tidal velocities.

	Tidal velocities	*Aver. Winter wind Surf./bottom*	*Typhoon 125 km/h Surf./bottom*
In/near the straits	150-300		
South. Y. Sea	5-25	3-6 / 1-2.5	80 / 6-18
North Y. Sea	25-50	4-7 / 1-3	80 / 15-20
Minamata Bay	4-5	3-6 / 1-3	80 / 15-25

Table 2. Order of magnitude of current velocities caused by tide or wind (in cm/s)

4. MASS-BALANCE OF MERCURY IN MINAMATA BAY

As already mentioned, the calculation of mass balance is always very important, as it includes evaluation of sources and sinks of mercury in the bay, which will help understand the whole process, predict its development in the future conditions and determine if any sanitation measures are necessary.

Fig. 6. shows sources and sinks of mercury in the bay, as estimated on the basis of measurements. The calculation is made for mercury in the water column (or whole water) which includes both dissolved and particulate mercury.
The sources/sinks of the Hg in Minamata Bay are the following:

1. Inflow with creeks from land.
2. Exchange with the Yatsushiro Sea. We shall consider the *net* outflow (outflow – inflow) as both phenomena are closely connected and it is difficult to distinguish between them. The resuspension from sediment (and settling) is also included in this term, as this only contributes to concentration of Hg in water column, which can be transported out of the bay.
3. Inflow of Hg from Fukuro Bay. Although this is an integral part of the Minamata Bay we consider it here as a separate bay, because measurements have shown that the Hg concentrations are higher in Fukuro Bay and thus it can represent a source of Hg for Minamata Bay.
4. Exchange with atmosphere.

At this stage there are not enough data to evaluate all of them accurately, but we will concentrate on the first two parameters, especially on the exchange of Hg with the Yatshushiro Sea, as this is the most important one. Only a rough estimation is given for the exchange with the Fukuro Bay. For the evaluation of exchange of Hg with atmosphere, measurements will be carried out during 2001.

Fig. 6. Parameters, influencing mass balance of mercury in the Minamata Bay

1. Inflow of Hg with creeks from land. Hyeken channel is the most important. Two measurements in 1999 (Horvat et al 2001) have shown that the Hg concentration is very near to 20.0 ngHg/L. The water discharge is normally Qw=3400m3/h (Akagi, personal communication). The mass of mercury inflow per year is calculated by simply multiplying the annual mass of water by Hg concentration, resulting in: $M1_n$= *0.6 kgHg/year.*
This quantity is very small, it can be neglected. But our experiences with sediment transport in rivers show, that usually most of the sediment transport is done during one or two high water periods. As much as 80, 90 or even 95 % of the annual sediment transport can be due to such short, but extreme events. We have no data on hydrology of the Hyeken channel. But even if we assume, that during flood conditions the transport of Hg is ten times higher as in normal conditions, we get a very rough value of

M1 = 6 kgHg/year,

2. Exchange with the Yatsushiro Sea. The study on mercury modelling in the Gulf of Trieste has shown that in a coastal sea, like Minamata bay, we should also distinguish two cases of Hg transport: *Normal conditions* and *Storm conditions*. Normal conditions are approximately the mean annual conditions, usually caused by tide or mean annual wind. They act permanently over the whole year, or most of the year. As in so averaged conditions current velocities are usually small, they cannot agitate the bottom sediments and cause resuspension. Storm conditions are short periods of very strong wind and/or inflow from rivers. In the second case, the current velocities are great, very often causing resuspension of bottom sediment into the water column. Therefore the concentration of sediment in water column becomes very high, transporting with them a big mass of particulate mercury.

Most of the data for the following calculation was obtained by measurements and with the help of above described 3D modelling of the whole Yatsushiro Sea.

A. Normal conditions.

HD simulations have shown, that during one tidal cycle the max. outflow velocity is approx. 4.7 cm/s, average velocity during one cycle 2.8 cm/s. The whole volume of water transported out of the bay during 1 cycle is about 6.0 million m3. As about 25% of this water returns back to the bay during the next cycle, approx. volume of water exchanged is about 4.5 x 10^6 m3. Taking into account average conc. of Hg in bay water, 4 ngHg/L, we get M_{2n} = 16.3 kg Hg / year.

The HD computations have also shown, that the tidal velocities (approx. 5 cm/s) are far too small to cause sediment resuspension, which could increase the outflow of mercury considerably. The critical velocities to begin resuspension of the bed sediments of the described size (depending also on water dept, and in a great degree on the height of waves) are of the order of 23 to 35 cm/s.

B. Storm conditions.

As we did not have a statistical evaluation of wind magnitude and direction during the year, in the first approximation we have assumed the following extreme conditions:

Wind force: 125 km/h (34.72 m/s), direction NW, duration 6 hours, 6 times a year. The strongest resuspension of sediment would occur along one part of the coast inside the bay, which is open to wind and waves from the NW. The opening to the Yatsushiro sea, between the Kojishima island and the peninsula south of it is approx. 1km long. But as the wind direction is from NW, and as the coast inside the bay is partly protected by the Kojishima island, we have taken the length of effective opening to be 400 m. Average depth inside the bay, near the attacked coast is about 7m.

Mean water velocity at the depth of 7m is 0.27 m/s. Wave height in the open part of the bay (also calculated by the HD model) would be 3.8 m. Concentration of resuspended sediment in water would be 0.63 kg/m3. The outflow of this sediment is only in the lower 3 meters of the opening (near the surface the flow is inward).

The flow of sediment through the area of 400x3 meters, with duration of 6 hours, 6 times a year would be 26.500 tons. With average concentration of Hg in sediment of about 4 ppm (measurements, Horvat et al 2001) the annual outflow of Hg is M_{2s} = 132 kg/year.

Thus the total outflow of Hg to Yatsushiro sea would be approx.

M2 = 150 kgHg/year.

We have tried to find some confirmation of these results from published literature. The following comparisons were possible.

Kudo et al. (1980) have published results of measurements of Hg in bed sediments of the Yatsushiro sea. The measurements were carried out in four consecutive years from 1975 to 78 at 27 sampling locations. They have found that between 1960 and 75 roughly 9 tons of Hg moved from the Minamata Bay into the Y.S. (or 0.6 tons/year), while during four years from 1975 and 78 almost 17 tons moved from the bay (5.7 tons/year). The authors examined the possible causes of so large outflow and its great increase in the years 1975-78. Their conclusion, that the tidal velocities are far too small to cause resuspension and increased sediment transport is right. They also consider that desorption of Hg from sediment is too slow to come into consideration. They discuss that maybe ship traffic, which increased considerably during last years, could be the cause of increased sediment outflow.

