NIMD

PROCEEDINGS OF

NIMD FORUM '97

Date: 30-31 July, 1997
Venue: National Institute for Minamata Disease,
Minamata City, Japan

Organized by National Institute for Minamata Disease, Japan

PROCEEDINGS OF

NIMD FORUM '97

Date: 30-31 July, 1997
Venue: National Institute for Minamata Disease,
Minamata City, Japan

Organized by National Institute for Minamata Disease, Japan

Preface

Mercury and its compounds occur naturally in the environment but their use by human kind can lead to high local concentrations which represent a serious risk to health. Although the hazard of mercury compounds has a long history, the most significant incidents from the scientific and epidemiological points of view have been those in Japan at Minamata and Niigata. In consequence, the Japanese experience allows the most rational analysis of the health risk to the community of methylmercury poisoning that can be made to date.

In October, 1995, more than 40 years after the Minamata disease was officially recognized, a political resolution was at last achieved. Japan has learned a modest lesson from the tragedy of the Minamata disease, and both the government and citizenry are enacting measures and systems to share Minamata's experience and technology-based improvements with the world in an earnest effort to make an international contribution.

One of the salient features of the NIMD Forum '97 is the opportunity for representatives of industry, government, and academia to meet at an international conference and to exchange ideas and information to extend international cooperation to the study of the dynamic aspects of mercury-related issues. Our NIMD was designated as a WHO collaborating Center in 1986. Fortunately, a Department of International Affairs and Environmental Sciences was added to our institute in 1996. We have already launched cooperative efforts with countries such as Brazil, China, Indonesia and Tanzania that are concerned about mercury pollution. This meeting was held in commemoration of the opening of the International Research Collaborating Facilities in NIMD.

The forum was indeed an excellent opportunity to bring together many of the very active and experienced researchers in this field. Keynote presentations by the World Health Organization (WHO), the International Atomic Energy Agency (IAEA) and the WHO Collaborating Center on Global Health Network (University of Pittsburgh) were intellectually stimulating and led to very meaningful discussion. Also fourteen presentations provided us with some knowledge, experience and information on the recent improvements in mercury-related problems.

It is my sincere hope that these Forum Proceedings will be useful in research efforts in all of these important areas as a stepping stone to expanding cooperative studies.

Yukio Takizawa, M.D.
Chairman
NIMD Forum '97 Organizing Committee
Director-General
National Institute for Minamata Disease

Welcome Address

Mr. Takao Ohnishi
Vice Minister, Environment Agency of Japan

Distinguished participants, ladies and gentlemen, it is my pleasure to be with you here and have an opportunity to say a few words at the opening of NIMD Forum '97. First of all, on behalf of the Environment Agency, I would like to thank all of you, especially those who have traveled a long way from abroad, for your participation to the forum.

It has passed forty years since the first case of Minamata Disease was reported in this region. During this 40 year period, a lot of effort has been put to solve the problem. Concerning the relief of the people suffering from the disease, political decision was made in 1995 to solve this issue ultimately. Effort has also been done to clean up polluted marine environment. Now the levels of mercury in fish and shellfish are below the standards.

On the other hand, we have to continue and strengthen our research in medical science and other scientific areas. Although we do not see evident mercury pollution in Japan anymore, environmental pollution by mercury is reported in other countries and it is concerned that some of them might be causing damage on the environment and human health.

The National Institute for Minamata Disease will reach the twentieth anniversary of its establishment next year. Taking into account these conditions on Minamata Disease and mercury pollution, it has to take on a greater role. The Institute will compile lessons from the research on Minamata Disease and disseminate the information to the world, in order that the tragedy we experienced in Japan will never occur anywhere in the world.

This Institute needs to strengthen information exchange with experts in the world to improve the quality of its studies. I do hope that this forum will be a valuable opportunity for this purpose.

Special Speech

Sukio Iwatare

Former Minister, Environment Agency of Japan

Thank you for your most kind introduction. I am very pleased to say a few words at the opening of this NIMD Forum '97.

As Dr. Takizawa, Director General of the Institute, mentioned a moment ago, forty years have now passed since Minamata disease was officially discovered, and with the final resolution proposed by the ruling and other parties, we have indeed reached a turning point.

As someone who has been personally involved, I have many different feelings now mixed with a sense of sadness. I visited Minamata last year in May to attend the Memorial Service for the souls of so many dear departed who fell victim to this disease. I was once more struck by the enormity of the suffering caused by Minamata disease.

As you know, today there are still many patients with Minamata disease in Japan who need treatment. And around the world there are not a few areas afflicted with mercury poisoning. Thus, the clarification of this situation and the elimination of this contamination are greatly to be desired.

When directly confronted by the gravity of this situation, we begin to realize the huge role this Center must play.

It is Japan's duty to make an international contribution by recognizing and empathizing with the pain inflicted on the minds and bodies of each and every one of you victims, and then make this painful lesson something the whole world can learn from.

Today, the second day of the Forum, is the first international milestone for this new Center.

It is my heartfelt hope that the success of this memorable Forum will mean "No more Minamatas" and serve as one of the Center's driving forces in the days to come. We look forward to lively discussion among all you participants.

Organizing Committee

Yuzo Suzu
Department of International Affairs and Environmental Sciences
National Institute for Minamata Disease

Yutaka Mori
Department of Administration Division
National Institute for Minamata Disease

Komyo Eto
Department of Clinical Medicine
National Institute for Minamata Disease

Akira Yasutake
Department of Basic Medical Sciences
National Institute for Minamata Disease

Ken-ichiro Miyamoto
Department of Clinical Medicine
National Institute for Minamata Disease

Editorial Board

Agenda

Welcome Remarks ***Yukio Takizawa***
National Institute for Minamata Disease (NIMD)
Takao Ohnishi
Vice Minister, Environment Agency
Sukio Iwatare
Former Minister, Environment Agency

Session I

Chairperson: ***Hiroo Kato*** (Former Director General, NIMD)

1) Prospects for Future Collaboration Between WHO and NIMD
Tord Kjellstrom
Office of Global and Integrated Environmental Health, WHO
2) Expectations for NIMD: Outbreak of Minamata Disease and the Responses of Company, Administration and Local Community
Sadami Maruyama
Department of Sociology, Faculty of Letters,
Kumamoto University
3) How to Use the Internet to Access Information on Minamata Disease
- Global Health Network - (Video)
Ronald E. LaPorte and ***Akira Sekikawa***
Department of Epidemiology, University of Pittsburgh, USA
4) National Institute for Environmental Studies (NIES) and Ethical Grounds of Its International Collaboration
Gen Ohi
Deputy Director General, NIES
5) Recent International Collaborative Research Activities on Mercury in NIMD
Hirokatsu Akagi
Department of International Affairs and Environmental Sciences,
NIMD

Session II

Chairperson: ***Tsuguyoshi Suzuki*** (Former Director General, NIES)
Makoto Futatsuka (School of Medicine, Kumamoto University)

6) Cooperative Studies on Mercury pollution Caused by Guizhou Organic Chemical Factory in Environment by China and Japan

Liya Qu

Guizhou Institute of Environmental Sciences, China

7) Environmental Research Activities by Environmental Management Center (EMC)

Bagus Bina Edvantoro

Toxic Substances Laboratory EMC, Indonesia

8) Mercury Biogeochemistry in the Amazon: History, State of the Art and Future Research Needs

Luiz Drude de Lacerda

Department of Geoquimica, Universidade Federal Fluminense, Brazil

9) The Mercury Problem and the Solutions Found in the Brazilian Amazon

Roberto C. Villas Bôas

CETEM/ CNPq - Center for Mineral Technology, Brazil

10) Analytical Quality Control for the Determination of Organomercury compounds in Environmental Samples

Milena Horvat

Department of Environmental Sciences, Jozef Stefan Institute, Slovenia

Session III

Chairperson: ***Nobumasa Imura*** (School of Pharmaceutical Sciences, Kitasato University)

Shoji Tsuji (Brain Research Institute, Niigata University)

11) IAEA Programs on Human Exposure to Mercury Pollution

Robert Parr

Nutritional and Health-Related Environmental Studies Section

International Atomic Energy Agency, Austria

12) Perspectives on Atmospheric Mercury and Its Fate in Aquatic Ecosystems

Donald B. Porcella

Electric Power Research Institute, USA

13) The Biochemistry and Genetics of Bacterial Mercury Resistance

Hidemitsu Hou

Faculty of Pharmaceutical Sciences, Setsunan University

14) Possibility of a Measurement Method of Toxicity to the Germ - Line by Methylmercury Exposure

Takashi Kuwana

Department of Basic Medical Sciences, NIMD

Session IV

Chairperseon: ***Chiharu Tohyama*** (Environmental Health Sciences Division, NIES)

Komyo Eto (Depertment of Clinical Medicine, NIMD)

15) Postnatal Effects of in Utero Exposure to Methylmercury; with Emphases on Co-exposure to Heat or Selenium Deficiency

Hiroshi Satoh

Graduate School of Medicine, Tohoku University

16) Effects of Methylmercury on Glutathione Metabolism

Akira Yasutake

Department of Basic Medical Sciences, NIMD

17) Role of Glutathione and γ-Glutamyltranspeptidase in Renal Uptake of Mercury Compounds

Akira Naganuma

Faculty of Pharmaceutical Sciences, Tohoku University

Closing Remarks ***Komyo Eto***

Contents

PROSPECTS FOR FUTURE COLLABORATION BETWEEN WHO NIMD

Tord Kjellstrom

Director, Office of Global and Integrated Environmental Health,
World Health Organization, Geneva, Switzerland

Abstract

The outbreak of Minamata Disease was an important landmark in the global history of environmental health impacts and public health science. This most unfortunate pollution catastrophe with hundreds of became a warning to the whole world of the serious effects of poorly controlled industrial development. By the establishment of the National Institute for Minamata Disease (NIMD) the community of the Minamata City and Japanese government agencies created the possibility to investigate in detail the health effects of methylmercury, the methods for treatment of the victims and approaches to preventing health impacts of mercury pollution in other places.

Mercury is still one of the most important environmental pollutants at global level and WHO is continuing to monitor the global situation in this field and to provide advice to countries that experience mercury pollution and related health effects. The continued collaboration from the NIMD in this respect is very much appreciated. Examples of countries from which significant mercury pollution has been reported include Brazil, the Philippines, and Tanzania. Often the source of mercury pollution is gold mining. NIMD support and advice in the development of chemical analysis method, research designs, biological monitoring, and diagnosis and treatment approaches for victims is of great value.

The most important health effect of methylmercury is brain damage, which can occur in children and adults as well as before birth id a pregnant woman is exposed to methylmercury. Other chemical pollutants, such as lead, organic solvents and certain pesticides, also cause brain damage. The expertise and research at NIMD could contribute to solving some of the remaining questions concerning the brain damage caused by different environmental pollutants. Some chronic brain diseases, such as Alzheimers disease, have been suspected to be at least partly caused by environmental factors. Epidemiological and bio-medical research at NIMD could help WHO identify important links between environmental pollution and brain disease. The research could also extend to all aspects of aging and health of the brain and the nervous system and their relationship to environmental exposures during earlier stages of life.

The WHO programmes on Environmental Health and Chemical Safety produce guidelines on how to protect health from environmental pollutants scientific assessments of the health impacts of different pollutants and training materials on different topics in environmental health. Often these materials are prepared by Collaborating Centers, such as NIMD, and the materials are finalized at expert meetings, often hosted by Collaborating Centers. WHO and its' Collaborating Centers also provide advice on how to deal with specific problems and we organize training courses, often for developing country participants, on different aspects of environmental health science. NIMD could make a significant contribution on WHO programmes through support of these activities.

Expectations for the National Institute for Minamata Disease (NIMD): Outbreak of Minamata Disease and the Responses of Company, Administration and Local Community

Sadami Maruyama

Professor, Dept. of Sociology
Faculty of Letters, Kumamoto University

Key words
compensation, local community, discrimination

Abstract

The main issues involved in the late Fifties, when the great Minamata incident occurred, may be summarized as follows.

1. Failure to control damage

   Although it had become clear that toxic substances had entered the food chain through fish and other marine life, the Kumamoto Prefectural government only asked for restraint on the part of fisheries in fishing and selling their catch. The pretext used was the Ministry of Health and Welfare's admonition as to the virtual impossibility of legally bringing fishing to a halt.

   In 1957, a research group at Kumamoto University indicated that plant wastewater discharge was the likely culprit (source of contamination). The Chisso Co. then decided to move their wastewater spillage point from Minamata Bay to the Minamata River mouth side, which only caused more contamination. The Japanese government did not stop the wastewater drainage so as not to pose a hindrance to its chemical industry plan.

   In mid-1959, the Kumamoto University study group set forth their organic mercury theory, but the wastewater tide was not stopped because the cause was said to be insufficiently clear. It was only in 1968, after the second outbreak, that the government published its official confirmation of Minamata disease.

2. Problem of compensating victims and fishing community

   Initially, Chisso Co. would not take responsibility for the damage caused and contracted to pay a small amount as a token of sympathy. But after the government confirmed officially that Chisso Co. was in fact the cause, patients sought just compensation, and an agreement was reached in 1973 following negotiations in and out of court. However, some patients were outside the compensation system.

3. Victims oppressed by local community

   Victims were first despised as having some kind of "strange disease"; they were rejected by the community and suffered discrimination. As the local image deteriorated, jealousy took root, tying the hands of the local community and government in dealing with Chisso. This only made the solution more difficult.

   Much of this remains to be elucidated in order to draw some lessons, and we count on NIMD to undertake this research. The results are bound to benefit not only Japan but developing countries, and provide valuable input for global environmental measures.

Preface

When the Minamata disease outbreak was confirmed, the measures required were prevention of its further spread and relief for its victims. But neither was done. It is fair to say that this neglect

in the late 1950s made a solution to the problem very difficult. Besides the outbreak of Minamata disease itself, we here examine the involvement of politics, government, society, and medicine, along with natural sciences, during the period. Looking to NIMD to make this a lesson for the future, we give here a comprehensive view of the times in retrospect.

1. Failure to control damage

(1) Failure to stop fishing operations

When it became clear that toxic substances had entered the food chain through fish and other marine life, fishing for the contaminated species should have been immediately stopped. However, the Kumamato Prefectural government merely requested fisheries to cut back on fishing and sales, falling back on the Ministry of Health and Welfare's claim that fishing could not be legally prohibited because there was no proof "that all fish and marine life in the bay are contaminated." Thus, no definite public warnings were given as to the danger of contaminated fish and marine life, and these contaminated catches continued to be distributed in the area so as to create further damage from Minamata disease.

(2) Failure to stop wastewater discharge

Even before the disease had been confirmed, fishery operations had begun to decrease in Minamata Bay. People engaged in fishing began to suspect some connection with factory wastewater discharge. Thus, when a study group at Kumamoto University reported that Minamata disease came from eating contaminated fish, and the source of this contamination was industrial wastewater discharge, the fishing community demanded the discharge be stopped, the water cleaned up and the sludge removed.

As early as 1957, the fishing community issued two demands to the Chisso Co.: put a stop to the discharge of the contaminated wastewater into the sea, and allow drainage into the sea only after processing by purification systems and demonstrating that all efflux is nontoxic.

The fishing community had struck upon a reasonable way to prevent contamination and assure safety. Had their demands been honored, the sources of the contamination would have disappeared and at least the subsequent wider damage avoided. Chisso, however, rejected these demands and made no substantial attempt to deal with the wastewater problem.

Then, in 1958, Chisso moved its discharge point for acetaldehyde from Minamata Bay to the mouth of the Minamata River to the north. No sooner was this done than Minamata disease patients appeared along the mouth of the river as well. And the Shiranui Sea became all the more contaminated. Chisso should have realized at this juncture that the causative substance was contained in the wastewater from its processing, but the discharge continued.

In 1959, panic spread along the coast of the Shiranui Sea. The fishing community clamored for Chisso to shut down its operations until cleanup facilities were completely in place, and launched a huge protest. On its part, the Ministry of International Trade and Industry (MITI) knew it had to find a resolution to this wastewater problem in a social perspective so as to allow Chisso to continue operating and minimize adverse effects on other chemical industries of this kind in Japan. Thus, MITI made Chisso stop all discharge at the mouth of the Minamata River, where more and more new patients were being found, and also required the company to complete its purification systems as early as possible.

In this situation, Chisso was forced to take some steps for all to see. The completion of its two wastewater processing facilities in 1959 ostensibly did this, but neither, as Chisso knew, could remove organic mercury compounds that had dissolved into the water.

Chisso advertised widely that the contamination was gone, even though the purported wastewater cleanup had no effect whatsoever on the actual contaminated substances. The fishing community and the community at large were deceived into believing the toxic substances were no longer being discharged, and so the fear of contamination disappeared. Instead of the contaminant problem, the issue then shifted to redress of damages among the fishing folk who had insisted the wastewater discharge be brought to a halt; compensation became the center of attention, and it was believed that the Minamata disease problem would be solved once and for all by resolving the compensation matters.

The government made no attempt to stop the wastewater discharge on the grounds that the source of contamination and the mechanism triggering it were not yet fully understood, and it did not wish to obstruct its plan for the national chemical industry sector by shutting down Chisso.

(3) Floundering efforts to confirm causes and political intervention

The research team at Kumamoto University, which had been requested to elucidate the origin(s) of the disease, was early to point out the factory wastewater discharge by Chisso for investigation. However, with no cooperation between the company and the government, there was no direct survey of the wastewater; instead, only a series of laboratory animal experiments was repeatedly conducted. To prevent the contamination from becoming widespread, the first step was thought to be proving that a substance contained in the factory discharge had entered the food chain through fish and marine life, and induced toxicity in the human body.

At that stage, if the discharge had been stopped, at least the subsequent spread of the contamination would be prevented.

Nonetheless, both the government and Chisso maintained their position that unless a specific substance is pinpointed as the cause, the true cause had not been identified. Researchers had to follow this logic and mainly repeated their trial-and-error testing of various toxic substances.

The research group, in their studies up till around the middle of 1959, finally announced that the causative substance was a kind of organic mercury compound. Since they could think of no other source of this compound than Chisso discharge, the patients and fishing community took Chisso to be the source of the contamination. It was time to stop the wastewater discharge in their view.

But Chisso and the government continued to argue that the cause can not be said to have been confirmed until the exact kind of organic mercury compound has been clearly identified, and the mechanism generating the disease has been carefully elucidated. Thus, the wastewater discharge was not halted right away.

Meanwhile, it was during the same year that Chisso's own in-house research, as it discovered later, made a considerable breakthrough with regard to the cause of Minamata disease. Cats administered wastewater directly from the acetaldehyde process developed the disease. This should have made it clear that wastewater from said process was indeed the source, and detailed identification of the causative substance would have been within reach. However, Chisso was careful not to disclose these findings since it would confirm that the company itself was the source of the contamination.

Thus, with the stage set for finally pinpointing the problem, in December of the same year the issue of compensating both patients and fisheries was settled, making it appear for all intents and purposes that the social problem of Minamata disease had been resolved. Thus, the social and political interest in the cause rapidly declined, and it was not until 1968, twelve years later, that the pathogenesis of Minamata disease was publicly confirmed.

It was because of the long years it took for the government to officially confirm the cause that victims failed to be justly compensated. And since government regulation of wastewater discharge did not go into effect, the second wave of Minamata disease resulted.