They did not consider the phenomenon of *storm conditions*. The energy of ship motors is for an order of magnitude smaller as the energy of typhoons. Analogue to the above calculations we can calculate the outflow from the bay in the time from 1975-78, when the dredging of the contaminated sediment has mostly not yet been done. The average sediment concentration in the bay was of the order of 70 ppm. The outflow of Hg in those conditions would be about 2.3 tons/year. This really falls into the order of magnitude as obtained by the measurements of Kudo.

Another evaluation was possible. From Fig. 4, taken from Rifardi et al (1998), we have done an evaluation of the mass of mercury in the Yatsushiro Sea at the time of measurements, i.e. 1996. Only an approximate calculation was possible, mainly because of not well defined depth of the bottom sediment, up to which mercury was still detected at each measuring station. However, the result was roughly 31 tons (±20 %). This would mean, that during the last 18 years (from 1978 to 1996) the outflow of Hg from the Minamata Bay was very small, roughly 0.06 tons/year. This is 2.5 times less than calculated by our assumptions, but as these were done for the most unfavorable conditions, we can be satisfied with the same order of magnitude obtained.

3. Inflow of Hg from Fukuro Bay. Fukuro Bay is a part of the Minamata Bay, its dimensions are about 700 x 500 m with the depth from 1 to 10 m. The strait between them is only about 100 m wide, its maximum depth being 10 m. The measured concentrations of total Hg in water was 5.5 ng/L (in 1999, Horvat et al 2001), and in bottom sediment (Akagi, 1997) about 7 ppm.

Although these concentrations are higher than in the Minamata Bay, because of the very narrow connection with the Minamata Bay, and because the bay is well protected from forming waves, the outflow of Hg can only be very small even during storm conditions. During a typhoon of 125 km/h, from the NW, we estimate the surface current velocity to about 40 cm/s, and bottom velocity (directed out of the Bay) of 10 cm/s. The water flow can be exchanged with the Minamata Bay over the deepest part of the connection channel of the cross section of about 200 m^2. If we assume that the storm would cause increased concentration of Hg in water (because of some resuspension of bottom sediment) to the order of 50 ngHg/L, a simple calculation shows that assuming 6 storms per year, each with the duration of 6 hours, we get a negligible Hg inflow from the Fukuro Bay to the Minamata Bay of

M3 = 0.13 kgHg/year.

More detailed simulations of the outflow of Hg from Fukuro Bay will be made after measurements of currents inside the Minamata Bay and in its vicinity will be carried out by the team of Nagasaki Univeristy.

5. CONCLUSIONS

1. Mathematical models are very useful tools at the study of the pollution of surface waters by different pollutants, including mercury. In the first case study (Gulf of Trieste), a 2D and 3D simulations helped understand the phenomena, while in the second study (Minamata Bay) the modelling simulations helped to evaluate the terms of mass balance, i.e. the mass of water and mercury, exchanged with the Yatsushiro Sea and Fukuro Bay.
2. The inflow of mercury to Minamata Bay from the Hyeken channel and from the Fukuro Bay is very probably negligible, of the order of some kilograms of Hg per year at maximum.
3. The outflow of Hg from Minamata Bay to Yatsushiro Sea, can mostly be effected during storms. The outflow is estimated to be 0.15 tons per year at maximum. This cannot represent any

important impact on the water quality of the Yatsushiro Sea. As before the clean-up actions in the Minamata Bay (dredging) the measurements have shown an outflow of the order of 1 to 5 tons Hg per year, the effectiveness of the clean-up works are again clearly confirmed.

4. Some additional measurements will be necessary, as e.g. measurements of atmospheric deposition. Also measurements of currents inside the Minamata Bay and in its vicinity will be carried out by the team of the Nagasaki University. The results of measurements will serve as boundary conditions for more detailed simulations of the phenomena inside the Minamata Bay.
5. By combining hydrodynamic and Hg cycling modelling, meaningful predictions for future behavior of Hg in both Minamata Bay and Yatsushiro Sea will be possible.

Acknowledgment

The main part of the research work, presented in this paper, has been carried out in the frame of collaboration Slovenia – Japan, and supported by the Ministry of Education, Science and Sport of Slovenia and by the Japanese Society for Promotion of Science.

References:

Horvat, M., Covelli, S., Faganeli, J., Logar, M., Mandic, V., Rajar, R., Širca, A., Zagar, D. (1999) Mercury in contaminated coastal environments, a case study: the Gulf of Trieste. *Sci. Tot. Envir.* **237/238**, 43-56.

Horvat, M. (1999). Mercury research in Slovenia: Current status of mercury contamination and research. *Proceedings of NIMD FORUM, 1999.* 94-115.

Hydrographic Department, Maritime Safety Agency (1978). *Charts of tidal currents in Simabara Wan and Yatsushiro Kai* (in Japanese). No. 6217, Maritime Safety Agency, Tokyo, Japan.

Kudo, A., Miyahara, S., and Miller, D.R. (1980). Movement of mercury from the Minamata Bay into the Yatsushiro Sea. *Prog. Wat. Tech.* **12,** 509-524.

Logar, M., Horvat, M., Akagi, H., Ando, T., Tomiyasu, T., and Fajon, V. (2001). Determination of total mercury and monomethylmercury compounds in water samples from Minamata Bay, Japan; an interlaboratory comparative study of different analytical techniques. Submitted to *Appl. Organomet. Chem.*

Rajar, R., Cetina, M., Širca, A. (1997). Hydrodynamic and Water Quality Modelling: Case Studies. *Ecological Modelling*, **101**, 209-228.

Rajar. R, (1998). Transport of Sediments and Radionuclides from Mururoa Lagoon into the Ocean. Chapter 3.2 in: *The Radiological Situation at the Atolls of Mururoa and Fangataufa,* main report, International Atomic Energy Agency, 177-179.

Rajar, R., Zagar, D., Širca, A., and Horvat, M. (2000). Three-dimensional modelling of mercury cycling in the Gulf of Trieste. *The Science of the Total Envir.* **260**, 109-123.

Rifardi, Oki, K., and Tomiyasu, T. (1998) Sedimentary environments based on texture of surface sediments and sedimentation rates in the South Yatsushiro Kai, SW Kyushu, Japan. *J. Sed. Soc. Japan*, **48**, 67-84.

Širca, A., Rajar, R.(1997). Modelling the effect of wind on average circulation and long-term contaminant dispersion in the Gulf of Trieste. *Acta Adriatica* **38**, (2), 45-59.