2. Compensation for victims and fishing community

Relief for the victims posed yet another problem for Chisso and the government. But since they failed to undertake the necessary measures, there had been a long confrontation between the victims and the company and government. At first, it was very difficult for Chisso to compensate victims. The company eventually gave in to a small solatium agreement but would not acknowledge its responsibility.

Yet later, when the government officially confirmed that Chisso was indeed the party responsible, the victims once more rose up to claim just compensation. Through the first trial in court and subsequent negotiations, in 1973 they finally succeeded in reaching an agreement with the company to establish the present compensation system, which incorporated some of their demands.

This was 17 years after Minamata disease had been officially recognized.

However, patients have to be officially recognized by the government as victims of the disease in order to be compensated by the present system. Therefore, victims who have not, for various reasons, been given certification of whose position has been rejected, have not been compensated as being outside the system.

3. Victims oppressed by local community

The local community put much pressure on the victims. The "strange disease" was simply unknown to people in the community, and it caused great fear. For them the disease was of unknown origin, but since it largely developed in families of the fishing community, they took it to be infectious. Patients became outcasts from the friendly communal life they had known, and families with such patients became isolated from the neighboring communities.

When it became clear that the disease originated from organic-mercury contaminated seafood, the fishing cooperatives in districts in which patients had not yet been "officially" recognized tried to hide their presence. This only served to further isolate patient families from the community.

Then, once Chisso became the target of the investigation, the company did all it could to suppress it. Chisso drew on the sense of solidarity among the local population who had allegedly benefited from its presence to make people look on the victims and their families as veritable enemies. Anyone seeking to investigate the company's responsibility, for whatever reason, was regarded as putting the commonweal at risk and therefore to be excluded as a kind of heretic or threat. Moreover, when Minamata disease surfaced, business declined and the place gained a bad image. People even began to feel victimized by the patients themselves and turned on them as if they were the perpetrators.

Since the local community failed to take timely and definite steps against Chisso, the cause of the problem, all of the measures taken by the company, the government, and the local community were too late. This only made the problem worse and more difficult to resolve.

Although we have summarized the major problems which occurred during the most important phase of the Minamata disease problem, much remains unresolved. The real work of extracting the meaning and drawing an overall lesson from the Minamata incident is just beginning from the standpoint of the natural and social sciences. This is the task we expect the NIMD to undertake.

The results will be reflected first of all in policies from the Japanese Government to assure the safety of its people. At the same time, it will go a long way toward assisting industrialization measures in developing countries and helping them deal with environmental problems on a global level.

National Institute for Environmental Studies (NIES) and Ethical Ground of its International Collaborative Research

Gen OHI, M.D., Ph. D.
Deputy director General, NIES

Abstract

From the standpoint of evolutionism, ethics may be construed as, at the group level, the strategic standards enabling the optimal continuation of the group, and at the individual level, behavior patterns that would maximize the survival of the individual as a constituent of the group. Ethical sentiment, then, represents the expression of the strategic standards for survival which are internalized in the individual and in the group. In many cultures, environment or nature has constituted the integral part or core of the basis for survival. This fact manifests itself in ethical sentiment as reverence for nature. In the 18th century Europe, however, the ideology of enlightening placed human rationality onto the supreme throne of the world.

This attempt to reorganize the world structure according to the design it draws invented the concept of civilization contrasting that of nature, which was denigrated to the position of the barbaric or uncivilized. Ever since, humans and their artifacts have proliferated with ever progressing environmental destruction. With the aforementioned understanding of the ongoing process and from the point of survival, it is clear that the international collaborative research can not confine itself to the domain of science and technology. It should address the reality of unharnessed greed and hubris combined with science-technology causing the current environmental problems.

Recent International Collaborative Research Activities in NIMD

Hirokatsu Akagi
National Institute for Minamata Disease

Abstract

Although environmental monitoring and control measures have become more rigorous in industrialised countries, an increasing proportion of environmental mercury pollution take place in the developing countries in recent years. In particular, a number of countries in the tropical belt of South America, Africa, and Southeast Asia have experienced a tremendous increase in uncontrolled, informal sector gold mining activities using mercury since late 1970s. While, in some developing countries mercury is still used in chemical industry for the production of acetaldehyde as well as caustic soda and chlorine.

In response to the requests from these developing countries for technical or research collaboration in relation to environmental mercury pollution, National Institute for Minamata Disease (NIMD) has been carring out several collaborative studies on mercury issues, supported by national and international grants since 1992. In this report an overview is given of the international collaborative research activities which have recently been undertaken or are underway in NIMD as well as the international scientific symposia or workshops organized so far by our Institute.

1. Introduction

The outbreak of Minamata Disease, which is one of the most tragic environmental pollution-caused health damages in Japan, originated in discharge of industrial wastes containing methylmercury from chemical plants, which polluted the surrounding environment and consequently incurred serious damages to human health and also to the local communities. National Institute for Minamata Disease (NIMD) was established in 1978 in Minamata City, Kumamoto Prefecture, with the purpose of conducting comprehensive medical research to improve medical treatment for victims of Minamata Disease and to contribute toward significant advances in measures against Minamata Disease while giving balanced consideration to its deep histrical background and social importance. In 1986, NIMD was designated as a collaborating Center of WHO, and furthermore the Institute was reorganized as new NIMD last year, with the aims of providing accumulated knowledge and expertise related to the mercury poisoning incidences in Japan for other countries, and making significant contribution in terms of international cooperation in the fields of the environmental health.

Because of the harmful properties of mercury compounds and the epidemic intoxication catastrophes in Japan as well as in Iraq, environmental monitoring and control measures have become more rigorous in many countries. However, in the developing countries, these pollution problems still remain. Particularly in recent years, beginning in Latin America in the 1980s, gold mining activities using amalgamation technique are widespread in tropical zones such as Latin America, Africa, and southeast Asia and the increasing release of mercury into the environment are causing great worldwide concern. This is the main reason for the international collaborative research started by the NIMD.

1. Recent International Collaborative Studies in NIMD

1) Study on Mercury Pollution Due to Gold Mining in the Amazon, Brazil[1-3](1993 - 1996)

This study has been undertaken in 1993 between our NIMD and Federal University of Rio de Janeiro in collaboration with Yokohama City University, Akita University and Tohoku University in Japan, as part of the Global Environmental Research Project in the Environment Agency of Japan. The main objectives of the study were to evaluate the actual extent of environmental mercury contamination and its health effects, as well as the pathways of mercury contamination, especially in methylated form, leading to human populations, mainly in the Tapajos river basin which is considered to be the oldest and most productive gold mining area in the Amazonian region. In this region, tremendous amounts of metallic mercury have been used for recovering fine gold from gravel through amalgamation and released into the surrounding environment since the early 1980s.[4,5] There are two main pathways of mercury contamination which can affect human populations in this region. First, occupational exposure of gold miners and workers in gold shops to inorganic mercury due to direct inhalation of mercury vapour during the gold recovery processes. Second, some parts of the mercury released into the river systems is methylated and ultimately bioaccumulated to a significant level in fish via the food chain, and thus people living along these rivers can be exposed to methylmercury through fish ingestion. People living near gold mining areas may be exposed to both inorganic and methylmercury simultaneously from the surrounding environment and through their diet.

Although a growing literature exists dealing with mercury contamination levels in various human and environmental materials taken from the main tributaries of the Amazonian region, it has so far been difficult to predict to what extent these people and biota are exposed to methylmercury that is formed by methylation of inorganic mercury released in the gold mining areas, since the reported data are based on the total mercury analysis. It is imperative that we obtain more information concerning mercury contamination in local populations and their environment from detailed mercury speciation studies. Thus, this study focused on the evaluation of the human and environmental exposure to methylmercury occurring as a

consequence of mercury pollution in this tropical aquatic ecosystems. For this purpose, simplified, accurate and highly sensitive analytical methods for the determinations of total mercury and methylmercury in various biological and environmental materials were first established in our laboratory and frequently verified with certified reference materials as well as through interlaboratory exercises. In order to estimate the actual exposure of local populations to methylmercury, human hair, blood samples from inhabitants as well as fish samples were collected from various fishing villages located at different distances from the main gold mining areas in the 2,000-km long Tapajos river basin. In addition, human hair, blood and urine samples were collected from gold miners and workers in gold shops in Itaituba and Alta Floresta cities, the main gold trading centers in this region. These human and fish samples were carefully analyzed for total mercury and methylmercury utilizing highly sensitive and reliable methods developed and modified in our laboratory.[1,2)]

It is well recognized that general population is primarily exposed to methylmercury through fish consumption, and scalp hair analysis is the most appropriate method for monitoring methylmercury intake in persons at risk. However, in the case of the Amazonian region a special consideration must be given to the evaluation of mercury levels in hair, because of the presence of inorganic mercury due to gold mining activities.

The results to date showed that the hair samples from gold miners and workers in gold shops had total mercury levels up to 113ppm, but the methylmercury levels were low and the average proportion of methylmercury to total mercury were only 5-14% in these groups, suggesting a large contribution of inorganic mercury from ambient air and/or sweat during the gold mining and burning of the gold-mercury amalgam. From these results it is apparent that the measurement of only total mercury in hair samples is not sufficient for evaluating the human exposure to methylmercury in areas, such as gold mining areas being continuously contaminated with inorganic mercury. In contrast, the results for total mercury and methylmercury levels in hair samples from people living in fishing villages showed that the inhabitants of all the fishing villages surveyed were continuously exposed to methylmercury at abnormally high levels rainging from 10 to 36ppm on average, with little confounding exposure to inorganic mercury. The inhabitants of fishing villages near the main gold mining areas were more exposed to methylmercury than those far downstream. About 3% of hair samples collected from 573 individuals were found to have methylmercury levels greater than 50ppm, the minimum threshold value for methylmercury intoxication, indicating high and widespread contamination with methylmercury. The methylmercury levels found in fish ranged from 0.1 to 3.8ppm and most of the fish samples from the upstream river had relatively high mercury levels exceeding the Brazilian permitted limit of 0.5ppm.

One purpose of this study was to evaluate more precisely the actual extent of human exposure to methylmercury due to gold mining. For this purpose, paired human hair and blood samples were collected from individuals in Jacareacanga, the upstream typical fishing village and

analyzed for total mercury and methylmercury.

The results showed that the hair and blood samples contained quite high levels of mercury and again almost all the mercury in the samples was in methylated form. As expected from the results of mercury analysis, a highly significant correlation between mercury in hair and blood (r^2 = 0. 87) was found with an average hair Hg/blood Hg ratio of 253. This figure was in reasonably agreement with the value of 250, which has been established in various populations exposed to methylmercury at fairly constant levels, suggesting that the inhabitants in fishing villages are continuously exposed to methylmercury mainly through fish diet.

2) Study on Environmental and Human Exposure to Mercury Due to Gold Mining in the Lake Victoria Goldfields, Tanzania[6,7] (1995-present)

Similar collaborative research has been undertaken in 1995 in collaboration with University of Dar es Salaam, Tanzania. In Tanzania, it is estimated that around 250,000 people are engaged in small scale gold mining using mercury amalgamation technique in three principal goldfields, namely the Lake Victoria goldfields, around Lake Victoria, the Lupa goldfields in the southwestern part of the country and the Mupanda mineral fields in the western part, and at least 6 tonnes of mercury are released into the environment anually from the production of about 4 tonnes of gold from small scale mining in various parts of the country. There is a potential risk of human exposure to mercury because of extensive use of mercury in gold recovery in Tanzania goldfields.

The purpose of the present study was to assess environmental and human exposure to mercury in the Lake Victoria goldfields. This study is the first that has dealt with biological monitoring of mercury contamination due to gold mining in Tanzania.

Two study areas, Mugusu and Nungwe Bay were chosen for biological monitoring of mercury contamination. Mugusu has been an active small gold mining area for around 8 years since 1988. Human hair and urine were collected in November 1995 from inhabitants of the Mugusu mine for monitoring of mercury exposure from amalgam burning. Nungwe Bay on the southwestern part of Lake Victoria is located about 10km from the Mugusu gold mine. The bay is essentially a drainage area for the Mugusu river and other rivers which might be contaminated with mercury due to gold mining activities. The bay is surrounded by Nyamwilolelwa village whose inhabitants are engaged both in fishing and farming, and fish is a major protein source in their diet. In addition to human hair and urine samples, fish samples were also collected in this area from different types of fish caught from Nungwe Bay by fishermen during days of sampling. All fish samples were transported and kept frozen until analyzed. These samples were analyzed for total mercury and methylmercury at NIMD, using new analytical techniques recently developed in our laboratory.[2]

Biological monitoring of environmental and human exposure to mercury in the Lake Victoria

goldfields has revealed low mercury levels in fish and human hair that seem to present bachground levels. Total mercury levels in 15 fish samples were in the range of 1.8 - 16.9ng/g wet weight(mean: 7ng/g). Lower mercury levels were found in Tilapia(mean: 2.4ng/g) and Kamongo(mean: 2.2ng/g) than the other fish species. Relatively high mercury levels were found in Soga(mean: 13.7ng/g) followed by Nile perch(mean: 9.7ng/g).

The proportion of methylmercury to total mercury in all fish samples was in the range of 63 - 97%(mean: 88%), which indicated that most of the mercury in the fish was in the form of methylmercury. Extremely low mercury levels in the fish samples in comparison with published data from other parts of the world suggest the presence of low background methylmercury levels in the Nungwe Bay area of the Lake Victoria and its catchment areas.

Total mercury levels in 22 human hair samples were in the range of 156 - 5,433ng/g(mean: 947ng/g) and the proportion of methylmercury to total mercury in hair from the inhabitants of Mug's mine was in the range of 7 - 69% (mean: 27%) , were as in the inhabitants of the Nunguwe Bay fishing village it was in the range of 21 - 82% (mean: 48%). The higher proportion of methylmercury in the fishing village population was consistent with the fact that the population is exposed methylmercury mainly through fish ingestion, though the mercury level in fish are quite low. Thus, gold mining activities seem, so far, not to have produced any significant increase in methylmercury level in the environment that could be reflected in high fish mercury content.

Urinary mercury levels in gold miners frequently exposed to mercury vapor were significantly higher with mean value of 241 ng/ml(Range: 129- 4ll ng/ml) than those in general mine population not occupational exposed to mercury(mean: 2.6ng/ml). Rotation of mine duties reduced mercury exposure levels and hence the risk of inorganic mercury intoxication in the gold miners.

In the present study, fieldwork was made possible through the University of Dar es Salaam Research Project on "Environmental Aspects of Mining and Industrialization in Tanzania" financed by the SAREC(Swedish Agency for Research Cooperation with Developing Countries) , Department of Swedish International Development Authority. Laboratory works were performed in NIMD under STA Fellowship in Science and Technology granted to Dr. J.R.Ikingura, University of Dar es Salaam, for 12 months by the Science and Technology Agency of Japan.

3) Study on Environmental Mercury Pollution in Baihua Lake, Guizhou Province, China[8)]
(1996 - present)

The Guizhou Organic Chemical Factory, which is located in central part of Guizhou Province, China, and has been producing acetaldehyde as well as acetic acid from acetylene using mercury as catalyst for more than 30 years. The industrial waste water containing mercury and

methylmercury has been discharged into the stream named Donmengiao river, neighboring the Factory, with only a single treatment for the same duration of the factory. The Donmengiao river, which is utilized as a source of irrigation in the region downstream from the factory, runs into Baihua lake. The water of the Baihua lake is decided to be a source of waterworks of Guiyang city, the capital of Guizhou Province. Therefore, farmers in the irrigated region is thought to be suffering mercury pollution, and the civil of Guiyang will sustain mercury pollution near future.

This research project between Guizhou Institute of Environmental Sciences and NIMD was launched in 1996 to survey the environmental pollution by mercury mainly in the regions of Donmengiao river and Baihua lake. The collaborative study of inspection for mercury pollution is still continuing with a view to obtaining more reliable analytical results on the environmental and human exposure to mercury in these areas.

4) UNHCR Project-Investigation into Suspected Mercury Contamination at Deder Bubu, Nookat, Kyrgyzstan[9] (1996)

This collaborative study requested by United Nations Commissioner For Refugees(UNHCR) has been conducted in 1996 in a village of Deder Bubu, Osh, Kyrgyzstan. The purpose of this study was to carry out an environmental health assessment of the refugees of Deder Bubu village, Kyrgyzstan in order to ascertain whether they are suffering from mercury poisoning. The village of Deder Bubu in Osh region of Kyrgyzstan is located 300 meters apart from an old open cast of mercury mine, which might be a source of environmental contamination of mercury in this area. It has a population of about 230, of which 80 are adults. Deder Bubu is also located about 4 km south of another mercury mine in Uluu Too with a population of approximately 230. Kojar is about 13 km south of the mine and the most of the population (approximately 6,000) were born there.

To assess suspected contamination with mercury, biological (human hair and urine) as well as some environmental samples (water and soil)were collected for mercury monitoring. Human hair and urine samples were collected from 105 inhabitants of Deder Bubu, 120 of Kojar and 90 of Uluu Too. At the same time, the information on each donor, eating habits, and so on, through questionnair from an epidemiological point of view. Sampling was conducted by the study group(Drs. J. Wakamiya and M. Sakamoto) in collaboration with staff members of the local Institute in Osh during the period of December 10-16, 1996. All these samples were transported and kept cool or frozen until analyzed.

Total mercury concentrations in human specimen were determined by cold-vapor atomic absorption spectrometry following burning of 50mg hair or 0.5ml urine, capturing of mercury in 0.3% potassium permanganate in 1N sulfuric acid and reducing to mercury vapor with stannous chloride. Total mercury in the other samples as well as methylmercury in hair samples were

analyzed using methods recently developed and routinely used in our laboratory[2].

In the residents of three regions, hair mercury levels were considerably low. Mercury levels were found to be highest in Deder Bubu refugees (mean: 1.545ppm), followed by Uluu Too residents (mean: 0.115ppm) and Kojar residents(mean: 0.068ppm). However, the hair mercury levels in the Deder Bubu refugees was not significantly($p > 0.05$) higher than the other regions. In the two hair samples from Dedry Bubu refugees, the concentrations were much higher at 89.3ppm and 44.4ppm. These samples were from female adults(24 and 43 years old, respectively). Those anormalous hair samples probably represent extreme cases of external contamination from the other sources, because they were confirmed to contain mercury mostly in inorganic form by their methylmercury analyses. The mercury levels in urine were also considerably low in three residents and showed a similar patern to hair mercury. The urine mercury levels in residents of Deder Bubu, Uluu Too and Kojar, were in the ranges of 0.05 - 12.5 (mean: 0.95μg/g creatinine), 0.15 - 5.76(mean: 1.21μg/g creatinine) and 0.01 - 2.64(mean: 0.28μg/g creatinine), respectively.

In order to estimate the kidney function, urine samples from inhabitants having much higher mercury levels in hair were determined for tB_2MG as well as NAG, and these general urinalysis revealed no abnormality.

On the other hand, the mercury levels in tap water which was used by Deder Bubu refugees and Kojar inhabitants were 0.50ng/L and 0.56ng/L, respectively. Except for water sample from the spring near Deder Bubu which showed 8.4ng/L, the other samples contained around 0.5ng/L, showing a background level. Soil mercury contents were in the range of 0.11 - 0.25μg/g dry wt in Deder Bubu, and the surface soil in Uluu Too and the surface soil of near the mercury mine in Uluu Too showed 1.97 and 1.20μg/g dry wt, respectively. However, the mercury level of the sediment in the natural spring near Deder Bubu was much higher at 55.4μg/g dry wt.

From these results, it was be concluded that the total mercury levels in hair and urine of Deder Bubu refugees, Kojar and Uluu Too inhabitants were lower than those of the general populations in European countries. The total mercury levels in water and soils in deder Bubu region were within the normal ranges observed in other parts of the world, but the levels in sediments at the natural spring near the refugees' camping site was abnormally high at 55.4μg/g on a dry weight basis, compared with other sampling sites surveyed. It should be noted that this concentration is not high as compared with that of other mercury mining areas.

No health effects due to mercury exposure was observed among Deder Bubu regugees, Kojar and Uluu Too inhabitants.

5) Others

There are two more Projects that we are planning to collaborate with other countries this year, one on the environmental mercury pollution from Chitagong Caustic soda Plant in Bangladesh,

and the other on the assessment of mercury pollution due to gold mining in West Kalimantan Province, Indonesia.

2. International Symposia/Workshops organized by NIMD

Another international collaborative activities in our NIMD are to organize scientific meetings on mercury inside Japan or abroad in coolperation with other countries.

As shown in Table. I , we have, so far, been organizing and holding several International Symposia or Workshops on mercury, including this NIMD Forum '97, almost every year inside and outside Japan.

Table I. **INTERNATONAL SYMPOSIA / WORKSHOPS ORGANIZED BY NIMD**

1. International Symposium on "Epidemiological Studies on Environmental Pollution and Health Effects of Methylmercury", October 2, 1992 (Kumamoto, Japan)
2. International Symposium on "Assessment of Environmental Pollution and Health Effects from Methylmercury", October 8- 9, 1993 (Kumamoto, Japan) - The Successive Workshop on WHO Consultation on Mercury Epidemiology Research" October 10-13, 1993 (NIMD, Minamata)
3. International Workshop on "Environmental Mercury Pollution and Health Effects in Amazon River Basin", November 30- December 2, 1994 (Rio de Janeiro, Brazil, jointly with SCOPE Workshop on Mercury)
4. International Workshop on "Mercury in the Environment: - Low Level Exposure and Its Potential Effects on Man - ", November 16, 1995 (Kumamoto, Japan)
5. International Workshop on "The Fate of Mercury in Gold Mining and Measures to Control the Environmental Pollution in Various Countries", November 25 - 26, 1996 (Jakarta, Indonesia)
6. NIMD Forum ,97, July 30- 31, 1997 in NIMD
7. International Workshop to be held in November 25- 30, 1997 in Manila, Phillippines (in preparation)

The first International Symposium entitled "Epidemiological Studies on Environmental Pollution and Health Effects of Methylmercury" was held on October 2nd, 1992 at Kumamoto National Hospital, Kumamoto, Japan, in coorporation with Agency for Cooperation in International Health. In 1993, the second International Symposium on "Assessment of Environmental Pollution and Health Effects from Methylmercury", was held jointly by WHO and our Institute on October 8-9 in Kumamoto. After the Symposium, the succssive Workshop was also held on October 10-13, 1993 in this Institute, here in Minamata under the same joint auspices of WHO and our Institute. At this Symposium, two major recent topics in the

epidemiological field of mercury pollution were discussed, one is on the Human Exposure to mercury Due to Gold mining, and one on the Prenatal Mercury Exposure. At the successive Workshop, the methodologies involved in conducting those epidemiological surveys were discussed, and a research protocol has been summarized.

The next International Workshop was held in Rio de Janeiro, Brazil, in December 1994, in collaboration with Federal University of Rio de Janeiro. In this connection, we should mention that another International workshop with aims similar to those of ours was also held by SCOPE at the same place in Rio de Janeiro on two days preceding our workshop and the two successive workshops were open to each other, so that the participants could share the fruits of both workshops. Our workshop focussed on health effects of mercury contamination from gold mining, while SCOPE concentrated on the ecological consequences. It was very productive and also valuable experiences. I should like to make special mention of the fact that the contributions rendered by Dr. Hiroo Kato, our former Director of this Institute, proved to be great important to accomplish this first workshop held abroad.

The third domestic International Workshop held in Kumamoto in November 1995, examined Mercury in the Environment, - Low level Exposure and Its Potential Effects on Man -, and was sponsored by Japan Public Health Association.

And then, International workshop on "The Fate of Mercury in Gold Mining and Measures to Control the Environmental Pollution in Various Countries, was held in Indonesia in November, last year, in collaboration with Faculty of Public Health - University of Indonesia. The workshop was sponsored by Science and Technology Agency of Japan. In addition to the information on mercury contamination from Brazil, Indonesia, Phillippines and Vietnam, the methodologies for mercury pollution research as well as the transformation of mercury in the ecosystems were also discussed in this workshop.

We are now preparing the next International Workshop to be held in Phillipines in forthcoming November.

References

1. Akagi, H., Malm, O., Kinjo, Y., Harada, M., Branches, F.J.P., Pfeiffer, W. C., and Kato, H. (1995) Methylmercury pollution in the Amazon, Brazil. *Sci. Total Environ.*, **175**, 85-95.

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

3. Akagi, H., Malm, O., and Branches, F. J. P. (1996) Human exposure to mercury due to gold mining in the Amazon, Brazil - A Review. *Environmental Sciences*, **4**(3), 199 - 211.

4. Lacerda, L.D., Pfeiffer, W. C., Ott, A.T., and Silveira, E.G. (1988) Mercury contamination in Madeira river, Amazon- Hg inputs to the environment. *Biotropica*, **21**, 91-93.

5. Pfeiffer, W. C., and Lacerda L.D.(1988) Mercury inputs into the Amazon region, Brazil. *Environ. Technic. Lett.*, **9**, 325- 330.

6. Ikingura, J.R. and Mutakyahwa (1995) Sources of mercury contamination and exposure in Tanzania. In Proc. Intern. Conf. Mining and the Environment in Eastern and Southern Africa, University of Dar es Salaam, Tanzania(in press).

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

8. Takizawa, Y. , and Sugihara, H. (1996) Mercury pollution from an acetaldehyde-producing factory in Guizhou Province, China. *Shigen Kankyo Taisaku*, **32**, No. 13, 1231-1235(in Japanese)

9. Sakamoto, M., Wakamiya, J., Nakano, A., Akagi, H., Aramaki, R. , Yamaguchi, M., and Takizawa, Y.(1997) NIMD report of collaborative study with UNHCR on Investigation into suspected mercury contamination at Deder Bubu village, Nookat, Kyrgyzstan.

Cooperative Studies on Mercury Pollution Caused by Guizhou Organic Chemical Factory in Environment by China and Japan

Liya Qu

Guizhou Institute of Environmental Sciences
184, Xinhua Road, Guizhou, China

In 1953, Minamata Disease broke out in Minamata City, Japan. The cause of that disease was verified, in 1959, to be an intoxication by the methyl-mercury in fish. The methyl-mercury accumulated in human body through biological concentration and food chain transfer. Great global attention had been paid to Minamata Disease because it is the first public nuisance in the world caused by environmental pollution. Guizhou Organic Chemical Factory employed a similar production process to that of Japan Nitrogen Fertilizer - Minamata Factory, in which mercury was used as a catalyst and finally caused the Minamata Disease. According to the information we got so far, Guizhou Organic Chemical Factory remains as the unique factory for this production process in the world. Guizhou Institute of Environmental Science has carried out some investigations on the water quality, soil and rice in the area of Guiyang City, the capitol of Guizhou province, from 1986 to 1990, details of the investigations have been introduced in May 1996, on the conference of "Environmental Studies on Mercury Pollution in the World" held by National Institute for Minamata Disease of Japan. According to the study, about 45,930,000 ton waste water is discharged every year to the environment with a mercury concentration of 0.0169 mg/l. The amount of mercury discharge to the environment, thus, can reach 780 kg. Since the factory has been running for as long as nearly 20 years and also that occupies a special geographical location (see below), we have to pay more attention on the problem of mercury pollution of environment in these area now than ever before.

In Guizhou Province, the largest artificial lake, Hongfeng Lake, is located at 4 km to the west of the Guizhou Organic Chemical Factory, and it is the first grade large scale reservoir in Maotiao River basin. It is the largest water source in Guiyang, with multi-functions such as flood diversion, hydropower generation, drinking water source, tourism, aquatic breeding and the water source for industry. In 1988, Hongfeng Lake was appointed as one of the key natural scenery reserves in China. Hongfeng Lake is

also the water intake source of Guizhou Organic Chemical Factory. About 7 km to the north of the factory, there is another artificial lake, Baihua Lake, which is the second grade large scale reservoir in Maotiao River basin and has almost the same multi-functions as Hongfeng Lake. The upstream of Guizhou Organic Chemical Factory is Hongfeng Lake, and the downstream is Baihua Lake. Waste water from the factory is directly discharged into the Maotiao River in between Hongfeng Lake and Baihua Lake, then flow into Baihua Lake, only after simple sedimentation and activated carbon treatment.

The water flowing into Baihua Lake mainly comes from Hongfeng Lake. Its catchment area is 299 km^2, reservoir volume is about 180,000,000 m^3 and flowing-down water amount is 500,000,000 m^3/y. The population in Maotiao River basin is about 665,000, of which 65% are engaged in agricultural activities, and about 300,000 people depend on Hongfeng Lake as their drinking water source. Baihua Lake mainly supply water for some villagers around the lake besides the residents in two districts in Guiyang. At present, Baihua Lake, which was appointed as the famous scenery spot in Guizhou Province in 1987, is one of the key aquatic products bases in Guiyang with a fish breeding area of 17,700 m^2. Therefore, it is crucial problem whether the level of mercury pollution by the factory is significant or not.

Keyword: Organic Chemistries Factory, acetic acid, mercury pollution

ENVIRONMENTAL RESEARCH ACTIVITIES BY ENVIRONMENTAL MANAGEMENT CENTER (EMC)

Bagus Bina Edvantoro*

Abstract

The center for environmental impact control facilities has been established in cooperation with Japanese Government to develop environmental research and technology as well as environmental monitoring studies since 1993. In recent years, however increasing wastes from industries, human settlements, urban activities, motor vehicles, agriculture and tourism are causing environmental pollution. Realizing that problems of pollution has caused the environmental degradation and human threats, so EMC has a relevantly important role in anticipating and overcoming the matters.

Related to the issue above, EMC has undertaken several programs to provide the information on the state of environmental quality and level of pollution at some specific areas. In the early stage (1994), EMC's staff carried out the research and development mainly in the field of environmental quality monitoring that focused on the river water and sediment at 10 provinces in Indonesia. The parameters emphasized in metals and organochlorine residue analysis (sampling was done twice a year during the rainy and dry season). From the analytical results, some parameters exhibited quite high concentration in sediments, such as Pb, Cu, Cr, Mn, Ni, at several sites , however most of the organochlorine pesticide residue was not detected.

In 1995, EMC conducted the monitoring activities at other five provinces which focused on various type of industries, such as Ni manufacturer, leather tanning , pulp and paper, fertilizer, plywood as well as organochlorine residue in agriculture. Parameters showing high concentration of pollutants at several places were Pb, Cr, Cu, and Ni, meanwhile most of the organochlorine residue and phenol were not detected. In May 1995, we had surveyed on mercury level along Kapuas river, West Kalimantan from gold mining activities. Most of mercury content in water, sediment, fish and hair samples were found.

During 1996, our program still emphasized in industrial discharges which was suspected to be toxic compound, such as Liquid Natural Gas, textile, gold mining, electroplating and steel manufacturer. Pollutants showing high level was Total Hg, Ni, Cr, Cu, Pb, Co and Ni at some places. In March 1996, under cooperation with UNU, Japan, we set up the project on environmental monitoring and analysis in the East Asian Region for measuring the organochlorine pesticide residue in rice and soil samples as well as PCBs in industrial area. Based on the analytical result, pesticide residue was found in trace concentration in soil samples only, while in rice was not detected. In October 1996, EMC collaborated with CITI, Japan in undertaking the environmental monitoring project along Cisadane river where there is industrial zone around this area. We collected sediment samples for determination of T-Hg, methyl mercury and PCBs. Total Hg was

discovered at most of sampling points with the range from 0.08 to 0.39 µg/g, PCB range from 0.01 to 0.14 µg/g, while methyl mercury was not detected.

In 1997/1998 fiscal year, the laboratory was developing skill and capability in other type of research, such as methodology development for determination of toxic compounds (PCB and organochlorine residue), toxicology (LD-50), hazardous waste characteristic and monitoring studies on specific activities, such as harbors , textile and paper industries. The projects above are still on the progress and we plan to continue some of them in the next fiscal year.

---

* Toxic substances laboratory, EMC
Complex PUSPIPTEK, Jl. Raya Puspiptek Serpong, Tangerang 15310, INDONESIA
Phone. +62-21-756-0562 ext. 1508 Fax. +62-21-756-3115

I. Introduction

The rapid development in the Republic of Indonesia during the successful First Long Term Development Plan created a considerable stress upon environment. Urban areas as well as rural regions now are facing with environmental problems due to the activities developed in the regions. Urban areas where economic activities are intensified and population are drastically increasing are suffering from water, air pollution and other nuisances. Adverse effects to human health may become a problem. On the other hand, in rural regions, excessive use of pesticide may cause serious pollution by its toxic components.

In this regard, The State Ministry for Environment was established in 1978 to pursue the objectives, and was engaged in improving laws and regulations relevant to environmental policies and to coordinate efforts with other ministries and agencies. To assist The State Ministry for the environment, the Government later on set up the Environmental Impact Management Agency (BAPEDAL) in 1990 to cater for the management of environmental impacts. Furthermore, the Government of the Republic of Indonesia in cooperation with the Government of Japan, designed the "Project for Establishment of the Environmental Management Center (EMC)" in 1993. EMC is expected to be the central institution for reference laboratory by further promoting research, development of environmental management techniques, information data analysis and training for officials and engineers in the government and the industry. EMC is anticipated to play a key role in the Second Long Range Development Plan from the view point of environmental control.

The objective of EMC project is to strengthen the scientific and technological base for environmental policy development and implementation. EMC undertakes, in the initial stage, research and development mainly in the field of environmental monitoring as well as training in the same area. EMC has primary function as national environmental reference laboratory, center for training related to environment and center for environmental information system. EMC should also contribute to international efforts for global environment as a national focal point of international and regional environmental cooperation programs conducted by international organization.

In the early stage (1994), EMC's staff carried out the research and development mainly in the field of environmental quality monitoring that focused on the river water and sediment at 10 provinces in Indonesia. The parameters emphasized in metals and organochlorine residue analysis (sampling was done twice a year during the rainy and dry season). In 1995, EMC conducted the monitoring activities at other five provinces which focused on various type of industries, such as Ni manufacturer, leather tanning, pulp and paper, fertilizer, plywood as well as organochlorine residue in agriculture. In May 1995, we had surveyed on mercury level along Kapuas river, West Kalimantan from gold mining activities. During 1996, our monitoring program still emphasized in industrial discharges which was suspected to be toxic compound, such as Liquid Natural

Gas, textile, gold mining, electroplating and steel manufacturer. In March 1996, under cooperation with UNU, Japan, we set up the project on environmental monitoring and analysis in the East Asian Region for measuring the organochlorine pesticide residue in rice and soil samples as well as PCBs in industrial area. Based on the analytical result, pesticide residue was found in trace concentration in soil samples only, while in rice was not detected. In October 1996, EMC collaborated with CITI, Japan in undertaking the environmental monitoring project along Cisadane river where there is industrial zone around this area.

This report emphasizes some of the findings from the research which have been carried out by EMC which includes Analysis of tributyltin (TBT) compounds in harbor sediment at Tanjung Priok (North Jakarta), Mercury analysis along Kapuas river, West Kalimantan, Environmental monitoring and analysis in the East Asian Region for organochlorine residue and PCBs as well as Environmental monitoring project along Cisadane river.

II. Materials and Methods

2.1. Mercury concentration along Kapuas river, West Kalimantan

2.1.1. Analysis of total mercury

Reagent :

HNO_3 (1+1)
H_2SO_4 (1+1)
5% $KMnO_4$
Hydroxylammonium hydrochloride
30% $SnCl_2$

Instrument and apparatus

Mercury analyzer
Reflux condenser
Waterbath
Analytical balance
Water purifier
Filter paper
Glassware

Sample preparation

Known amount of sample was decomposed with acid digestion by using sulfuric acid and nitric acid at 95°C for about two or three hours. Potassium permanganate solution

was used as oxidizing agent which was added at 40°C. When purple color did not disappear within 10 minutes after addition of permanganate, the decomposition process was completed. The excess amount of Potassium permanganate was neutralized by adding the Hydroxylammonium hydrochloride in drop wise. Sample then was filtered and put into 100 ml volumetric flask and adjust up to the mark with distilled water. 100 ml of this solution was taken for determination of total mercury concentration by using 10 ml of 30% Stannous chloride as a reduction agent.

2.2.2. Analysis of Methyl mercury in human hair sample

Reagent

2 N HCl
6 N HCl
Conc. HCl
Benzene
1% Cysteine

Instrument and Apparatus

GC-ECD
Recipro-shaker
Centrifuge
Analytical balance

Sample preparation

Approximately 1-2 g of hair sample was weighed and cut into tiny pieces. Hair sample was mixed with benzene solvent to extract some organic compound. L-cysteine-sodium acetate was used to extract conjugation of organo mercury-cysteine compound into water layer from benzene extract. Extract solution was acidified and shaken with benzene to reextract methyl mercury into benzene layer. Volume of benzene layer was adjusted to 5 ml for methyl mercury determination.

2.2. Analysis of Tributyltin (TBT) compounds in harbor sediment at Tanjung Priok (North Jakarta)

Reagents

I N HCl-MeOH: EtAc (1:1) mixture
10% NaCl solution
Hexane: EtAc (2:3) mixture
n-Hexane
Ethanol
Hexane : Cyclohexane (1:1) mixture

Na_2SO_4 anhydride
Propyl magnesium bromide (Grignard reagent)
1 N H_2SO_4
5% Ether-hexane solution
Nitrogen gas (industrial use quality)
Standard solution

Instrument and apparatus :

GC-FPD (Gas Chromatograph Flame Photometric Detector)
Waterbath
Rotary evaporator
Nitrogen purging system
Grignard reaction system
Water purifier
Glassware
Analytical balance

Sample preparation :

10 grams homogenized sediment sample was taken into 250 ml Erlenmeyer flask with stopper and added 70 ml 1 N HCl-MeOH : EtAc (1:1), then shaken for 30 minutes. This solution was filtered and put into 250 ml separating funnel, then wash the filter with 30 ml extract solvent. 100 ml 10% of NaCl solution was added to the filtrate and 25 ml EtAc : Hexane and then shaken for 15 minutes. Separated water and solvent layers, in the water layer was added 25 ml EtAc : Hexane, then shake and solvent layer was collected. 100 ml Hexane was added in the solvent layer, stand for 20 minutes, and wash three or four times with 50 ml NaCl solution. Pass the solution to Na_2SO_4 anhydrous column and 10 ml hexane to drying column, then concentrate with rotary evaporator at 40°C until 1 ml. The test solution was transferred into test tube, under N_2 stream blew it until nearly dry and added exactly 10 ml EtOH. 5 ml solution was taken into 100 ml separating funnel, added 15 ml 1 N HCl-MeOH and 15 ml 10% NaCl solution, then mix. Add 5 ml Hexane : Cyclohexane (1:1) and shake for 15 minutes.