Širca, A., Rajar, R., Horvat, M., Harris, R. C. (1999). Mercury transport and fate in the Gulf of Trieste (Northern Adriatic)-a two-dimensional modelling approach. *Environ. model. softw.* **14**, 645-655.

Širca, A., Horvat, M., Rajar, R., Covelli, S., Zagar, D., and Faganeli, J. (1999). Estimation of mercury mass balance in the Gulf of Trieste. *Acta Adriatica*, **40**, 75-85.

Atmospheric Cycling of Mercury in Polar Regions

R. EBINGHAUS[1]*, CH. TEMME[1], H.H. KOCK[1], A. LÖWE[2]
AND W.H. SCHROEDER[4]

[1]GKSS Research Centre Geesthacht, Institute for Coastal Research/Physical and Chemical Analysis, D-21502 Geesthacht, GERMANY,
[2]University of Jena, Institute of Inorganic and Analytical Chemistry, D-07743 Jena, GERMANY
[3]Alfred-Wegener-Institute for Polar and Marine Research, Columbusstr., D-27568 Bremerhaven, GERMANY,
[4]Meterological Service of Canada, Downsview, ON, CANADA

INTRODUCTION

It is well known that the Arctic ecosystem is impacted by anthropogenic and natural emissions of mercury into the atmosphere. Air currents are believed to be a major pathway for entry of mercury into the arctic environment (Boutron et al., 1998).

The background concentration of total gaseous mercury (TGM) in the lower troposphere of the northern hemisphere is generally around 1.7 ng m^{-3} and 1.3 ng m^{-3} in the southern hemisphere respectively. Due to its fairly long atmospheric residence time atmospheric mercury is distributed on a global scale.

Recently, highly time resolved TGM measurements in the Canadian Arctic have shown that TGM levels are significantly depleted during the months after polar sunrise. A possible explanation may be a sequence of oxidation, adsorption and deposition of TGM leading to an increased input of atmospheric mercury into the Arctic ecosystem [*Schroeder et al.*, 1998]. Detailed studies on this phenomenon have been reported by Canadian and North-American groups, showing that the surface snow is impacted by increased Hg-deposition during the Arctic springtime. As one consequence of these findings, some of the circumpolar nations have initiated supplementary studies in Norway and Russia.

The Arctic TGM depletion is strongly correlated with ground level ozone concentrations. It is suggested that Cl and Br atoms are involved in a chemical reaction with ozone and that the depletion of total gaseous mercury is due to a reaction between gaseous mercury and BrO molecules [*Boudris and Bottenheim*, 2000]. Ozone depletion events have also been observed in Antarctica [*Wessel et al.*, 1998] however, highly time resolved TGM measurements have not yet been reported for the antarctic region.

In the period from January 2000 to February 2001 highly time resolved measurements of TGM with an integration time of 15 minutes have been carried out at the German Antarctic Research Station Neumayer, operated by the Alfred Wegener Institute for Polar and Marine Research. The site is located 70°39’S, 8°15’E, on the Ekströmisen, about 8 km from the Atka Bay.

METHODS

Two Tekran gas-phase mercury vapor analyzers (Model 2537A) have been installed at Neumayer Station in January 2000. The accuracy and precision of this instrument has been assessed during field intercomparisons at an urban/industrial site and at a remote marine background location [*Schroeder et al.*, 1995, *Ebinghaus et al.*, 1999].

The analyzers have been installed in the Neumayer Air Chemistry Observatory, which is approximately 1,5 km south of the main site. The prevailing wind directions are from the east. Northern wind directions are very rare, ensuring that the data are not influenced by contamination from the base station. The Tekran analyzers were operated with a 15 minute sampling frequency. The sample inlet is located 6 m above the snow surface on top of the Air Chemistry Observatory. The air is pulled with a flow rate of 1.0 L min^{-1} through a heated Teflon line about 10 m in length. To ensure the accuracy of the measurements the following sampling protocol is used at Neumayer: For quality assurance two analyzers are operated in parallel for one week. When the correspondence of the two Tekran analyzers has been tested and quantified one analyzer starts measuring TGM for the next three weeks, the other one is turned off. The next cycle will follow after three weeks as described above. After polar sunrise in August 2000 both analyzer have been in operation in parallel between antarctic springtime and the end of the measurement period.

RESULTS AND DISCUSSION

The results of TGM measurements for the time period January to November 2000 are presented in **Figure 1**. The TGM data can briefly be characterized by three different periods:

1. Between January and February 2000 and December to February 2000/01 TGM concentrations were highly variable with pronounced diurnal patterns and daily average concentrations between 0.6 and 1.8 ng $^{-3}$. During these episodes, TGM and ozone concentrations are frequently anti-correlated.