2.3. Environmental monitoring project along Cisadane river, Tangerang, West Java

2.3.1. Analysis of total mercury in sediment sample

The analytical method is the same procedure as written in 2.1.1

2.3.2. Analysis of PCBs

Reagent

KOH-EtOH
Hexane
Na_2SO_4 anhydride
Glasswool
Wakogel

Instrument and apparatus

GC-ECD
Reflux condenser
Waterbath
Recipro-shaker
Centrifuge
Rotary evaporator

Sample preparation

10 grams of sediment sample is placed into round bottom flask and 50 ml KOH-EtOH is added then reflux for about one hour in waterbath. When cool, 50 ml hexane for residue analysis is added and shaken vigorously for 15 minutes. The test solution is filtered with glasswool. Filtrate is put into separating funnel and shake it again thoroughly. Hexane is taken with pipette. While, water layer is added with hexane and shake it again. The hexane layer is combined and washed with water for three times. This hexane layer is added with hexane solvent and lets it pass through column which is filled with sodium sulfate and then concentrate it until the final volume reach 5 ml. Clean-up with Wakogel in column. This portion of solution is analyzed with GC-ECD under specified condition for PCBs determination using GC-ECD.

2.3.3. Analysis of Methyl mercury in sediment samples

Reagent

HCl
CuCl
Benzene
NaCl
Distilled water
L-cysteine
Na2SO4 anhydrous

Apparatus and instrument

GC-ECD
Shaker
Centrifuge
Analytical balance
Glassware
Rotary evaporator

Sample preparation

10 grams of sediment sample was placed in the centrifuge tube then added with HCl and CuCl. Shake for about five minutes. 20 ml of benzene was mixed and shaken for five minutes. Benzene layer in the test solution was added with NaCl and wash with water for three times. Benzene was also added to water layer. L-cysteine solution was poured to benzene layer and shaken for 10 minutes. Water layer in this solution was added with HCl and benzene, then shaken again for five minutes. Sodium sulfate anhydrous was used to absorb water in sample solution. This portion of solution is analysed with GC-ECD under specified condition for methyl mercury determination.

2.4. Environmental monitoring and analysis in the east asian region : Indonesia

2.4.1. Analysis of PCBs in sediment sample

The analytical method is the same procedure as written in 2.3.2, except the analytical instrument using GC-MS.

2.4.2. Analysis of organochlorine residue

Reagent

Acetone
NaCl
Hexane
Distilled water
Na2SO4 anhydrous
Floricil

Apparatus and Instrument

GC-MS
Shaker
Centrifuge
Analytical balance
Glassware
Rotary evaporator

Sample preparation

Known amount of sample was mixed three times with 50 ml acetone and shaken for 30 minutes. Add 50 ml acetone again to the residue and combine these acetone layer then concentrate it until the final volume was 30 ml. Transfer this part of solution to 1 L separating funnel and mixed with 300 ml NaCl solution and 100 ml hexane, then shaken for five minutes. Add 100 ml hexane to the water layer and combine the hexane layer. Wash the hexane layer two times with 100 ml distilled water. Pass the hexane layer through Na2SO4 anhydrous and concentrate it until the final volume was 5 ml. Fractionate this solution with floricil column (3 fractions 30 ml each). This solution was ready to be determined with GC-MS.

III. Results

3.1. Result of mercury concentration along Kapuas river in West Kalimantan can be seen in the following :

3.1.1. Analysis of total mercury in water, sediment and fish samples

No.	Sampling points	Water		Sediment		Fish	
		No	conc.(ppb)	No	conc.(ppm)	No	conc.(ppm)
1.	Kapuas river, Sintang	1	1.1	1A 1B	0.17 0.20	1a 1b	0.37 0.26
2.	Melawi river, Sintang	2	0.9	2	0.13	2a 2b	0.23 0.17
3	Kapuas river, Sepauk	3	10.0	3	0.15	-	-
4.	Sepauk river, Sepauk	4	1.1	4L 4R	0.20 0.24	4a1 4a2 4b1 4b2	0.19 0.23 0.08 0.09
5.	Kapuas river, Kampung sungai batu	5	1.2	5L 5R1 5R2	0.15 0.13 0.15	5a1 5a2 5b1 5b2	0.23 0.24 0.10 0.11
6.	Kapuas river, Pontianak	6	0.8	6R 6L	0.29 0.33	6a 6b 6c 6d1 6d2	0.31 0.43 0.15 0.25 0.29

Note :
a,b,c,d : different individual of fish samples
L : left side of the river
R : right side of the river

3.1.2. Analysis of methyl mercury in human head hair samples :

No.	Sample no.	Sampling location	Data record	Conc. of Me-Hg (ppm)
1.	1	Sintang	Female, age 20, community	0.19
2.	4A	Sepauk	Male, age 40, 3 yrs stayed	1.99
3.	4B	Sepauk	Female, age 30, indigenous	1.89
4.	4C	Sepauk	Female, age 50, indigenous	1.89
5.	4D	Goldrefinery, Sepauk	Male, age 40, 8 yrs worked	2.24
6.	C1	Bogor	Female, age 26, EMC staff	0.29
7.	C2	Serpong	Male, age 35, EMC staff	0.44

3.2. Analysis of Tributyltin (TBT) compounds in harbor sediment at Tanjung Priok (North Jakarta)

No.	Sample no.	Sampling point	Conc. of TBT (ppm)
1.	S.1	Basin I, center	0.102
2.	S.2	Basin I, mouth	0.113
3.	S.5	Basin III, center	0.204
4.	S.6	Basin III, mouth	0.176
5.	S.8	Outside west breakwater	n.d.
6.	S.9	Mouth of breakwater	0.022
7.	S.10	In front of a dry dock	0.193

3.3. The average concentration of each parameter at four sampling points along Cisadane river can be shown as follows :

No.	Sampling date	Sampling point no.	Parameter		
			Total Hg (µg/g)	Methyl Hg (µg/g)	PCBs (µg/g)
1.	October 8, 1996	I	0.08	< 0.01	< 0.01
		II	0.10	< 0.01	0.07
		III	0.19	< 0.01	0.07
		IV	0.39	< 0.01	0.05
2.	November 6, 1996	I	0.16	< 0.01	0.01
		II	0.17	< 0.01	< 0.01
		III	0.20	< 0.01	0.02
		IV	0.18	< 0.01	0.01
3.	December 3, 1996	I	0.08	< 0.01	< 0.01
		II	0.10	< 0.01	0.05
		III	0.15	< 0.01	0.14
		IV	0.13	< 0.01	0.09

3.4. Environmental monitoring and analysis in the East Asian Region

IV. Discussion

Based on the mercury survey due to gold mining activity along Kapuas river, West Kalimantan, we can observe that average concentration found in surface water was 1.02 ppb which ranges from 0.8 to 10.0 ppb (refers to proceedings of the International Workshop on the fate of mercury mercury concentration along Kapuas river, West Kalimantan). Generally speaking, the surface water along Kapuas river indicated that mercury used for gold mining processing had contaminated the river. Even though, the mercury content is still under compliance, we shall take into account the increase of mercury pollution at this particular area. Mercury concentration in sediment sample ranges from 0.13 to 0.33 ppm with average which ranged from 0.29 and 0.33 ppm. Mercury discharged from gold mining activity was flown by the river

and then it concentrated and accumulated at the bottom of the sediment of the river eventually. This was the possible reason why this particular point exhibited the high amount of mercury. Mercury concentration in the fish samples collected along Kapuas river ranged from 0.08 to 0.43 ppm with average value 0.22 ppm. According to the provisionally acceptable concentration of mercury in fish and shellfish in Japan, total mercury concentration must be lower than 0.4 ppm. It was rather difficult to determine whether the people consumed fish will suffer the diseases similar to Minamata. Mercury enters to the human body little by little. Men or women who continuously eat contaminated fish for 20 or 30 years will probably be affected in the future. Based on the analytical result demonstrated that people live near the mining or gold refinery showed the higher value than other sampling points due to mercury contamination in their hair sample with mean value was 2.0 ppm. Other hair sample taken far away from mining site exhibited the mean of less than 0.5 ppm. Mercury could enter to the human body through absorption, inhalation and digestion. Concentration of 2 ppm found in hair sample from the people live near the mining site or gold refinery had not shown the serious level yet (1).

According to result of analysis of Tributhyltin compound in harbor sediment, TBT was found in almost all sampling points, not only inside the breakwater but also outside of it. Tanjung Priok harbor in Jakarta city contains TBT with concentration value ranges 0.022 to 0.204 ppm. The data showed that the sample from Basin III, mouth of the river contains high value of TBT in sediment. The sample taken from outside of west breakwater contains low value of TBT in sediment (not detected). The concentration of TBT in front of dry dock was relatively higher than other sampling points because some activities like repainting the bottom of the ship. Although the concentration of TBT is still below the acceptable limit value, but we have to take into account the possibility of this compound to deposit in human body (4).

From the result of environmental monitoring project (Total mercury, methyl mercury and PCBs) in sediment samples along Cisadane river, Tangerang, North Jakarta, we could see that the mean of mercury concentration measured in surface sediments from four sampling points ranges from 0.08 to 0.39 μg/g. The contour lines for mercury content shows that the concentration were highest (0.39 μg/g) at downstream and lowest (0.08 μg/g) at upstream. The high mercury concentration around downstream may indicate the sediment deposition in this area. The wide range of contaminants from the industrial or domestic waste discharges to the river and it carries away then accumulates to the downstream ultimately. According to industrial data, there are a number of potential sources of mercury contamination along the river, such as the factory of electrical equipment, metal plating, chemicals and paints which could possibly contribute the mercury inputs to the river body. This amount may increase the mercury content of the local sediments because most trace metals entering the aquatic environment become associated with the bottom sediment. Methyl mercury in all sampling points is not found by GC-ECD with the detection limit 0.01 μg/g. Percentage of recovery for Me-Hg analysis in sediment samples is 113% averagely which ranges from 104 to 122%. It shows the analytical procedure and instrument as well as technical work is highly accepted for Me-Hg determination in this type of sample. The result of PCBs analysis in all sampling sites ranges from 0.01 to 0.14 μg/g. Although the percentage of recovery is low, but the repeatability of analytical work is good. From the analytical result, it is known that sediments taken from all sampling sites were already contaminated with a wide variety of PCBs. The concentration of PCBs is relatively low, particularly when compared with other references because there is no evidence of potential PCB use in the factories based on the field survey. The potential source of PCB contamination might generated from the waste of used diesel oil from the vehicle service stations (2).

It is noted from the result of organochlorine residue analysis in rice samples collected in agricultural area around Krawang, Klaten, Lamongan which includes lindane, heptachlor, fenitrothion, malathion, chloropyrifos, aldrin, dieldrin, endrin and pp'DDT are not detected as well as in soil sample at the same area and the same parameters, except for lindane and pp'DDT are found in Kerawang. We can not find the residue in rice samples probably because the farmer do not use the organochlorine pesticide anymore for pest control, but other kind of types. Besides, the mechanism of organochlorine absorption to the rice will be a critical factor involving the concentration of organochlorine residue (3).

V. Acknowledgement

I would like to express my grateful appreciation to the organization and individuals from Japan who have contributed to the successful work, especially for CITI, UNU and NIMD. Special thank is also addressed to JICA expert who has provided a lot of valuable inputs in completing the projects.

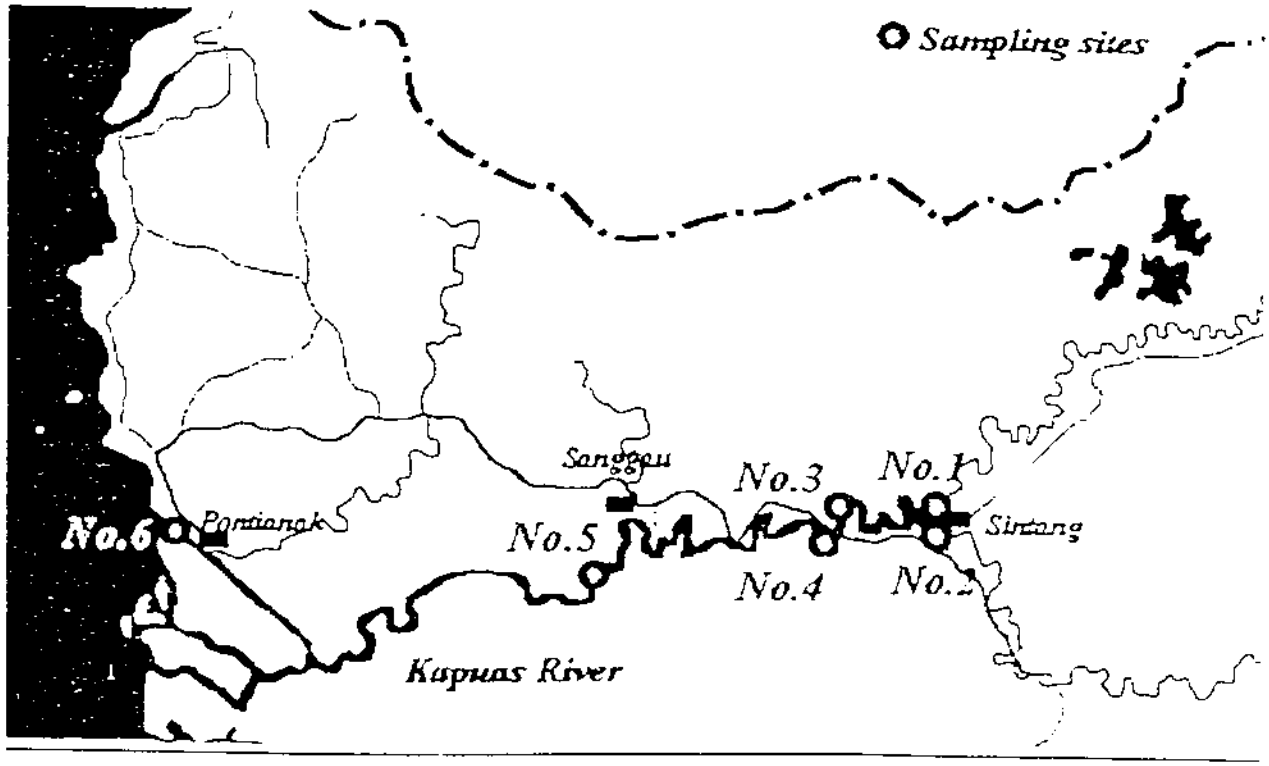
VI. References

1) Edvantoro, B.B. et. al. (1995). Mercury Concentration Along Kapuas River in West Kalimantan inProceedings of the International Workshop on The Fate of Mercury in Gold Mining and Measures to Control the Environmental Pollution in Various Countries. 1996. National Institute for Minamata Disease , Japan and Faculty of Public Health- University of Indonesia.

2) Edvantoro, B.B. et. al (1997). Report on Environmental Monitoring Project (Total mercury, Methyl mercury and PCBs) in sediment along Cisadane river, Tangerang .Environmental Management Center

3) Environmental Management Center. (1996). Environmental Monitoring and Analysis in Indonesia. UNU Project on : Environmental Monitoring and Analysis in the East Asian Region : Technology Transfer and Environmental Governance

4) Lahtiani. S. (1996). Analysis of Tributhyltin (TBT) Compounds in Harbor Sediment in Papers for International Seminar on Resources, Production, Environment and Trade. (1997) vol. 1. published by Ministry of Industry and Trade. Project on Training in Industrial Pollution Prevention Technology. Japan International Cooperation Agency.



Sampling points in Kapuas river, West Kalimantan.

Sampling points of mercury concebtration survey along Kapuas river, West Kalimantan

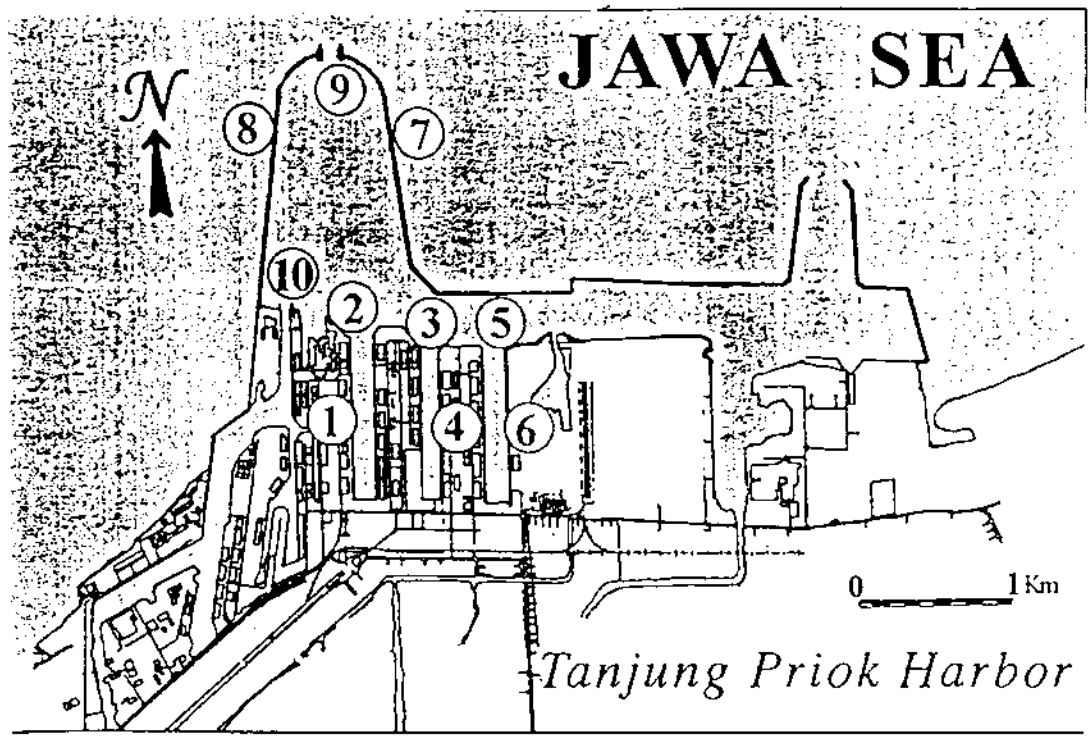
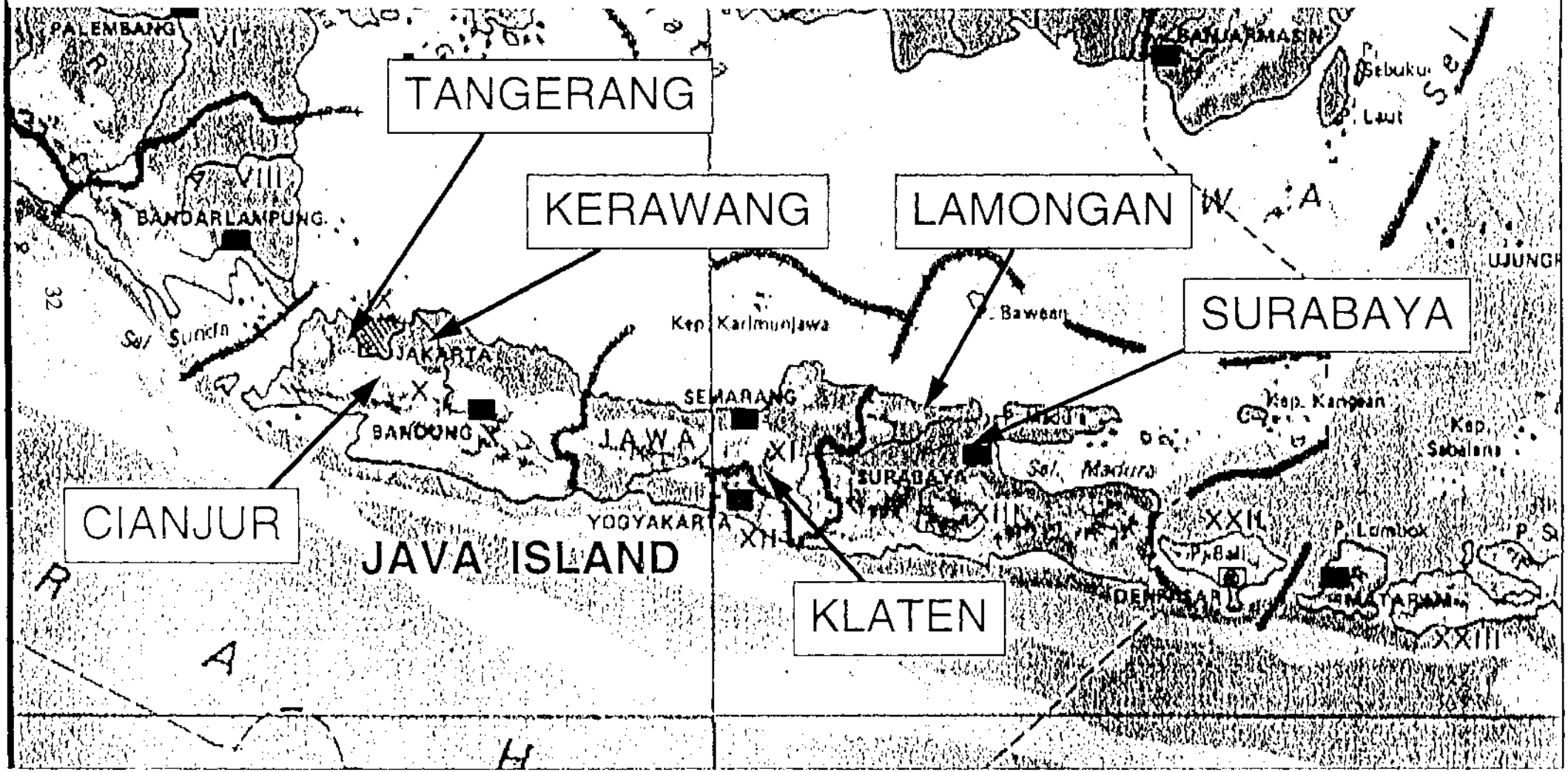


Fig. 3. Map of the sampling points.