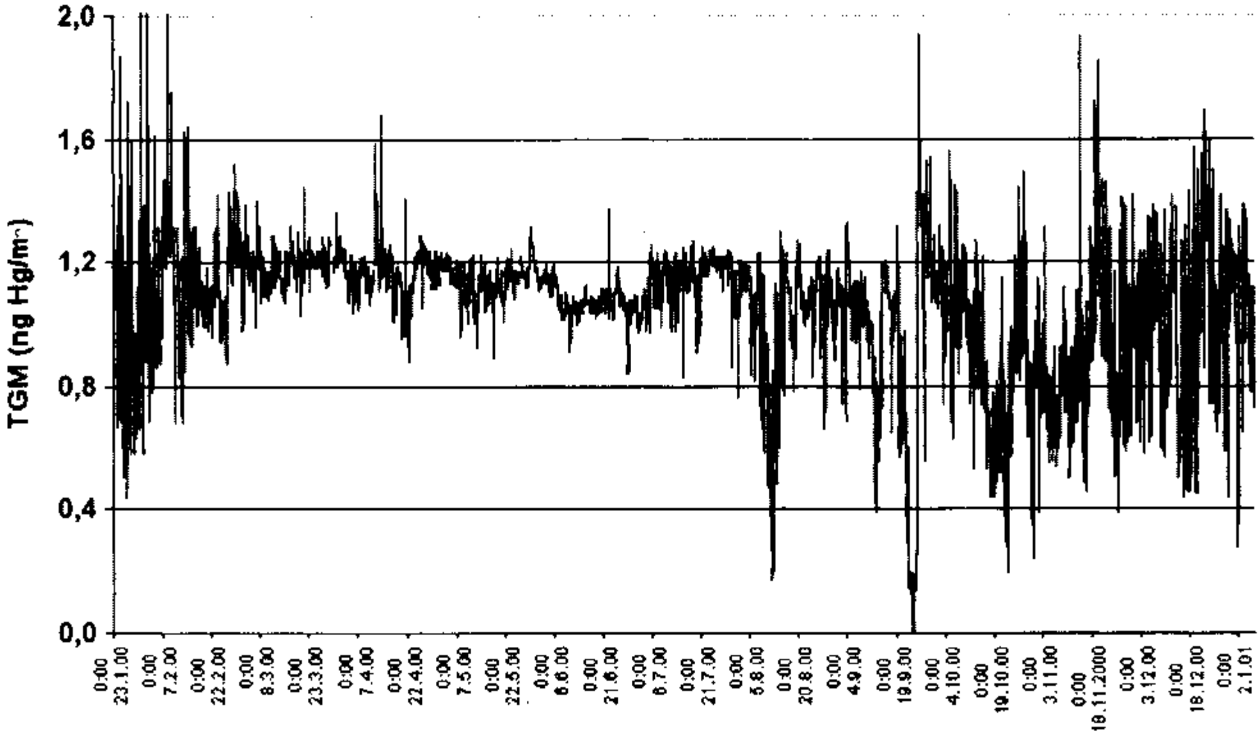


Figure 1: Annual time series of 1 hour averaged TGM at Neumayer, Jan 2000 - Jan2001

2. Between March and July 2000 the TGM concentrations remained on a fairly constant level of about 1.2 ng m^{-3}.

3. Between August and November 2000 several depletion events have been detected with minimum daily average concentrations of about 0.1 ng $^{-3}$. This TGM concentration pattern shows a strong positive correlation with ozone. (**Figure 2**).

The annual time series measured at Neumayer Station gives clear evidence that mercury depletion events (MDE) do occur in the Antarctic. It could also be shown that MDE coincides with ozone depletion in the lower troposphere and with the enhanced occurrence of BrO radicals in the Antarctic atmosphere, measured from the satellite borne GOME instrument and at Neumayer Station as BrO column densities.

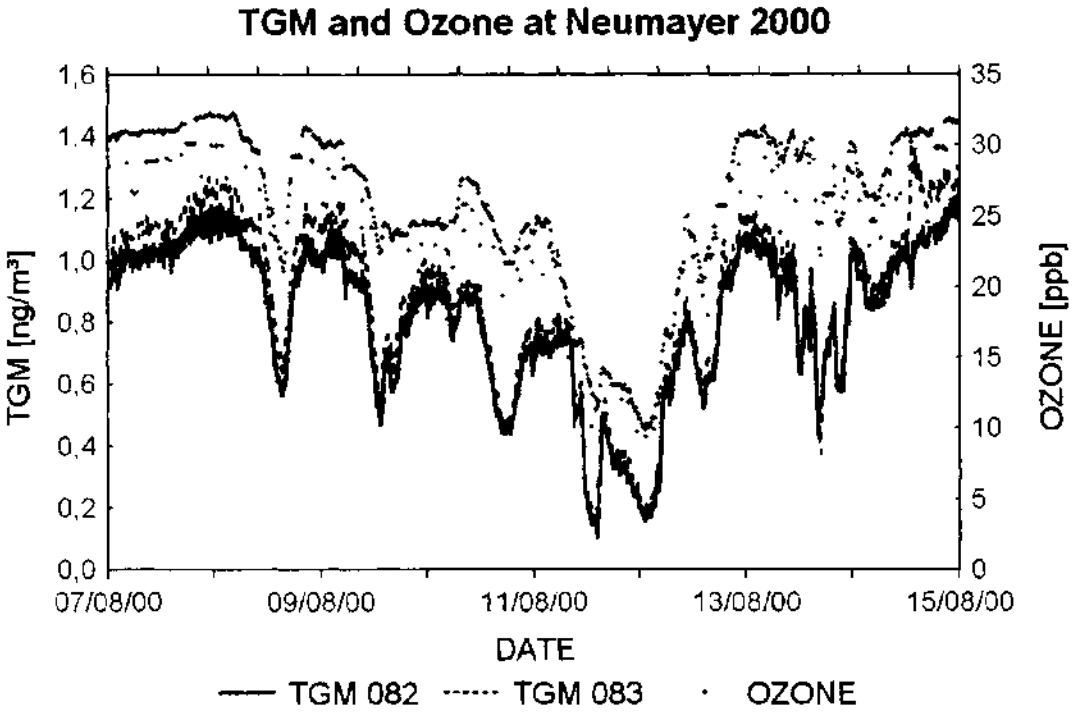


Figure 2: Ozone and TGM concentrations during the first MDE in August 2000 (TGM 082 and TGM 083 are two different Tekran analyzers operating in parallel since the beginning of August).

Statistical analysis of the first depletion event between August 7 - 15 has shown a strong positive correlation (r=0,9205) between ozone and TGM as reported earlier for the Canadian Arctic (Schroeder et al., 1998). The maximum in the crosscorrelation function of ozone and TGM during the first big depletion event in August 2000 occurs at a lag of zero (**Figure 3**). Thus it appears that the suggested reaction between ozone, Br and the resulting BrO and gaseous mercury

(i) must be a very fast reaction which is completed on-site within less than 15 minutes within the sampled air-mass or

(ii) has already been finished before the depleted air parcels are advected to the measurement location.