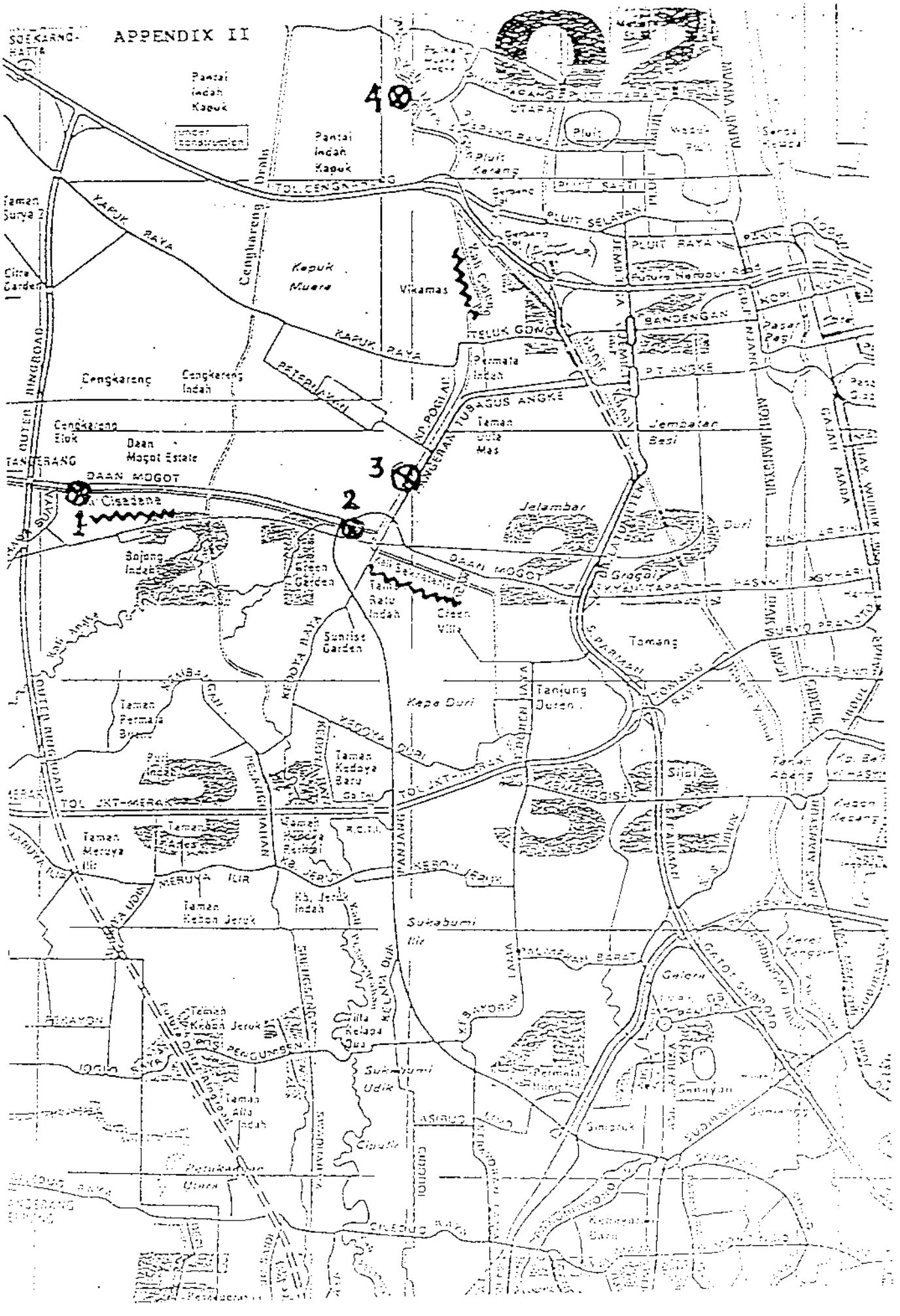
Sampling points of analysis of tributhyltin compounds in harbor sediment at Tanjung Priok

SAMPLING SITE



SAMPLING POINTS OF ENVIRONMENTAL MONITORING PROJECT

APPENDIX II



SAMPLING SITES OF ENVIRONMENTAL MONITORING PROJECT
(total mercury, methyl mercury and PCBs) ALONG
CISADANE RIVER

CONSIDERATIONS ON THE BIOGEOCHEMISTRY OF MERCURY IN AMAZON SOILS

L.D. Lacerda

Dept. Geoquímica, Universidade Federal Fluminense, Niterói, 24020-007, RJ, Brazil

Abstract

Important biogeochemical processes controlling the Hg cycle in the Amazon region are discussed. Mobilization of Hg emitted from gold mining is mostly due to atmospheric emissions and further deposition. Once deposited on soils, Hg is mostly affected by changing land use, which enhances re-emission of the deposited Hg to the atmosphere and erosion of Hg-rich soil particles to rivers. Basin soils also control, to a certain extent, the amount of Hg exported to rivers and eventually its availability to aquatic biota. Flooded soils, both from river flood plains and flooded forest soils for reservoir construction, provide exceptional conditions for Hg methylatiuon and therefore, also play a key role in the Hg accumulation in fish and eventually in humans, rather than the bulk Hg load to a given area. Under a future scenario of intense changes in the natural functioning of Amazon ecosystems, increasing Hg mobilization from soils is expected and time delayed effects of Hg contamination may occur, even if control of present Hg sources is eventually atchieved.

Key words: *Biogeochemistry, Hg, Amazon soils, Delayed Effects, Amzon Ecology*

Introduction

Mercury amalgamation was the major gold and silver mining technique in Latin America until the beginning of this century. This technique is believed to have emitted to the environment over 200,000 tons of Hg from 1540 to 1900, mostly in the silver production in Spanish Colonial America (Nriagu 1994). The exaustion of the rich silver deposits and the introduction of cyanidation in gold mining by the late 1800's, reduced to near cessation the use of this technique. In the last two decades however, due to increasing gold prices and hardening of social-economic condition in the developing world, this technique has been resuscitated, in particular in countries of tropical Asia and Latin Amercia. At the Amazon basin in particular, this technique represents a simple, cheap and reliable option for gold production, ready to be used by a growing population lacking proper jobs in most developing countries of this region (Lacerda *et al.*, 1995). Present annual emission of Hg to the environment from this activity to the Amazon basin may reach up to 200 tons, being Brazil, Venezuela, Guiana and Colombia the largest emitters (Lacerda, 1997a).

Following extensive surveys on the Hg concentrations in many natural compartments in the Amazon basin, including fish and humans (see Lacerda & Salomons (1997) for a review), increasing evidence shows that human risks may be not directly related to Hg loads to a given area, but rather to some key biogeochemical processes contyrolling the mobility and bioavailability of Hg (Lacerda & Salomons, 1997; Roulet & Lucotte, 1995; Forsberg *et al.*, 1997). Therefore, a proper assessment of the potential contamination of Amazon natural resources and its population, is dependent not only on accurate Hg determinations in environmental compartments, but mostly on a reasonable knowledge of the biogeochemistry of the complex Amazon ecosystems, in particular on the biogeochemical

reactions controlling the Hg cycle and availability to fish in this environment. In the present paper I will discuss some theorethical framework of the biogeochemical cycle of Hg which may affect the present and future contamination of the region. It is of key importance to note that, regardless of the future trends in emissions, the amount of Hg already deposited in Amazon ecosystems, which also includes the Hg from the old Spanish silver mining operations, may, under certain circunstances, be remobilized to ecological cycles. The accelerating land use changes in the region may also accelerate Hg remobilization processes, and particular characteristics of some Amazon environments may create ideal conditions for Hg methylation and accumulation through the food chain.

Mercury uses and emissions

A typical characteristic of the Amazon gold mining is the low efficiency of the process due to the intuitive use of Hg. Average Hg emission factor from this activity is approximately 1.3 kg of Hg per kg of gold produced (Lacerda *et al.*, 1995). Most (55 to 70%) of the Hg is emitted to the atmosphere during the burning of the Hg-Au amalgam, whereas from 30 to 45% is emitted to river banks, soils and drainages. Emissions from amalagam burning are mostly of Hg^0-vapor, whereas from gold purification, a fraction is also emitted as Hg-rich particles and possibly as Hg^{2+} and HgO (Marins et al., 1991; Marins & Tonietto, 1995). For example, Marins et al. (1997), studying the speciation of Hg in rural and urban air, showed that over rural sites, Hg^0-vapor makes up to 99% of the total Hg content in air. In urban air however, this species of Hg may range from 70 to 99% of the total Hg content in air, confirming the existence of other Hg species in the emission from gold purification.

Direct emissions to rivers, soils and tailings constitute basically of metallic Hg^0 - liquid. The dominance of the atmospheric emission results in relatively large atmospheric deposition rates, typically ranging from 40 to 200 μgHg. m^{-2}.$year^{-1}$, over areas surrounding gold mining sites (Lacerda & Salomons, 1997). As a result, Hg contamination of soils is common. Another source of Hg re-emission to the atmosphere is the mine tailings left by ancient and recent gold and silver mining operations. Degassing from tailings range from 50 to 1,500 $\mu g.m^{-2}.yr^{-1}$, much higher than over mineralized areas containing cinnabar deposits, where degassing rates can reach up to 60 $\mu gHg.m^2.yr^{-1}$ (Rasmussen, 1994). These rates however, are similar to those measured over Hg mining tailings, such as in Almadén, Spain, where degassing may reach extremely high values of up to 2,600 $\mu gHg.m^{-2}.yr^{-1}$ (Ferrara & Marseti 1994).

Apart from gaseous Hg, tailings may also emit heavily contaminated dust particles to the atmosphere. Extremely high Hg concentrations have been reported in this compartment. For example, Hg concentrations in soil dust collected at small Amazon towns, where large amounts of gold are commercialized, reach up to 36 $\mu g.g^{-1}$ and 250 $\mu g.g^{-1}$ of dust (Malm *et al.*, 1991; Thornton *et al.*, 1992, respectively), representing na enrtichment factor of 100 to 1,000 times the average Hg concentrations found in nearby soils. This compartment shows a very short residence time in the atmosphere. However, it has been reported as a significant source of human Hg exposure, through inhalation, in gold mining centers in Central Brazil (Hacon *et al.*, 1997).

Emission and re-emission of Hg to the atmosphere is of key importance to the Hg cycle in the Amazon region, since it provides rapid oxidation of Hg^0 under the atmospheric conditions of the Amazon, in particular during the dry season when a 5-times enrichment of ozone and a 100-times increase in atmospheric suspended particles due to forest burning and dust ressuspension, facilitate the oxidation processes. Therefore, most Hg reaching Amazon soils is Hg^{2+} and since the Hg species directly released into rivers and soils and left in tailings are associated with residual minerals or as

elemental Hg^0 , and even amalgamated with gold grains (Ching & Hongxiao, 1985), atmospheric Hg is probably the sole source of potentially methylated Hg to Amazon ecosystems.

The average atmospheric deposition of Hg over the Amazon region has been estimated by some authors (Lacerda, 1997b). Lacerda et al. (in prep.) based on the distribution of Hg in sediment cores collected in remote lakes at the Carajás Mountains in SE Amazon, found pre-colonial Hg deposition to be of circa 5.0 $\mu gHg.m^{-2}.yr^{-1}$, whereas modern deposition rates to the same lakes reach approximately 12.0 $\mu gHg.m^{-2}.yr^{-1}$. Other authors estimated Hg deposition rates by considering the emission to the atmosphere calculated from gold production and dividing by the total Amazon area. Although these are in general much more variable, the results are comparable to those measured in remote Amazon lakes, ranging from 8.0 to 13.0 $\mu gHg.m^{-2}.yr^{-1}$ (Forsberg et al., 1997). However, when deposition rates are measured close to mining sites, either from direct collection of bulk atmospheric deposition or using indirect methods such as lake sediment cores, they are much higher. In general, deposition rates range from 60 to 110 $\mu gHg.m^{-2}.yr^{-1}$ (Lacerda et al., 1991; Tumpling et al., 1995).

Taking into consideration the assumptions made above, Hg contamination of the Amazon environment is typically from a diffuse source (mostly from atmospheric deposition), with relatively small load to area ratio. This results in a regional rather than local aspect to the problem. Local contamination by Hg^0-metallic, although very high in some sites, such as in areas influenced by mine tailings, is probably of secondary importance, due to the very low bioavailability of this form of Hg, and therefore presents a low risk factor. However, these sites may be much more significant as a secondary source of Hg, through volatilization of the deposited Hg to the atmosphere.

Fate of mercury in Amazon forest soils

Considering the potential large-scale dispersion of atmospheric Hg in gold mining areas, it is expected that soils of large areas should also be contaminated. However, notwithstanding the importance of such compartment for the understanding of Hg cycling in the Amazon region, few studies have dealt with it.

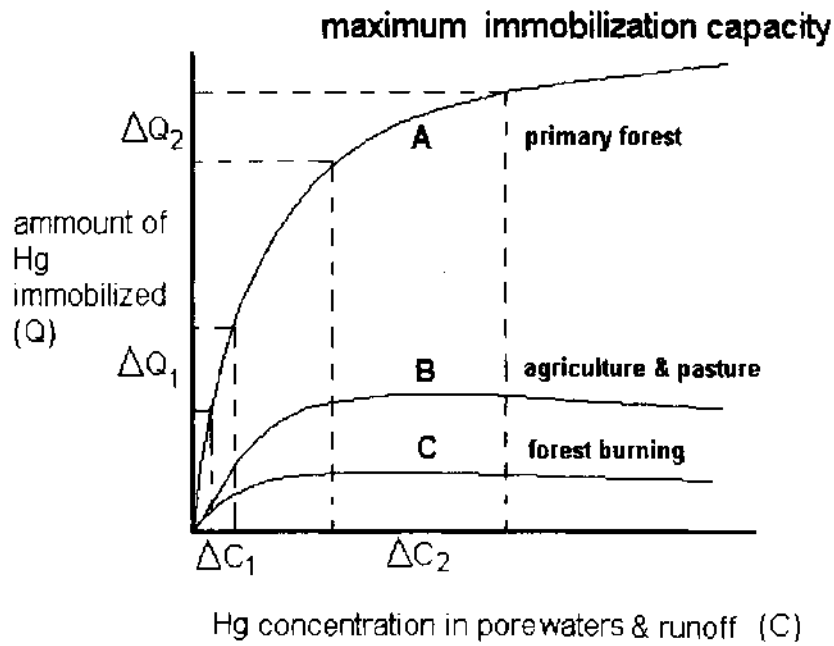
Forest soils from the Madeira River watershed, Northwestern Amazon, were analysed by Lacerda et al. (1995) and Malm et al. (1991) and showed Hg concentrations ranging from 35 to 300 $\mu g.kg^{-1}$ and 30 to 340 $\mu g.kg^{-1}$, respectively. The highest values were found close to river sections where intense mining takes place. These authors associated the high Hg content found in the top soil, with atmospheric deposition and with the high (up to 27%) organic matter content of the top soil. In another study in Poconé, Central Brazil, soil samples from a 10,000 km^2 area under the influence of various mining sites, showed very low Hg concentrations, being smaller than 30 $\mu g.kg^{-1}$ (which is considered the local background Hg concentration), in 70% of the analysed samples. In circa 30% of the samples, Hg concentrations ranged from 30 and 100 $\mu g.kg^{-1}$. Close to mining sites however, Hg concentrations reached 270 $\mu g.kg^{-1}$ (Lacerda et al., 1991). In another large survey of forest soils in Alta Floresta, Southern Amazon, covering na area of over 5,000 km^2, Hg concentrations ranged from 20 to 210 $\mu g.kg^{-1}$ (Souza, 1997). In French Guyana, forest soils presented maximum Hg concentrations of 320 $\mu g.kg^{-1}$ (Roulet & Lucotte, 1995), wheres in the Venezuela, forest soils showed average Hg concentrations of 103 $\mu g.kg^{-1}$ (Sherestha & Quilarte, 1989). Recently Hg concentrations ranging from 120 to 240 $\mu g.kg^{-1}$ and 200 $\mu g.kg^{-1}$ were reported for the Negro River and Tapajós basins (Forsberg et al., 1997).

Temperate forest soils in general, show Hg concentrations between 70 and 300 $\mu g.kg^{-1}$ (Mitra, 1986), but can reach up to 880 $\mu g.kg^{-1}$ in German forests and 190 to 240 $\mu g.kg^{-1}$ in Scandinavian forests (Godbold, 1994). The values reported for the Amazon forest soils, with the

expection of those reported for French Guyana, seem to be much lower, in particular what is considered the regional background Hg concentrations, which consistentely falls below 50 $\mu g.kg^{-1}$ in most Amazon soils. Rather than the total Hg concentrations or loads to a given area however, the behavior of Hg in the Amazon soils is the most important variable controlling the Hg cycling in the Amazon ecosystems.

The behavior of a functioning Amazon forest soil in relation to the immobilization and mobilization of Hg is described in figure 1.1, modified and adapted from Stigliani (1995). For any given point on the curve, the vertical axis shows the quantity of Hg immobilized by the soil, i.e. the bulk accumulation in soil compartments. The horizontal axis shows Hg amount in mobilized phases, in the case of Amazon soils, mobilized phases are mostly dissolved species in soil pore waters and runoff. The slope of the line at a given point ($\Delta Q/\Delta C$) gives the accumulation capacity of the soil to Hg.

Figure 1. General curve describing the hypothetical behavior of Hg in Amazon soils, modified and adapted from Stigliani (1995). A - pristine primary forest, B - agriculature, pasture and secondary growth forest soils, C - forest removal.



At the beginning of the contamination process the immobilization capacity is higher (see $\Delta Q1/\Delta C1$), as the contamination proceeds, immobilization capacity decreases as in $\Delta Q2/\Delta C2$, until a maximum accumulation capacity is reached where immobilization tends to zero, meaning that the soil is saturated and no Hg can be immobilized (Stigliani, 1995). At this point any extra load of Hg will rapidly be mobilized through soil pore water and runoff and may reach fluvial systems. However, it is quite possible that even before reaching this threshold, Hg concentrations would already be higher than the standard values for the protection of wildlife, and may become a health risk for humans through inhalation of vapor and particles.

Since the processes involved in the immobilization capacity and therefore of the maximum accumulation capacity of a soil, will include biogeochemical properties of a given site, soils under

different uses will show different maximum accumulation capacity. Also, it seems that the immobilization of Hg in Amazon soils is tightly linked to the ecosystem health. In particular on the maintenance of the complex cycle of nutrients and water between the soil and the forest Once the pristine conditions are changed (e.g. through deforestation, changing into agriculture or pasture, road clearing etc.), which accelerate Hg vaporization and favor soil mass tranfer by runoff, larger amounts of Hg will be mobilized and with faster rates of transfer.

Fate of mercury in Amazon agriculture soils

Drastic land use changes are occurring in the Amazon forest during the last three decades. In particular deforestation, reaching rates ranging from 7,000 to 21,000 km^2 per year. These large deforestation rates cause increasing soil erosion and eventually increase sedimentation rates in natural and man-made aquatic ecosystems (Forsberg *et al.*, 1989; Skole & Tucker, 1993; Lacerda, 1995). Conversion of forest to pasture has a significant effect on Hg deposited in soils. Through increasing albedo and therefore soil temperatures and decreasing canopy roughness, re-emission of deposited Hg is enhanced, since most of the Hg present in the soil is accumulated in surface horizons (Grigal et al., 1994). If deforestation is reached through forest burning, atmospheric conditions are also changed, in particular by increasing ozone and suspended particles concentrations which accelerate the oxidation of elemental Hg^0 vapor to ionic Hg^{2+} (Lacerda & Salomons, 1997). This decreases the residence time of Hg in the Amazon atmosphere, augmenting Hg deposition close to sources, under a chemical form (Hg^{2+}) ready for methylation.

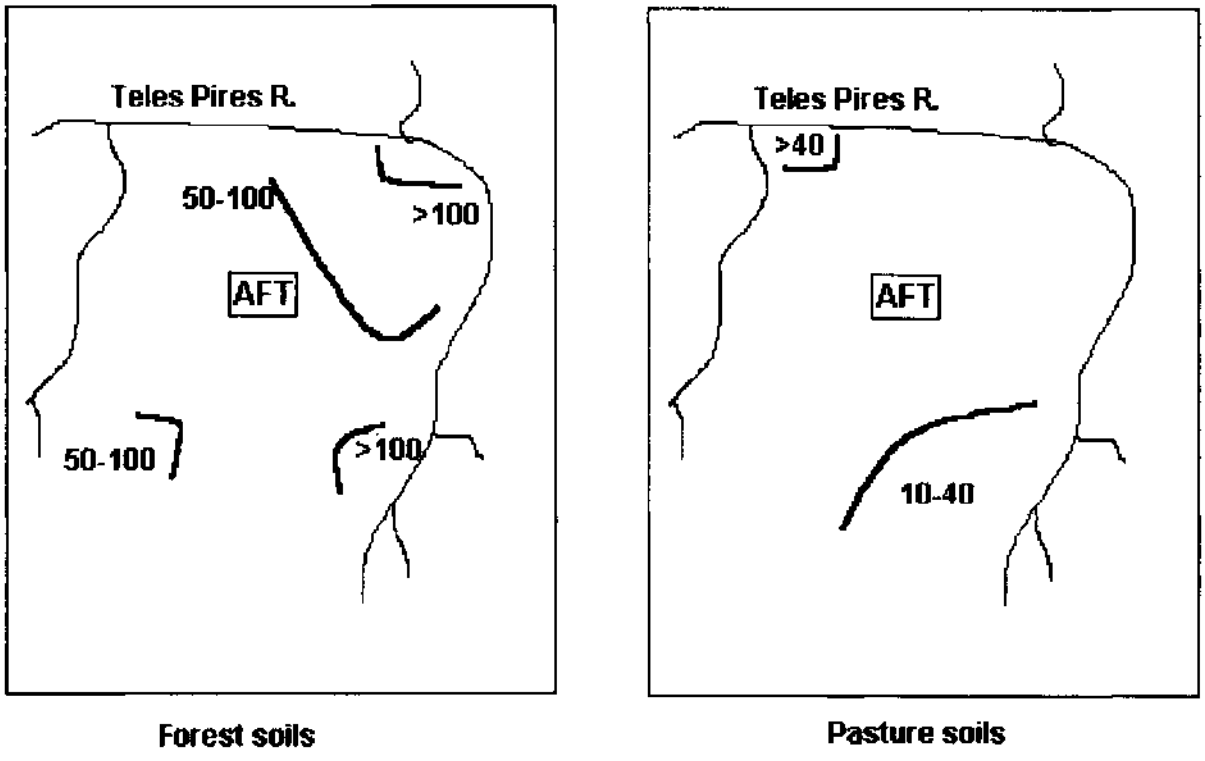
Agriculture practices reduce soil organic matter content in tropical environments, whcih regulates Hg retaintion in forest soils (Grigal et al., 1994). These practices will also increase soil erosion, accelerating the transport of deposited Hg to water courses, and may result in fish contamination. Soil erosion due to deforestation in the Amazon basin may also affect the soil Hg burden accumulated at least since the Spanish silver mining. At least for certain areas, mobilization of the soil burden due to soil erosion, has been suggested as the major source of Hg to fish.

An example of the effect of changing land use onto the Hg distribution in surface Amazon soils is shown in figure 2. It shows iso-concentration curves representing Hg distribution in surface soils, based on pair of soil samples collected simultaneously in forest and pasture areas in Alta Floresta, Southern Amazon. It is clear from comparing the curves that, although located at the same relative distance to sources, soil Hg content is much lower than forest soil. Forest soils showed Hg concentrations ranging from 27 to 200 $\mu g.kg^{-1}$, whereas pasture soils ranged from 10 to 42 $\mu g.kg^{-1}$. This suggests that larger Hg interception may occur over forests due to more roughness of tree conopy surfaces, and changing wind circulation. But more important, it shows that Hg is being re-emitted from pasture soils very efficiently, reducing Hg content by a factor of 2 to 100 relative to forest soils (Lacerda & Salomons, 1997). Similar results were encountered by Grigal et al. (1994) in East Central Minessota landscape, USA, and seems to be a general characteristic of pasture soils compared to forest soils.

Estimates of Hg residence times in forested soils reach over 1,000 years. Therefore, soils are in general considered as a sink to Hg. However, changes in soil use, such as in those soils shown in figure 2, may have reduced Hg residence time to a few days to months in pasture areas. Mercury re-emission from these pasture soils will also affect increasingly larger areas. The increasing of the Hg residence time in the soil will be a function of the time necessary to a pasture or agriculture soil, to re-aquire its forest characteristics, and in general this time span is in the order of 100 years, for temperate soils (Grigal et al., 1994). For tropical Amazon soils, this is mostly unknown. However,

since the entire structure and nature of the soil is dependent on the presence of the forest itself, this time may be much longer.

Figure 2. Mercury concentrations ($\mu g.kg^{-1}$) in surface soil samples, collected at the same sites under forest and pasture in Alta Floresta, Southern Amazon, modified after Lacerda & Salomons (1997). (AFT - town of Alta Floresta).



The behavior of Hg in Amazon soils profiles, which could help estimating Hg residence time were only studied in two remote sites in French Guyana and Southern Para State, Brazil. The results of these studies showed a significant accumulation of Hg in surface horizons just below the litter layer, due to higher organic matter content and a secondary accumulation peak at Fe-rich horizons (Aula *et al.*, 1994; Roulet & Lucotte, 1995). Roulet & Lucotte (1995) suggested that Hg, after crossing the surface organic-rich horizon, is leached through the soil column associated with humic substances and accumulates in Fe-rich horizons at relatively shallow depths, ranging from 20 to 30 cm, in a process of long term accumulation which results in high Hg concentrations regardless of existing Hg sources. Laboratory experiments, however, showed that once organic matter is absent from the soil column, inorganic Hg is rapidly leached to deeper horizons or to underground waters (Semu *et al.*, 1987). Therefore, a generalization on the behavior of Hg in Amazon forests is still far from reaching, in particular on the average residence time of Hg.

Fate of Hg in Amazon flooded soils

Apart from forest soils, aquatic environments also receive a significant fraction of the total anthropogenic Hg input. Amazon rivers are characterized by large flood plains areas where soils are inundated 4 to 8 months per year, during the rainy season. While in major large Amazon rivers,

dilution will renders low Hg concentrations, accumulation in flood plain soils and oxbow lakes may result not only in high Hg concentrations but also in increasing reactivity of Hg chemical species, since these environments are typically highly productive, with intense bacterial activity and with strong interaction with the forest itself. These areas are also key environment for local fish communities which use them as nursery grounds and for feeding directly on forest products during flood periods.

Artificial flooding of river basins to create power plant reservoirs has also been a major land use change in the Amazon region. Presently this form of land use covers over 5,000 km^2 in the Amazon. These reservoirs are created by the flooding of forest areas without the removal of the forest biomass. Reservoirs have a poor water circulation, high residence time and reducing conditions in the its bottom waters with extremely high rates of microbial activity. These reservoirs accumulate Hg carried in associated with river suspended matter and from atmospheric deposition, which can be significant due to the surface of most Amazon reservoirs. Mercury methylation rates are from 3 to 5 orders of magnitude higher in artificial reservoir than in most Amazon rivers. Also, when creating a reservoir, changes in food chain structure result in the dominance of top carnivorous fish species, which accumulates higher Hg concentrations than lower trophic level species. And since fisheries become a major use of these new flooded areas, the potential transfer of Hg to humans is highly enhanced.

In Canada, elevated Hg concentrations have been measured in fish from artificial reservoirs. Various authors (see review in Lacerda & Salomons, 1997) found higher concentrations in top carnivorous species after few years of creating the reservoir. For example, various studies found a two-fold increase of Hg concentrations (from c.a. 0.6 to c.a. 1.2 $\mu g.g^{-1}$ w.w.) in the muscle tissues of Pike (*Esox lucius* L.) after 4 years of the reservoir formation. They related that to higher methylation rates under the new conditions provided by the reservoir, i.e. increasing organic matter content, increasing bacterial activity and remobilization of Hg formerly present in soils. Similar results have been presented for other areas (Olgivie, 1981; Simola & Lodenius, 1982; Nuorteva et al., 1979, among others).

In French Guyana, Roulet & Lucotte (1995) suggested that a large fraction of Hg present in flooded soils is released to the water column upon flooding, due to the reduction of Fe hydro-oxides rich soil layers, also enriched in Hg. Aula et al. (1994) also suggested that a fraction of the Hg responsible for the extremely high concentrations found in fish of the Tucuruí reservoir is derived from flooded soils. Average Hg content of carnivorous fish (*Serrasalmus nattereri*) in this reservoir reach 2.9 $\mu g.g^{-1}$d.w., whereas other top carnivorous such as alligators, showed up to 19 $\mu g.g^{-1}$d.w. of Hg in liver.

Limnological conditions prevailing in Amazon reservoirs and flood plain areas also increase the bioavailability of Hg to fish. These waters are frequently acidic and presents high dissolved organic carbon concentrations. Dissolved organic carbon and pH are reported as the key variables controlling Hg concentrations in predatory fish. Dissolved organic carbon facilitates the transport o Hg from soils to rivers and its accumulation in aquatic environments, also a large fraction of Hg is kept in solution associated with organic compounds. Low pH reduces Hg losses to the atmosphere and favors methylation and bioaccumulation of Hg in aquatic food chains. Since Hg content in fish-eating human populations is a function of Hg in fish and intake frequencies, these variables also seem to control Hg burden in humans (Forsberg et al., 1997). High Hg concentrations in hair from humans living along the Negro River and the Tucuruí reservoir, both areas relatively far from any gold mining site, presented very high Hg content, even higher than those measured in mining sites (Forsberg et al., 1997; Aula et al., 1994).

Biogeochemical considerations

Although there is information available on the cycling of metals in temperate forest ecosystems to infer and predict some of their impacts, the tropical ecosystem differs in too many ways to apply this knowledge without critical evaluation. The cycling of inorganic substances in the Amazon forest is very efficient. Biological interactions at the root-soil level (micorhiza) and at the atmosphere-canopy level (epiphylae and high biomass and diversity of epiphytes), are extremely efficient for the uptake of nutrients from atmospheric precipitation and fast incorporation into the plant biomass (Jordan et al., 1980). In temperate forests this pathways of nutrients is negligible. In temperate ecosystems the larger nutrient reservoir is the soil, whereas in tropical forest it is the plant biomass itself. Even when these nutrients are eventually returned to the soil as plant litter, fast decomposition rates mediated by root-associated fungi, recycle them very fast avoiding large losses to sub-soil and out of the ecosystem (Benzing 1981; Nadkarni, 1984).

The major pathway for Hg in the Amazon is the atmosphere. Mercury vapour is ready absorbed by plant leaves from the atmosphere (Braune & Fang, 1978). Therefore, it is likely that it will follow the same pathways as nutrients do. If this occurs in tropical rain forest trees, it is a major difference between the fate of Hg in temperate ecosystems, in which immobilization in soils and in other slow cycling compartments dominate. In most temperate ecosystem when sources of a given contaminant are controlled, one can expect that through time it will eventually accumulate in long term sinks, and that concentrations in cycling compartments will constantly decrease, unless the capacity of the sink is exceeded or external changes occur (time-delayed responses). Through efficient recycling in Amazon ecosystems, Hg (and other contaminants) just move from one cycling, biological compartment to another, increasing the probability of organification and accumulation in high trophic levels animals. The possible accumulation of pollutants in the biomass (tropical system) instead of sinks like soils (temperate systems), makes it much more difficult to manage the system once pollutants have entered it.

Unfortunatelly there is no database yet to test this hypothesis. The few data from surveys of Hg concentrations in soils generates soil burdens at least conflicting. Most soil Hg data, outside the influence of a mining operation falls between 10 to 220 $\mu g.kg^{-1}$, resulting in a total amount of Hg accumulated in the top 10 cm of soil ranging from 5.0 to 110 $g.ha^{-1}$. Data on Hg distribution in the forest biomass are still more scarce, but very preliminary results give a Hg burden in the biomass of Amazon forests ranginfg from 6 to 10 $g.ha^{-1}$ (Lacerda, 1995). Therefore, it can be either similar or one order of magnitude lower than the soil burden.

Biological diversity in most tropical ecosystems is strongly dependent on the structure of food webs, which are in general controlled by top predators. It is exactly in these animals that Hg should accumulate, and where its toxic effects should appear first. Therefore, once high trophic levels species are affected, whole food chains can also be affected resulting in rapid changes in communities structure. It is likely that fish communities would be one to be highly affected. Fish, apart from forest biomass itself is the major valuable natural resource in the Amazon for the local population. Therefore any changes in such important resources may led to significant economic constrains for the region.

The picture presented on the fate of Hg in a tropical system needs more validation and more research. However, the important differences with temperate climates is the high turnover and the biomass as the main reservoir of nutrients (and probably of pollutants). This fact makes it very difficult to manage the system when pollutants enter its intricate element cycles. Once they have entered it, it will be more difficult to control them compared with the temperate climate ecosystems.

The question whether Hg (and other pollutants) in the Amazon or in any other tropical ecosystem presents a potential "chemical time bomb" with its associated time-delayed and

spatially-displaced responses has to be answered in the positive sense. However three remarks have to be made to be put this conculsion into the context of the more detailed experience from temperate climates:

- the "chemical time bomb" in a tropical ecosystem will be much more difficult to control due to the predominant role of the biomass as a sink.
- the existence of a "chemical time bomb" has to be substantiated with more research. Not with isolated studies, not with multi-disciplinary research but with integrated research with the functioning of the ecosystem as its focal point.
- if ever the Hg released in the Amazon, develops its health effects as in other contaminated sites, the dependence of the local population on the region's natural resources, will certainly results in a regional disater

Acknowledgements

This paper is part of a conference given by the author at the national Insitute of Minamata Disease (NIMD), during its Forum'97. I thank the NIMD for provinding the neceessary funds for that visit, in particular Prof. H. Akagi, Chjief of International Affairs of NIMD. Thanks are also due to the Conselho nacional de Desenvovlvimento Científico e Tecnológico (CNPq-Brazil), for providing continuous support for my field research in the Amazon.

Reference

Aula, I., Braunsweiller, H., Leino, T., Malin, I., Porvari, P., Hatanaka, T., Lodenius, M. and Juras, A. (1994) Levels of mercury in the Tucuruí reservoir and its surrounding area in Pará, Brazil. *In*:. Mercury Pollution: Integration and Synthesis. (Watras, C.J. & Huckabee, J.W., Eds.), pp:21-40 Lewis Publ., Boca Raton, Louisiana/USA.

Benzing, D.H. (1981) Mineral nutrition of epiphytes: na appraisal of adaptative features. *Selbyana*, **5**, 219-223.

Browne, C.L. and Fang, S.C. (1978) Uptake of mercury vapor by wheat: na assimilation model. *Plant Physiol.* **61**, 403-433.

Ching, L and Hongxiao, T. (1985) Chemical studies of aquatic pollution by heavy metals in China. *In*: Proc. Workshop Environmental Inorganic Chemistry, (Irgolic, K.J & Martell, A.E. Eds.), pp 359-371, San Miniato/ Italy.

Ferrara, R. and Maserti, B.E. (1994) Mercury degassing rate in some mediterranean areas. *In*: Procc. 2nd International Conference on Mercury as a Global Pollutant. Whistler, B.C., Abstract **8B**.

Forsberg, B., Godoy, J.M., Victoria, R.L. and Martinelli, L.A. (1989) Development and erosion in the Brazilian Amazon. A geochronological case study. *GeoJournal*, **19**, 402-405.

Forsber, B.R. et al. (1997) **Personal Communication**

Godbold, D. (1994) Mercury in forest ecosystems: Risk and research needs. *In*: Mercury Pollution: Integration and Synthesis (Watras, C.J. & Huckbee, J.M. Eds.), pp:295-303. Lewis Publ., Boca Raton, Louisiana/USA.