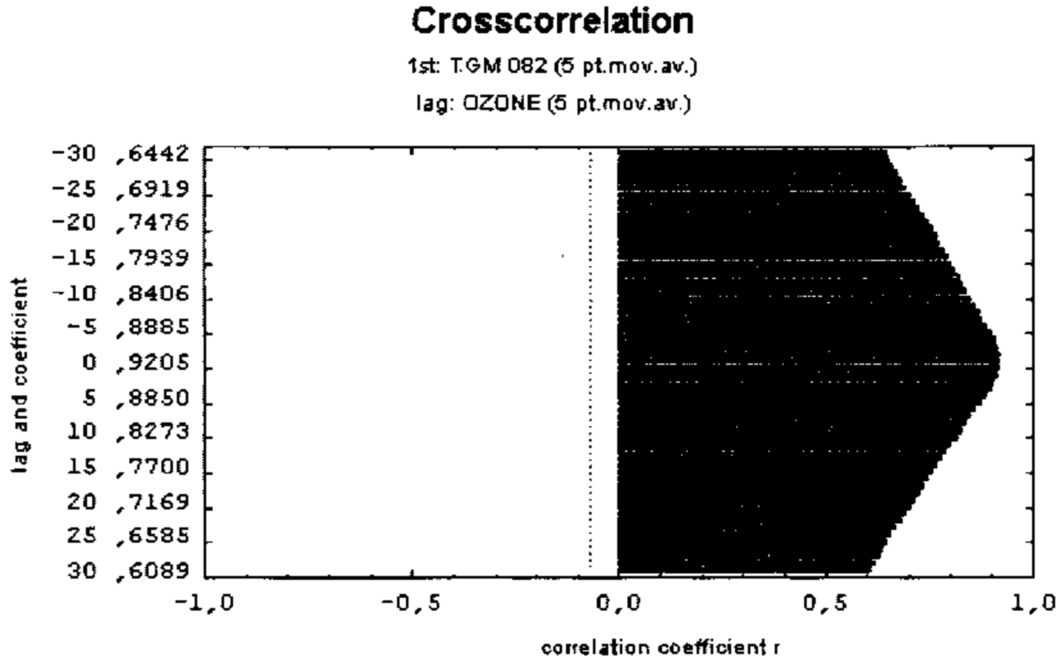


Figure 3: Crosscorrelation function of TGM (from one analyzer) and ground level ozone concentrations during the first depletion event in August 2000. The highest correlation coefficient is found at a lag of zero.

A previous study has given evidence that enhanced BrO concentrations could be detected at Neumayer when the advected air mass has remained relatively long over the sea-ice surfaces (Friess et al., 2000). Further studies are necessary to explain the MDE identified during our measurements in the Antarctic.

The data obtained at Neumayer will be supplemented by results from Canadian and North American groups obtained at the Arctic research stations Alert and Barrow.

Acknowledgements: The authors thank G. König-Langlo and R. Weller of the Alfred Wegener Institute for their assistance in providing the meteorological and supplementary chemical data for the Neumayer Station.

REFERENCES

BOUDRIS, H., BOTTENHEIM, J.W.:, Cl and Br atom concentrations during a surface boundary layer ozone depletion event in the Canadian high Arctic. *Geophys. Res. Lett.*, 27 (4), 517-520, (2000).

BOUTRON, C.F., VANDAL, G.M., FITZGERALD, W.F. AND C.P. FERRARI: A forty year record of mercury in central Greenland snow, *Geophys. Res. Lett.*,25, 3315-3318, (1998).

EBINGHAUS, R., JENNINGS, S.G., SCHROEDER, W.H., BERG, T., DONAGHY, T., GUENTZEL, J., KENNY, C., KOCK, H.H., KVIETKUS, K., LANDING, W., MÜHLECK, T., MUNTHE, J., PRESTBO, E.M., SCHNEEBERGER, D., SLEMR, F., SOMMAR, J., URBA, A., WALLSCHLÄGER, D., XIAO, Z.: International field intercomparison measurements of atmospheric mercury species at Mace Head, Ireland. *Atmospheric Environment*, 33, 3063-3073, (1999).

FRIESS, U., WAGNER; T., PLATT, U.: Spectroscopic measurements of atmospheric bromine oxide at Neumayer Station, Antarctica, Poster presentation, Institute for Environmental Physics, University of Heidelberg, (2000).

SCHROEDER, W.H., KEELER, G., KOCK, H.H., ROUSSEL, P., SCHNEEBERGER, D., SCHAEDLICH, F.: International field intercomparison of atmospheric mercury measurement methods. *Water Air Soil Poll.*, 80, 611-620, (1995).

SCHROEDER, W.H., ANLAUF, K.G., BARRIE, L.A., BERG, T., LU, J.Y., STEFFEN, A., SCHNEEBERGER, D.R: Arctic springtime depletion of mercury. *Nature*, Vol. 394, 331-332, 23 July (1998).

WESSEL, S., AOKI, S., WINKLER, P., WELLER, R., HERBER, A., GERNANDT, H., SCHREMS, O.: Tropospheric ozone depletion in polar regions. A comparison of observations in the Arctic and Antarctic. *Tellus*, 50B, 34-50, (1998).

MERCURY ENVIRONMENTAL AND HUMAN CONTAMINATION IN BRAZILIAN AMAZON; AN OVERVIEW.

Olaf Malm
Laboratório de Radioisótopos EPF, IBCCF,
Universidade Federal do Rio de Janeiro 21949-900, Rio Brasil. olaf@biof.ufrj.br

Key Words: Mercury, Amazon, Air, Urine, Fish, Hair.

Abstract

Gold mining is an economic activity that is coming and going from time to time and from place to place, nowadays mainly over undeveloped and developing countries. A gold rush happens in South American Amazon during the last 25 years. It was occurring mainly in Brazil and nowadays is more spread over other countries like Venezuela, Colombia, Bolivia, French Guyana, Guyana, Ecuador and Peru. Primary Gold mining process use mercury (amalgamation) is known for more than 5,000 years. From mid 1980's, environmental research has been carried out in impacted Amazon rivers, later followed by human exposure studies. Main river basins studied were the Tapajós, Madeira and Negro, but also some man-made reservoirs and areas in central Brazil. Due to more stable aquatic systems special attention will be given to human made reservoirs. Results of our investigations performed with fish and human hair samples will be summarised.

Introduction

Mercury was extensively used in Spanish Colonial South America during colonial time along nearly 300 years, with an annual input 4 times higher than in the recent mining activities but along 300 years). Along the 1980's, Brazil was first gold producer (around 100 to 200 tons per year) in South America and in 1988 (283 tons) second in the world. It is estimated that at least 2 thousand tonnes of mercury has been released to the environment. (Table 1)

TABLE 1

ESTIMATED MERCURY INPUT TO THE ENVIRONMENT DUE TO GOLD AND SILVER MINING.

	Period	Total input (tonnes)	Annual input (tonnes per year)
Spanish Colonial America	1554-1880	196,000	600
Brazilian Amazon	1979-1994	2,300	150

The results show high variation, perhaps related to biological diversity, biogeochemical differences in the river basins and seasonal changes. High mercury values also occur in some areas with no known history of gold mining. The results available document a considerable impact on environmental mercury concentrations and frequent occurrence of human exposure levels that may lead to adverse health effects.

Results

Different samples from distinct goldmining areas have helped understanding the tropical mercury cycle and identified human exposure pathways and possible risks.

As a general result in the case of mercury vapor, resulting from burning or reburning of gold amalgams, the main human exposed group are the gold dealers in the shops rather than the gold miners (*garimpeiros*), who work outdoors. In the case of methylmercury, the riverine populations constitute the group at risk, as they depend on fish as their main source of protein.

The first critical human exposure to mercury results from the burning of the amalgam, when a significant fraction of mercury emissions are occurring mainly to the atmosphere.

Table 2 summarises the main data on mercury in air from studies.

Table 2

MERCURY CONCENTRATION ($\mu g.m^{-3}$) IN AIR FROM URBAN, RURAL OR OCCUPATIONAL EXPOSURES.

Origin / Situation	Average	N	Range	Reference
Rondônia - SW Amazon				
• Urban, far from reburning areas	-	7	<0.02 - <0.66	Malm *et al.*, 1991
• Urban, nearby reburning areas	2.80	8	0.45 - 7.50	
• Occupational exposure during reburning in the dealers shops	71.40	7	17.50 - 107.20	
Poconé City (urban area) - Central Brazil				
• Urban, nearby reburning areas	-	10	<0.14 - 1.86	Marins *et al.*, 1991
Alta Floresta City - South Amazon				
• Occupational exposure during reburning in the dealers shops	5.14	86	0.07 - 40.60	Hacon, 1995
• Urban, far from reburning areas	0.61	152	0.01 - 5.79	

Very high air mercury concentrations are found when burning amalgam in open air. Fortunately this occurs at a low frequency (about once a week) in connection with gold mining. High mercury air values were found also in indoor areas in the gold dealer shops even without reburning activities. In our studies (1992) an average value of 71.50 $\mu g.m^{-3}$ was obtained, i.e., above the 50 $\mu g.m^{-3}$ maximum limit established by WHO (WHO,1991) for occupational exposure. A more recent study (Hacon *et al.*, 1996) showed lower average values, perhaps due to decreased production rates.

Inhaled metallic mercury is readily absorbed through lungs (more than 85 %) and after some time in blood stream, part is oxidised and accumulated in the kidneys (WHO, 1991). The mercuric ion is excreted through urine, which is the best indicator of metallic mercury exposure and inorganic mercury body burden (WHO, 1991).

Reburning of amalgams (bullion) in the gold dealer shops, in cities and villages, contaminates indoor areas and the vicinities. The highest average values were observed in urine samples from people working in indoor conditions with little ventilation or in reburning rooms with air conditioning. Urine from gold shop workers contain mercury mostly in inorganic form (Akagi et al. 1995a). Higher values were also observed in gold traders when compared with miners (Cleary et al, 1994) in several goldmining areas along the Tapajós basin.

On 78 urine samples collected from gold shop workers from Santarém some showed extremely high concentrations in urine (up to 1200 μg/l), very much above the limit of 50 μg/l established by WHO (1991) during the years 1991 and 1992 (median value 115μg/l), but in 1994 (with median value of 5.2μg/l) the concentrations were considerably lower. The reduction of mercury values is a likely consequence of mining decline thus reducing the number of primitive gold shops but perhaps also a fruit of recommendations by a local physician, Dr. Fernando J.P. Branches. In Alta Floresta the values were significantly lower (up to 70 μg/l and median value of 11.7 during the period from 1991 to 1995) but this is a young city with newer, and better ventilated shops (Malm et al. 1997).

Fish is the main source of metilmercury to humans. Piscivorous or carnivorous fish from several goldmining areas show higher mercury concentrations followed by fish from lower trophic levels such as omnivorous, detritivorous and herbivorous species. The former should be monitored as indicators of aquatic system contamination as well as for public health reasons. There are around 200 different fish species of commercial importance in each river basin. Fishing activities vary significantly according to the season (Malm *et al.*., 1995a; Lebel *et al.*., 1997) as well as fish fat and protein contents (Val and Almeida-Val, 1995).

Fish from pristine areas usually present levels lower than 0.2 μg mercury.g^{-1} w.w. in rivers. In contaminated areas predatory fish can reach levels of 2 to 6 $\mu g.g^{-1}$ or even more. The piscivorous and carnivorous group from main mined Amazon river basins usually present mercury average values above the 0.5 µg.g^{-1} maximum limit established by the Brazilian Legislation (Brasil, 1975). Table 3 summarises mercury data on carnivorous and piscivorous fish from Amazon areas.

Table 3

MERCURY CONCENTRATIONS IN CARNIVOROUS OR PISCIVOROUS FISH FROM DIFFERENT AMAZON AREAS

Origin	Number of species	Number of samples	Average (ng.g^{-1}).	Range (ng.g^{-1}).	Reference
• Madeira River	50	370	850	165 - 3920	Malm *et al.*, 1997
• Madeira River	22	154	665	60 - 3960	Silveira, E.G, 1997
• Madeira River	-	251	638	- 11500	Barbosa *et al.*, 1995
• Tapajós River	23	118	498	25 - 5960	Malm *et al.*, 1997
• Tapajós River	12	212	499	46 - 2200	Uryu, 1996
• Tapajós River	19	73	511	132 - 1354	Lebel *et al.*, 1997
• Tapajós River	09	85	723	120 - 3580	Hacon, 1996
• Negro river	18	113	780	226 - 4231	Malm *et al.*, 1994
• Tucuruí Reservoir	8	121	1300	200 - 5900	Porvari *et al.*, 1995
• Balbina Reservoir	6	27	371	49 - 1103	Malm *et al.* 1996b,

In some piscivorous/carnivorous fish species, a tendency for increasing mercury concentrations with increasing fish weight was observed. Even for this group, a high variability was observed even in the same species of fish collected at the same time in the same net (Meili *et al.*., 1997). A mercury seasonal variability was suggested for some piscivorous species but more data is still needed for several species in different river basins or reservoirs.

There are around 200 different fish species of commercial importance in each river basin. Fishing activities vary significantly according to the season (Malm *et al.*., 1995a; Lebel *et al.*., 1997) as well as its fat and protein content (Val and Almeida-Val, 1995).

Human hair is accepted as the best indicator for assessment of contamination in populations exposed to metilmercury (WHO, 1990). Table 4 presents some main results on mercury in human hair samples from several areas. Again in general there is good agreement between the different investigations, and the total number of samples is reasonable representative.