Grigal, D.F., Nater, E.A. and Homann, P.S. (1994) Spatial distribution pattern of Hg in na East Central Minesota Landscape. *In*: "Mercury Pollution: Integration and Synthesis" (Watras, C.J. & Huckbee, J.M., Eds.), pp:305-312.. Lewis Publ., Boca Raton, Louisiana/USA.

Hacon, S.S., Rochedo, E.R.R., Campos, R.C., Rosales, G. and Lacerda, L.D. (1997) Risk assessment of mercury in Alta floresta, Amazon basin - Brazil. *Water, Air & Soil Pollut.*, **97**, 209-216

Jordan, C.F., Golley, F,B., Hall, J. and Hall, J. (1980) Nutrient scavenging of rainfall by the canopy of an Amazonian rain forest. *Biotropica*, **12**: 61-66.

Lacerda, L.D. 1995. Amazon mercury emissions. *Nature*, **374**, 20-21.

Lacerda, L.D. (1997a) Global emissions of mercury from gold and silver mining. *Water, Air & Soil Pollut.*, **97**, 209-221.

Lacerda, L.D. (1997b) Atmospheric mercury and fish contamination in then Amazon. *Ciência & Cultura*, **49**, 54-57.

Lacerda L.D., Marins R.V., Souza, C.M.M., Rodrigues, S., Pfeiffer, W.C. and Bastos W.R. (1991) Mercury dispersal in water, sediments and aquatic biota of a gold mining tailings drainage in Poconé, Brazil. *Water Air & Soil Pollut,*. **55**, 283-294

Lacerda, L.D., Malm, O., Guimarães, J.R.D., Salomons, W. and Wilken, R-D. (1995) Mercury and the new gold rush in the South *In*: Biogeodynamics of Pollutants in soils and Sediments, (Salomons, W; Stigliani, W.M., Eds), pp 213-245, Springer Verlag, Berlin/Germany.

Lacerda, L.D. & Salomons, W. (1997) Mercury from Gold and Silver Mining: A Chemical Time Bomb. Springer Verlag, Berlin, 184p.

Malm, O., Pfeiffer, W.C. and Souza, C.M.M. (1991) Main pathways of mercury in the Madeira River area, Rondonia, Brazil. *In*: Proc 8th Intern Conf Heavy Metals in the Environment, pp: 515-518, Edingburgh/UK.

Marins, R.V., Imbassay, J.A., Pfeiffer, W.C. and Bastos, W.R. (1991) Contaminação atmosférica de mercúrio em area produtora de ouro no distrito de Poconé, Mato Grosso, MT. Proc. I Inter. Simp. Environm. Studies Tropical Forest. Manaus, **1**, 199-203.

Marins, R.V. & Tonietto, G.B. (1995). An evaluation of the sampling and measurements techniques of mercury in the air. *In*: Proc. 5th Brazilian Geochemical Congress, Niterói, Analytical Geochemistry Section, **1**, 1-4.

Marins, R.V., Andrade, J.B., Perereira, P.A., Paiva, E.C. and Paraquetti, H.H.M. (1997). Evaluation of sampling techniques for speciation of vapor and particulate Hg in the assessment of atmospheric pollution in the Amazon. *The Analyst* (**submitted**).

Mitra, S. (1986). Mercury in the ecosystem. Transtech. Publ., Switzerland.

Nadkarni, N.H. (1984) Epiphyte biomass and nutrient capital of a neotropical elfin forest. *Biotropica*, **16**, 249-256.

Nuorteva, P.; Lodenius, M. & Nuorteva, S.L. 1979. Decrease in Mercury levels of *Esox lucius* (l.) and *Abramis farenus* (L.) (teleostei) in the Haneenkyro water course after the phenylmercury ban in Finland. *Aquilo. Ser.*, **19**, 97-100.

Ogilvie, D.M. (1981). Mercury clean-up in Canada's lake St. Clair. *Ambio*, **10**, 350-351.

Simola, H. and M. Lodenius (1982) Recent increase in mercury sedimentation in a forest lake attributable to peatland drainage. *Bull. Environm. Contam. Toxicol.*, **29**, 298-305.

Nriagu, J.O. (1994) Mercury pollution from the past mining of gold and silver in the Americas. *Sci. Tot. Environm.*, **149**, 177-182.

Rasmussen, P.E. (1994) Current methods of estimating atmospheric mercury fluxes in remote areas. *Environm. Sci. Technol.*, **28**, 2233-2241.

Roulet, M. and Lucotte, M. (1995) Geochemistry of mercury in pristine and flooded ferralitic soils of tropical rain forest in French Guiana, South America. *Water, Air Soil Pollut.*, **80**, 1079-1088.

Skole, D. and Tucker, C.J. (1993) Tropical deforestation and habitat fragmentation in the Amazon: satellite data from 1978 to 1988. *Science*, **260**, 1905-1910.

Souza, M. (1997) Distribuição de mercúrio em solos de floresta e pasto na região de Alta Floresta, Mato Grosso. Dissertação de Mestrado, Universidade Federal Fluminense, Niteroi, Rio de Janeiro/Brazil.

Stigliani, W.M. (1995) Global perspective and risk assessment. *In*: Biogeodynamics of Pollutants in Soil and Sediments. (W. Salomons & W.M. Stigliani, Eds.), pp. 331-344,Springer Verlag, Berlin/Germany.

Thornton, I., Cleary, D. and Worthington, S. (1992) Mercury contamination in the Brazilian Amazon. A cooperative Research study conducted by Gedebam, Brazil e Sol 3 (Switzerland and Luxemburg), p 45.

Tumpling, W.V.; Wilken, R.D. and Einax, J. (1995) Mercury contamination in the northern Pantanal region, Mato Grosso, Brazil. J. Geochem. Explor., 52, 127-134.

Mercury from gold production in Brazil: problems and solutions to date

Roberto C. Villas Bôas*, Laura Barreto* and Ricardo Melamed*

CETEM - Centro de Tecnologia Mineral, Rua 4, Quadra D, Cidade Universitária, Ilha do Fundão, CEP 21941-590, Rio de Janeiro, Brazil

1. Introduction

Due to its geological fate the rainforest areas of the world are a rich compartment for secondary to primary gold ore deposits. Thus, colluvial, alluvial and near the surface ore bodies are all scattered in these regions, promoting a nice business environment for the "*garimpeiros*".

For this reason, mercury is widely utilized in gold extraction since it readily amalgamates and the resulting amalgam is easily broken by fire. At these operations, amalgamation and firing, sources of mercury releases to the environment are present; when mercury is introduce to amalgamate it is seldom handled in a close-circuit; the same being true when it is released from the amalgam, generally at open air.

The problems regarding elemental and other forms of mercury in the environment and local population are all well discussed and documented in the literature, see for instance, LACERDA and SALOMONS (1992), BARRETO (1993), SILVA (1995), AKAGI et al. (1996), and VILLAS BÔAS (1997), for the brazilian case.

2. Legal aspects

The Brazilian Mining Law, approved in 1967, defines the profile of the "garimpeiro" as a professional who works the outcropping deposits (typically alluvium, alluvium and colluvial deposits) manually (with the help of tools). Ideally, he should be an individual professional without economic and technical resources, who would make "garimpo" mining his means of subsistence. Because of this technical and economic limitation, the damage to the mineral reserves, even in the case of ambitious mining practice (predatory mining), would be negligible.

The most recent Brazilian Constitution (1988), favors the "garimpeiro" - even in detriment to the constituted mining activity - according to many - and gives the Federal Government the power to establish areas and conditions for the "garimpo" activity (Art. 21, XXV and Art. 174, paragraphs 3 and 4). The aim is to encourage the "garimpeiros" to associate in cooperatives, and to do so, gives them priority for prospecting and mining the deposits that could be exploited by the "garimpo"- in areas where they are already working at in other areas that may be legally determined.

Up to 1988, there was no reference made whatsoever in any legal document, to the "garimpo", as a mining activity with rights and responsibilities, rather than a mining activity always subordinate to the prospecting and mining systems. The Constitution raised the "garimpo" activity to mining system "status", recognizing it as an economically profitable and socially desirable activity.

* Corresponding authors:
Fax: 5521 260-2837; E-mail: villasboas@cetem.gov.br; lbarreto@cetem.gov.br, rmelamed@cetem.gov.br

The "Regime de Permissão de Lavra Garimpeira" ("Garimpo" Mining Permit System) was instituted as a result of these constitutional provisions and can basically be defined as the system to be applied to the alluvium, alluvium, colluvial or other deposits as defined by the DNPM ("Bureau of Mines") that may be mined without the need for previous prospecting work. This law can only be applied inside well-defined areas.

The "Garimpo" Mining Permit introduced a new mining system, with rights and responsibilities, defining the difference between the Concession and the permit systems as: the type of deposits that may be worked by the "garimpo", the individual work, and the absence of mineral prospecting studies. The cooperative was chosen as the type of organization, because in the constitutional legislator's evaluation, this would hasten the social and economic development of the "garimpeiros" and make environmental preservation feasible.

These distinctions between the systems are, in fact, strictly partial, which means, for example, alluvium, alluvium and colluvial mineral deposits can be the subject of concessions under the mining concession system. On the other hand, the "Garimpo" mining permit, although a simplified mining system, may require mineral prospecting studies. The difficulty of distinguishing between the two systems has led inevitably to persistent conflicts between the two main economic agents: mining companies and "garimpeiros".

Law No. 7805/89, which instituted the "Garimpo" Mining Permit, mainly aims to discipline "garimpo" activity. However, the concept of a simplified system was affected by the difficulty of establishing a homogeneous picture of the role of the "garimpo" activity in the mineral and even in the national scenario. The result conceptions of the "garimpo" activity, which in practice brought about an overload System, according to corporate reasoning, and ignoring that of the "garimpo".

All these incongruities and evident contradictions denote the difficulty of adversely affecting the mining sector because of the increasing importance of the "garimpo" activity in recent years.

The priority given to the co-operative over other systems led the legislator to exclude the small and medium-size mining companies, meaning that a large part of the "garimpo" activity has evaded legal control. Such a rationale is much more business-oriented than cooperative or individual since "garimpo owners" are commonly known as "garimpo entrepreneurs". It seems necessary that the small and even medium-sized companies be recognized in the Brazilian mining scenario, not only because of the "garimpo", but principally for their own sake.

Reference to the small and medium-sized mining companies, means different rights and responsibilities from those of the so-called mining companies. This means a company with simplified legalization processes, taxed according to its size, although without losing its identity as a mining enterprise.

Equating the cooperative to the mining company is much more an enigma than a solution because, for logical and legal reasons, a cooperative is, and will always be, a cooperative and a company will always be a company. There are several types of companies, but they can never be mistaken for a cooperative.

Two points stand out in the current regulations: the priviledge of not having to carry out previous prospecting studies and doubts about the size of the area for the "garimpo".

Regarding the first point, both in the 1968 legislation and the current Law, one of the basic differences between the "garimpo" activity and mining companies was, and still is, precisely the non-existence or demand for mineral prospecting studies. This is not incidental, nor does this mean that the "garimpo" activity is being favored. The legislator's reasoning was to recognize the special nature of the "garimpo" activity due, essentially, to the type of deposit that can be mined. These are defined by law and are the alluvium, alluvium and colluvial deposits.

Regarding the second point, the size of the "garimpo" area, prevailing legislation determines that the "garimpeiros" are not allowed an area larger than 50 ha, and in spite of this restriction, this area is considered large, apparently without any plausible justification.

In short, this is a good reason, without knowing whether an area is larger Hanna 50, 100 or 200 ha, for having large areas. "Garimpo" is an activity where prospecting studies are not required for the reasons mentioned above; therefore it does not have previously delimited mines or deposits: the limits and ore contents are uncertain and are defined as the work progresses.

It would make no sense, for example, if a cooperative requested a mining permit, which is presently a very complex process, and after one month's work has to abandon the area because the deposit is not in the requested area, or because it is not economically feasible.

Obviously, in large areas this may also occur but on a smaller scale, and as part of the risk involved in the mining activity in general. It seems that an exaggerated limitation (e.g. 50 to 100 ha) in the case of the "garimpo" and specially in the Amazon region, will make it an extremely high risk activity, making in impractical or leading to illegal practice, as currently occurs.

In the case of "garimpo", some concepts and beliefs must be clarified. One of them refers to "garimpo" phenomenon itself. What is the reason for the existence of "garimpo" in Brazil? Generally, there is only one answer, whether from the progressive or conservative sectors: the reasons are exclusively social. The serious economic crisis in Brazil has brought to the "garimpo" a large number of unemployed people with no schooling or professional qualifications, who dedicate themselves to this activity as a last choice. Hence, if the social problem is solved, the "garimpo" problem would be solved.

Looking at the problem from another angle, there are geological reasons which motivated the appearance and increase of "garimpo" activity in Brazil. As long as these reasons persist, there will be "garimpo" activity in Brazil, regardless of the social reasons. These social reasons may aggravate the situation, but in themselves will never be determinant. This has to be proved not only empirically, but also technically and this means that if this is true, the solution is not outside the mineral sector and that the solution to the existing conflict between miners and "garimpeiros" must come from the mineral sector itself.

The attitude taken by current legislation shares the same idea: the creation of a new mining system is a clear example although, as explained above, this is still contradictory and incipient.

An aspect of the utmost importance in the solution to the "garimpo" problem is to know if is it possible to reconcile "garimpo" activity with environmental preservation? The answer to this question is crucial, since there is a progressive and inexorable movement in the direction of eliminating activities which are potentially and inexorable movement in the direction of eliminating activities which are potentially and inevitably polluting. Certain activities can be considered as causes of greater environmental impacts than others and would be the "naturally polluting". To eliminate such impacts requires the development of technology and investment in the production processes so that these activities would become economically impractical.

In activities that are essential to mankind, the economic cost/environmental improvement ratio may be counterbalanced by subsidies, exemptions and other forms of economic and non-economic incentives. However, the tendency in the activities defined here as naturally polluting is their transformation, when possible, from a technological and economic point of view, or their elimination.

In this aspect, is "garimpo" a naturally polluting activity? The answer to this question is complex, because in involves a complete analysis of the work methods and relations, the technology used, the environmental impacts, among other relevant aspects of the "garimpo" activity in itself, meaning that the answer at this moment must come from reflections based on discussions of the matter, rather than from results of studies on it.

Politically speaking, the matter is addressed in another way, considering that the "garimpo" activity intrinsic nature could be described as disorganized, and consequently detrimental to the mineral sector (the ore would not be suitably mined), to the environment and to society. Nevertheless, in innumerable "garimpo" sites throughout Brazilian territory, including the States of Amazonas, Roraima, Pará, Goiás, Amapá, Acre, Tocantins and Mato Grosso, to mention only the most important, there are people working according to determined methods; the objectives and

structured. At a "garimpo" it is immediately apparent who is in command, and it is easy to discover which task each "garimpeiro" is responsible for. This is also the case for the methods and instruments which are used for extracting the ore, or even how and by whom a certain deposit was found, what classes of "garimpeiros" exist (much more will be revealed to those who are interested and ask properly).

It is often assumed that mining companies are characteristically organized, while the "garimpo" activity is characteristically disorganized. There are disorganized companies as well as organized "garimpos" and, of course, the opposite is also true.

If there is a disorganized characteristic in the "garimpos" this is due to the fact that the cooperative's legal nature, does not fit in with the "garimpo's" reality, nor that of the "garimpo" workers, because they are neither partners, nor individual workers, but someone else's employees. Any effort at regulating the "garimpo" must keep in mind the question of adapting the law to the "garimpo" reality. When the distortion of the work is mentioned, this refers to a "garimpo" system that has not existed since the sixties, although this is the concept of the Código de Mineração (Mining Code) and also, in part, of the recent law. This concept is perhaps responsible for the current conflicts between "garimpeiros" and miners, which have led mining to become impractical in several regions of the country.

Disorganization is therefore not an intrinsic characteristic of the "garimpo". What are then the characteristics of the "garimpo"? What are the differences between a mining company's activity and the "garimpo" activity? The answers to all these questions are found in the law; but are they satisfactory? These questions and answers could help understand the complex reality of the "garimpo" and the regulation of this activity.

If disorganization is not a "garimpo" attribute, and a conciliation of the "garimpo" activity with environmental preservation is possible, it remains to briefly present the environmental regulations that apply to "garimpo" activities. In the first place it should be noted that there are no substantial differences between the regulations applied to the Permit System and those applied to the other Mining Laws.

The previous Constitution (1967) on which the Mining Code was based, did not foresee environmental rules that would cover the activity of the different economic agents; hence, the Mining Code deals with this matter in a sporadic way, and only regarding one point or other. The eighties are particulary important for Brazilian environmental legislation. A set of rules and new concepts, like that of environmental preservation were introduced in the 1988 Constitution, as well as in subsequent common law. The 1988 Constitution puts a great emphasis on the environment and requires that class action may void any act harmful to the environment. The Amazon Forest, the Mata Atlântica, the Serra do Mar, the Mato Grosso wetlands (Pantanal) and the Coastal Zone were declared Protected National Properties.

This legislation applies to all economic activities, including mining, although some constitutional principles had been established for the mining activity (these were demands that were previously established by law). Among them are: all activities that may cause any environmental degradation must, before being established, be preceded by an environmental impact study; responsibility for recovering the degraded environment is required from the miners; and physical or corporate agents responsible for conduct and activities considered to be harmful to the environment are subject to penal and administrative sanctions, regardless of the obligation to repair the damage caused.

On one side, the 1988 Constitution defines the exclusive competence of the Federal Government to legislate on mineral deposits, mines and other natural resources, on the other, it establishes the competence of the Federal Government, of the States and of the Federal District (DC) to legislate on the preservation of nature, protection of the soil and mineral resources and protection of the environment and pollution control. Accordingly, federal control of prospecting and mining of mineral resources, must observe the federal environmental legislation and the Normas Suplementares Estaduais Específicas (Specific State Supplementary Rules).

The "garimpeiro", as is the case of the miner, must request Environmental Licensing from the Órgão Estadual Ambiental (State Environmental Department) or from the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA - (Brazilian Environment Institute) (Hermann, H.; Fornasari Filho, N.; Loschl Filho, C., Universidade Estadual de Campinas, unpublished data).

Environmental licensing depends on an Environmental Impact Study - EIA. The Environmental Impact Report (RIMA) must reflect the conclusions of the for presenting details about the project and its environmental impact as well as to discuss the RIMA.

3. Sampling areas and results

The sampling areas where data were collected and solutions proposed for the minimization of mercury released to the environment, are: POCONÉ (Lat. 16° 16'S and Long. 56° 36'W), ALTA FLORESTA (Lat. 9° 52'S and Long. 56° 05'W), ITAITUBA (Lat. 4° 16'S and Long. 55° 59'W), PEIXOTO DE AZEVEDO (Lat. 10° 12'S and Long. 55° 03'W), and RIO PRETO (Lat. and Long.).

3.1. *Sediments, Air, Gold Shops, Tailings*

Background and sediment mercury concentrations and measurements in local air, gold shops and urban areas, were made, as well as Au/Hg ratio and the content of mercury in processed tailings determined.

These measurements and determinations were reported in the literature, see VILLAS BÔAS (1997), VEIGA et al. (1991), RODRIGUES FILHO and MADDOCK (1997), FARID (1992), BRAGA and ARAÚJO (1995), and ARAUJO and SANTOS (1995), all from CETEM. Tables 1, 2 and 3 summarize these findings.

Table 1 - Mercury concentrations in sediments, air, gold shops and tailings.