Table 4

TOTAL MERCURY CONCENTRATIONS IN HAIR FROM DIFFERENT AMAZON AREAS

Origin	Number of samples	Average (µg.g^{-1})	Range	Reference
• Madeira river	169	8.98	0.22 - 71	Malm *et al.*, 1996
• Madeira river	242	17.2	- 303	Barbosa *et al.*, 1995; Boischio *et al.*, 1995
• Tapajós river	432	16.76	0.7 - 176	Malm *et al.*, 1996; Akagi *et al.*, 1995b
• Tapajós river	96	13.2	1.0 - 142	Lebel *et al.*, 1997
• Kayapó Indians	419	8.00	- 37	Barbosa *et al.*, 1995
• Negro river	154	75.5	- 171	Forsberg *et al.*, 1994
• Tucuruí reservoir	125	35	0.9 - 240	Leino & Lodenius, 1995
• Balbina Reservoir	58	5.78	1.15 - 26	Malm *et al.*, 1995
• Yanomami Indians	162	3.61	1.40 - 8.14	Castro *et al.*, 1992

Several groups present average values within the range associated to a risk to pregnant women (WHO, 1990). At the same time a high variability (as in fish) was observed in hair mercury even within a family or a group living in the same household. In general, a relation between hair mercury and fish ingestion is observed (Grandjean et al, 1993) but variability in the same family maybe high. High variability was also observed along strands of human hair (Akagi *et al.*, 1994, Kehrig *et al..*, 1997) from the same house what could be explained by seasonal changes but also by high variability observed in fish.

Discussion

Recent mercury emissions in the Amazon are important because of the new environmental circumstances of mercury releases rather than because of their magnitude. The complex ecosystem associated with tropical rain forest river basins with its enormous biodiversity is the new challenge for the understanding of mercury cycle and evaluate mercury health risks.

In Brazil estimation of releases of mercury per (area or) river basins does not correlate with the levels found in biota. The Tapajós river basin has been prospected for a longer time than the Madeira (30 and 15 years, respectively), but mercury concentrations in fish of the same species and weight range are higher in the latter basin. Elevated concentrations are found in the Tapajós river basin only in its upper reaches. The Negro river with no significant history of gold gold mining activities presents quite high values in fish samples.

Since no input of mercury is known for sure, the high levels of mercury in the Negro river samples could be due to certain natural processes contamination some centuries ago. Where the mercury released during Spanish Colonial time is being nowadays is still a question. The importance of forest burning on mercury emissions has also been a matter of discussion (Veiga, 1997).

Changing in fish alimentary habits in riverine populations is a factor that must be considered in human risk evaluation. Nevertheless clinical evaluation (if possible showing changes from the past) must support any decision on this respect. Attention must be paid to avoid political and dishonest bad use of this information.

Atmospheric mercury investigations are necessary for better understanding of the dispersion and deposition of mercury in the surroundings of urban and rural areas in Amazon (Malm *et al.*, 1996a). Further, better knowledge on mercury methylation and the rate controlling factors is essential for understanding food chain contamination in this tropical environment.

Concentration of metilmercury in sediments, water and fish is probably influenced by several parameters, such as mercury concentration, microbiological activity, organic matter, presence of methyl group donors, pH, redox potential and O_2 among others.

The experiments done so far indicate detectable methylation in bottom sediments of rivers such as the Madeira and Tapajós, and higher rates in forest streams. The highest methylation rates were recently found in environments that are typical of tropical aquatic systems, such as the surface of seasonally flooded forest soils and specially, the root zone of the "floating meadows" formed by the aquatic vegetation, were up to 35 % of added mercury chloride was converted to metilmercury after short term experiments (Guimarães *et al.*, 1997).

The high variability of mercury values observed in carnivorous or piscivorous fish is making it difficult to generate advice on fish ingestion to the local population. Maybe a small number of top predator species could be blacklisted, but decisions must be made area by area and reviewed regularly.

High high-mercury values were observed in several individual cases as well as in some populations. If neurological damage occurs (Lebel et al., 1996), its extent deserves to be documented to provide a deeper understanding of health risks and as a basis for dietary recommendations.

References

Akagi,H., Kinjo,Y., Branches,F.J.P., Malm, O., Harada, M., Pfeiffer,W.C. and Kato,H. (1994) Methylmercury pollution in Tapajós river basin, Amazon. - *Environ. Sciences* 3(1),25 -32

Akagi H, Malm O, Branches F J P, Kinjo Y, Kashima Y, Guimaraes JRD, Oliveira RB, Haraguchi K, Pfeiffer WC, Takizawa Y and Kato K (1995a) Human exposure to mercury due to goldmining in the Tapajos River basin, Amazon, Brazil: Speciation of mercury in human hair, blood and urine. Water Air Soil Pollut 80:85-94.

Akagi, H., Malm, O., Kinjo, Y., Harada, H., Branches, F.J.P., Pfeiffer, W.C., and Kato, H. (1995b). Methylmercury pollution in the Amazon, Brazil. *Sci. Total Environ.* 175(2), 85-95.

Barbosa, A.C., Boischio A.A. East, G.A., Ferrari,I., Gonçalves, A., and Silva, P.R.M. and Da Cruz, T.M.E.(1995) Mercury contamination in the Brazilian Amazon. Environmental and occupational aspects. *Water Air and Soil Pollution*, 80, 109-121.

Boischio, A. A., Heenshel, D., and Barbosa, A. C. (1995). Mercury Exposure Through Fish Consumption by the Upper Madeira River Population Brazil-1991. *Ecosystem Health* 1(3), 177-192.

Brasil (1975). Ministério da Saúde, Resolução No. 18/75 da Comissão Nacional de Normas e Padrões para Alimentos. *In* "Diario Oficial da União" Brasilia 9 de Dezembro de 1975. Seção 1 pp.16378

Castro, M.B., Albert, B., and Pfeiffer, W.C.. (1991) Mercury levels in Yanomami indians hair from Roraima, Brazil. *Proc. 8th Intern. Conf. Heavy Metals in the Environment.* Ed. Cep Consultants, Edinburgh, UK, 1: 367-370.

Cleary, D, Thornton, I, Brown. N., Kazantzis, G., Delves, T., Worthington, S. (1994) Mercury in Brazil. Nature - Vol. 369, 613-614.

Forsberg, B.R., Forsberg, M.C.S., Padovani, C.R., Sargentini, E. and Malm, O. (1995) High levels in fish and human hair from the rio Negro (Brazilian Amazon): Natural background or anthropogenic contamination. Proc. Int. Workshop on Environmental mercury Pollution and its health effects in the Amazon river basin, pp33-40 (Eds.) Nat.Inst. for Minamata Disease/ Federal University of Rio de Janeiro.

Grandjean, P., Cardoso, B., Guimarães, G. (1993). Mercury poisoning. Lancet 342:991.

Guimarães, J.R., D, Malm, O. & Meili, M. (1997) mercury methylation in sediments and floating meadows of a tropical lake of the Pantanal wetland, Brazil. *Sci. Total Env.*, in press

Hacon, S., Artaxo, P., Campos, R.C. Conti, L.F.and Lacerda L.D. (1995) Atmospheric Mercury and trace elements in the region of Alta Floresta in the Amazon Basin. *Water ai and Soil Pollution.* 80:273-283.

Kehrig, H.A., Malm, O., and Akagi, H. (1997). Methylmercury in hair samples from different riverine groups, Amazon, Brazil. *Water Air Soil Pollut.* "in press".

Lacerda, L. D. and Salomons, W. (1997). The Environmental Impact of Mercury from Gold and Silver Mining. *Springer Verlag.* (In press) 300pp

Lebel, J., Mergler, D., Lucotte, M., Amorin, M., Dolbec, J., Miranda, D. Arantès, G., Rheault, I., and Pichet, P. (1996) Evidence of early nervous system dysfunctions in Amazonian populations exposed to low-levels of methylmercury. *Neurotoxicology* 17(1): 157-168.

Lebel, J., Roulet, M, Mergler, D., Lucotte, M. and Larribe, F., (1997) Fish diet and mercury exposure in a riparian amazonian population. *Water, Air and Soil Pollution*, (In press).

Leino, T. and Lodenius, M. (1995). Human Hair Mercury levels in Tucuruí Area, State of Pará, Brazil. *Sci. Total Environ.* 175(2): 119-125.

Malm, O., Akagi, H., Guimarães, J.R.D., Bastos, W.R., Costa, L.S. & Torres, J.P.M. (1996b) Data on mercury and methyl mercury in environmental and human samples from new study areas in Brazil: Balbina Reservoir and Poconé (Pantanal wetland) *International workshop on the fate of mercury in gold mining and measures to control the environmental pollution in various countries*, Jakarta, Indonesia, 25-27 November 1996, Abs 2-3.

Malm, O., Branches, F.J.P., Akagi, H., Castro, M.B., Pfeiffer, W.C., Harada, M., Bastos, W.R., and Kato, H. (1995a). Mercury and Methylmercury in fish and human hair from the Tapajós river basin, Brazil. *Sci. Total Environ.* 175(2) 141-150

Malm, O., Castro, M.B., Bastos, W.R., Branches, F.J.P., Guimarães, J.R.D., Zuffo, C.E., and Pfeiffer, W.C. (1995b) An assessment of Mercury pollution in different goldmining areas, Amazon Brazil. *Science of Total Environment.* 175(2) 127-140

Malm, O., Fonseca, M.F., Hissnauer, P.M., Bastos, W.R and Pinto, F. N. (1996a) Use of epiphyte plants as biomonitors to map atmospheric mercury in a gold trade center city, Amazon, Brazil. *Fourth International Conference on Mercury as a Global Pollutant*, Hamburg, Aug. 1996.

Malm, O., Guimarães, J.R.D. and Pfeiffer W.C. (1993) Accumulation of metallic mercury and natural amalgams findings in Madeira river basin bottom sediments, Amazon. In: Abrão, J.J., Wasserman. J.C. and Silva-Filho, E.V.(eds.) *Proc. of Perspectives for Environmemtal Geochemistry in Tropical Countries*. Niterói, Brasil. p. 391-393.

Malm, O., Guimarães, J.R.D., Castro, M.B., Bastos, W.R, Viana, J.P., Branches, F.J.P., Silveira, E.G., and Pfeiffer, W.C. (1997). Follow-up of mercury in fish, human hair and urine in Madeira and Tapajós basins, Amazon, Brazil, *Water Air Soil Pollut.* 97: 45-51.

Malm, O., Pfeiffer, W.C. e Souza, C.M.M. (1991) Main pathways of mercury in the Madeira river area, Rondonia, Brazil. *Proc. of the VIII International Conference Heavy Metals in the Environment*, Edimburg, UK. p.515-518.

Malm, O.; Castro, MB; Branches,FJP; Zuffo, CE; Padovani, CR; Viana, JP; Akagi, H; Bastos, WR; Silveira, EG; Guimarães, JRD;and Pfeiffer (1994) Fish and human Hair as biomonitors of mercury contamination on Tapajós, Madeira and Negro river basins, Amazon, Brasil.- Proc. Int. Workshop on Environm.Mercury pollution and its Health effects in Amazon River Basin. R.J. 30 nov.-2dez. p. 25 - 32

Marins, R.V., Imbassahy, J.A., Pfeiffer, W.C., and Bastos, W.R. (1990) Preliminary study on mercury contamination in the urban atmosphere of a gold producing area in Poconé, Mato Grosso (MT) In: *First Int. Symp. Environm. Stud. Tropical Humid Forests*, Manaus, Brazil. 6p.

Pfeiffer, W.C. and Lacerda, L.D. (1988). Mercury Inputs into the Amazon Region, Brazil. *Environ. Technol. Lett.* 9, 325-330.

Pfeiffer, W.C., Lacerda, L.D., Salomons, W. and Malm, O. (1993) Environmental fate of mercury from goldmining in the Brazilian Amazon. *Environ. Rev.* 1:26-37.

Porvari, P. (1995). Mercury levels of fish in Tucuruí hydroelectric reservoir and in river Mojú in Amazonia, in the state of Pará, Brazil. *Sci. Total Environ.* 175(2), 109-117.

Uryu, Y. (1996) Mercury and fish in the Brazilian Amazon: A study in the Tapajós River. Master thesis. University College, London, UK.

Val, A. L., and Almeida - Val, V.M.F. (1995). The Amazon Ichthyofauna. *In* "Fishes of the Amazon and their Environment" (S.D. Bradsaw, W. Burggren, H.C. Heller, S. Ishii, H. Langer, G. Neuweiler and D.L. Randall Eds), pp. 28-66. Springer-Verlag Berlin Heidelberg.

Veiga, M.M. (1997) Introducing New Technologies for abatement of Global Mercury Pollution, Phase II: Latin America. *Report prepared for UNIDO.* 70p.

WHO (1990). Methylmercury. *Environmental Health Criteria* 101, *Geneva*, World Health Organisation.

WHO (1991). Inorganic Mercury. *Environmental Health Criteria* 118, *Geneva*, World Health Organisation.

PROCEEDINGS OF

NIMD Forum 2001

平成13年3月31日発行

発行　環境省　国立水俣病総合研究センター
Published by National Institute for Minamata Disease

〒867-0008　熊本県水俣市浜4058番18号
TEL: 0966-63-3111
FAX: 0966-61-1145

4058-18 Hama, Minamata City, Kumamoto 867-0008, Japan
TEL: +81-966-63-3111
FAX: +81-966-61-1145

E-mail address: kokusai@nimd.go.jp
Web Address: http://www.nimd.go.jp

March 2001

問い合わせ先：国際・総合研究部
Inquiry: Department of International Affairs and Environmental Sciences