Area	**Mercury**[a]					
	Background (ppm)	Sediments Cn/Bn[b]	Air ($\mu g/m^3$)	Gold Shops ($\mu g/m^3$)	Au/Hg	Tailings (ppm)
Poconé	~ 0.10	1,5 to 24	0,14 - 1,68	~ 100	1:1,5	1 - 25
Alta Floresta	~ 0.07	1,5 to 48	up to 5,8	up to 41	1:1,5	5 - 134
Itaituba	~ 0.15	1,5 to 24	up to 6,6	> 9,9	N.A.	47

[a] from several field samples of CETEM;

[b] Cn/Bn accounts for the ratio between the concentration of Hg in the minus 74μm fraction, Cn, and Bn, the background value of Hg in the same fraction.

Table 2 - Field results for Peixoto de Azevedo [c].

Source	**Hg input**	**Recovery**	**Losses**	**Air**	**Water**
Garimpo do Melado	16 kg/month	3,20 kg/month	12,8 kg/month	7,7 kg/month	5,1 kg/month

[c] BRAGA and ARAÚJO.

Table 3 - Number of Gold Shops [c]

Area	N° Gold Shops	Year
Alta Floresta	12	1995
Peixoto Azevedo	19	1995
Itaituba	06	1995
Matupá (P.A.)	07	1995

3.2. *Physico-chemical interaction of mercury species with river sediments*

For the study of the mechanisms of physico-chemical interactions of mercury with river sediments, to access the relative mobility of mercury species, the following data were gathered by MELAMED et al., from Rio Preto, RJ, as shown in Figure 1, where mercury species where analyzed by cold vapor atomic absorption spectrometry with a gold trap, being:

Hg^T ≡ the total mercury, was determined by oxidizing all forms of Hg in the supernatant with bromine chloride (BrCl), before reduction with stannous chloride ($SnCl_2$).

Hg^A ≡ the mercury comprising dissolved metallic and inorganic-Hg, was determined by reduction of all Hg in the supernatant with ($SnCl_2$).

Hg^X ≡ mercury in the form of organic complexes was calculated from the difference between Hg^T and Hg^A.

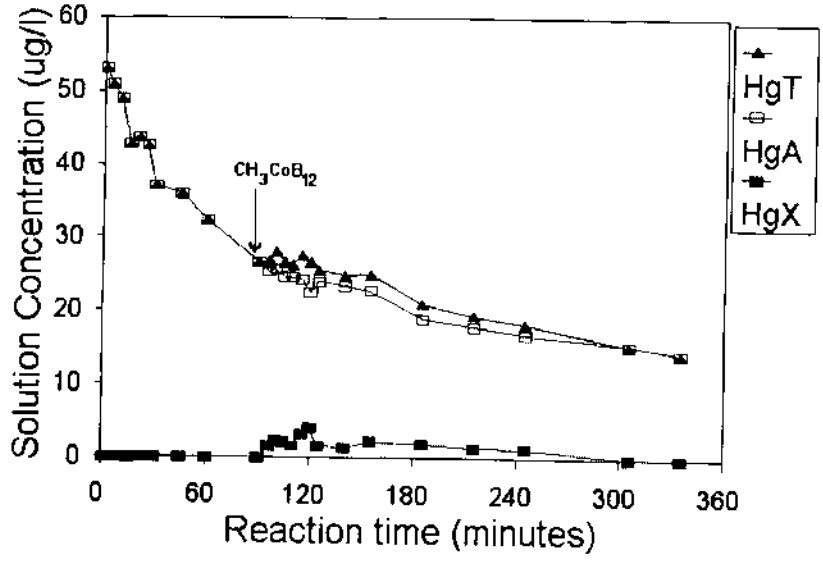


Fig. 1 - Hg adsorption kinetics on Preto river sediments. Arrow indicates time of input of methylcobalamin.

These results show Hg adsorption kinetics in this system, and the effect of the addition of methylcobalamin (CH_3CoB_{12}), a methylating agent, at 90 minutes reaction time. Initially, the rate of adsorption was relatively fast, and about 50% of Hg(II) in solution was taken up by the sediments. Then, upon addition of the methylating agent, a temporary decrease in adsorption rate was observed, reflected by a relative stabilization of Hg^T and Hg^A solution concentrations, as well as a concomitant increase in Hg^X. This decrease in Hg adsorption rate after the addition of CH_3CoB_{12} is attributed to the relative lower affinity of Hg^X for the sediment surface, suggesting a

lower affinity of methyl mercury for sorption at the sediment/solution interface as compared to $HgCl_2^0$.

MELAMED et al. (1997) also showed the increased solubility of Hg^0 in the presence of humic acid through a dissolution-complexation mechanism (Figure 2) and that with increased humic acid content, the concentration of Hg^T at the sediment surface increases because Hg^0 solubility is enhanced in the presence of dissolved humic acid (Figure 3). However, Hg^A is more reactive with the sediment surface than the soluble complexed formed (Hg^X). Hg^X was the main form of mercury in solution. Dissolved organic acids in natural systems are expected to enhance the solubility of Hg^0 and the mobility of Hg.

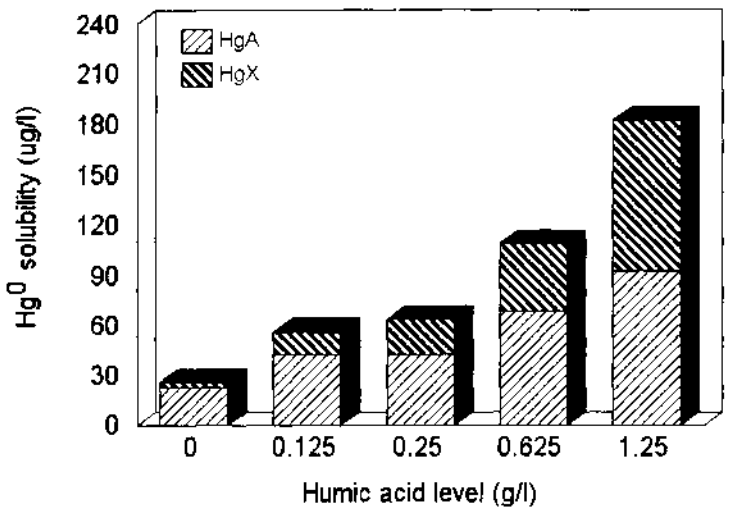


Fig. 2 - Effect of humic acid level on Hg^0 solubility.

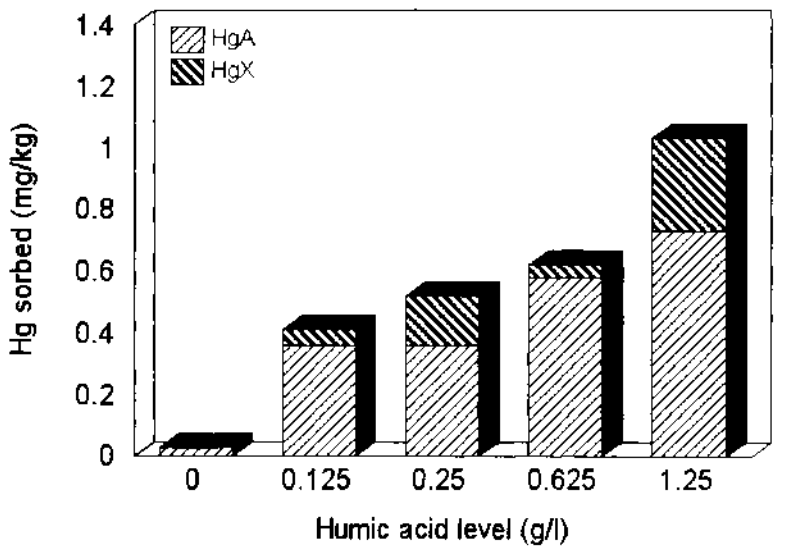


Fig. 3 - Effect of humic acid level on Hg sorption by Preto river sediments.

3.3. *Mercury dispersion in Alta Floresta (Apud NOBRE, 1996)*

Figure 4 shows the atmospheric dispersion of contaminants, after 7 days from the emission, at the level 925 hPa, from a source with constant intensity, active for 5 days, beginning in 20/08/95 and located at Alta Floresta. At the seventh day, it can be observed that the dispersion was directed to the west as well as to the north and south directions, certainly due to the effect produced by the Andes. Thus, in a few days, contaminants such as mercury, emitted from the Alta Floresta region, may deposit in relatively distant hydrological basins.

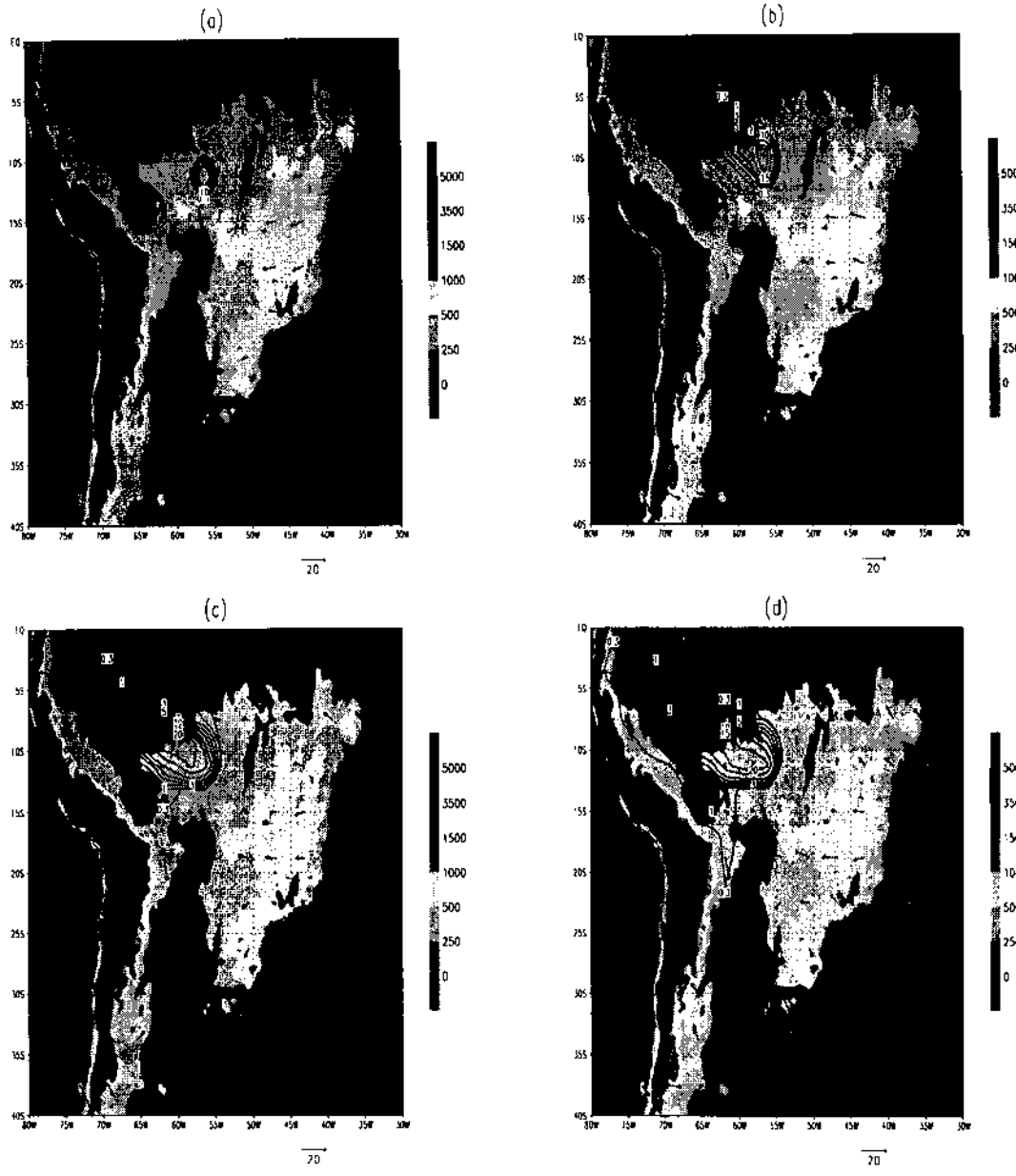


Fig. 4 - Mercury dispersion in Alta Floresta, considering a constant source of emission of 3.5 units initiated at 20/08/95. (a) first day; (b) second day; (c) seventh day; (d) ninth day after the beginning of the emission.

4. Solutions to minimize mercury in the environment

Problems with mercury release in small scale, secondary gold ore processing do not occur solely in the Brazilian wetlands and in the Amazon Basin, but are widespread in the pacific Rim, Latin America, and Africa, which together are responsible for the production of thousands to tonnes of gold per year. The conception, design and implementation of adequate methods for mercury utilization, monitoring and release mitigation are required in all of these areas.

The solutions to the problem were divided into two categories: (1) the existing problem; and (2) future avoidance of the problem. To tackle both requires involvement of the local communities (Unions, Rotary's, County Officials, the public etc.) to have any chance of success, i.e., the solutions are to be established by consent ! HOFFMANN (1994) has described the project goals achieved with participation of the local people.

Accredited analytical procedures were used (WILKEN, 1991) to sample the soil, water, air. Results were then discussed in several meetings with local community involvement and societal commitments were reached in other to mitigate the problems associated with Hg releases. These commitments were: (1) closed circuit utilization of mercury in the concentration/amalgamation steps; (2) burning of the amalgam in retorts in the field, and use of fume hoods in gold dealers' shops; and (3) confinement of processed material in specially build settling ponds. These measures were taken both for the present problems and proposed to avoid future problems. For the present problems, remediation measures were also taken regarding mercury fixation and/or recovery as below.

4.1. *Immobilization of Hg*

Mercury can be fixed by sulphur in polysulphides. This method, however, which can be utilized for the inactivation of Hg in solid masses, has been criticized on the grounds of the equilibrium constants for the several Hg-S bonds. No field tests were conducted. However, laboratory testing was performed utilizing polysulphite solutions, obtained from a mixture of sulphur flowers and soda ash, i.e., commercial grade sulphur and sodium hydroxide. The results, in terms of the actual degree of fixation are still pending because of difficulty in analyzing HgS below 1 ppm (WILKEN, 1991).

4.2. *Recovery of Hg*

Whenever possible, mercury has to be recovered. One method tested by CETEM is that of electrooxidation (VEIGA et al. (1991), SOBRAL and SANTOS (1995)). Its main feature is generation of hypochlorite ions by oxidation of chloride ions to elemental chlorine that in a aqueous media results in hypochlorite. Such a process may be viewed as na electrolytic segregation process, because small amounts of NaCl are intermixed with the resulting residue ("ore") in na aqueous pulp that is electrolyzed. The general reactions may be written as:

$$Hg + 2ClO^- + 4Cl^- + 2H_2O \Leftrightarrow Cl_2 + HgCl_4^{2-} + 4OH^- \quad (1)$$

and

$$2Au + 7NaCl + NaClO_3 + 3H_2O \Leftrightarrow 2NaAuCl_4 + 6NaOH \quad (2)$$

the dissolved gold being electrowon jointly with mercury.

The data in Fig. 1 has been recorded by CETEM.

Mercury recovery from tailings was conducted by installing and electrooxidation pilot demonstration unit in which up to 92% Hg recovery was achieved for a 6 h electrolysis time, in a 100 g/L NaCl solution, with na average energy consumption of 177 kWh/t, at pH 6-7, from tailings containing 6.8 mg/kg of Hg, producing a final solid material with 0.5 mg/kg of Hg. The dissolution of mercury may be viewed as:

$$2NaOCl + Hg^0 + 4HCl \rightarrow 2Na^+ + HgCl_4^{2-} + 2H_2O + Cl_2$$

and mercury being deposited as elemental mercury.

A series of pilot plant runs were conducted in the location of Rio do Rato, Itaituba region in order to decrease the energy consumption of the electro-oxidation process.

The results of this field campaign are shown in Figures 5 and 6:

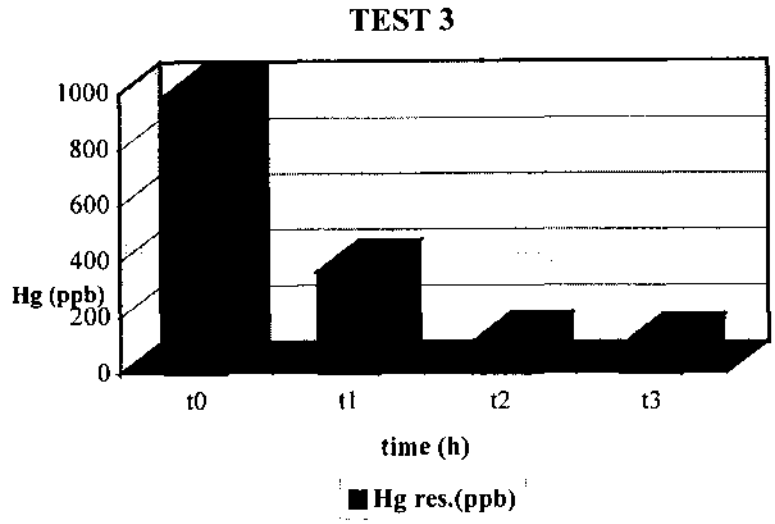


Fig. 5 - Mercury concentration in residue during electroleaching, i_a = 0,8 A/dm^2; NaCl 49.1 g/dm^3; HCl 17 mL; time 4 h; 1550 rpm.

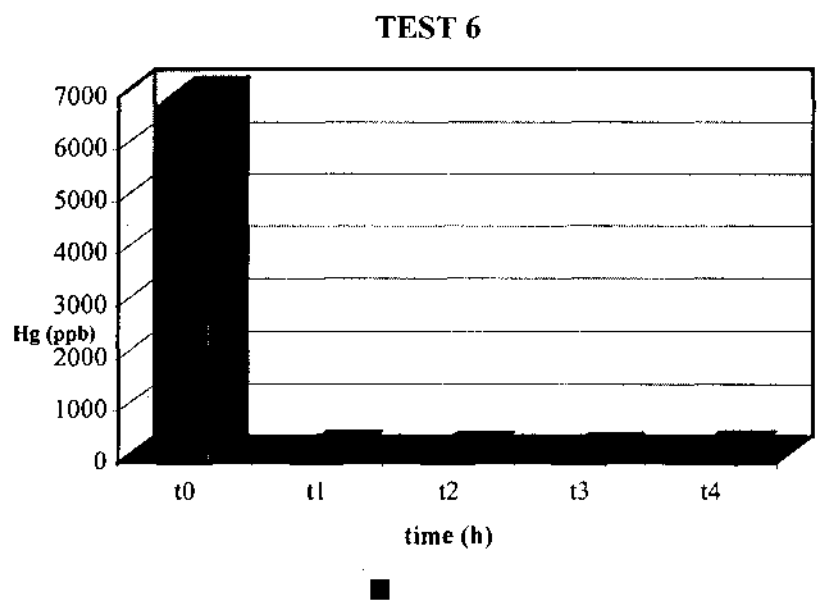


Fig. 6 - Mercury concentration in residue during electroleaching, i_a = 0,8 A/dm^2; NaCl 49.1 g/dm^3; HCl 25 mL; time 4h; 1550 rpm.

4.3. *Effect of Ca on the counteraction of humic acid-induced solubility of Hg^0*

Preliminary tests utilized Ca to revert the effect of humic acid on the enhancement of Hg^0 solubility (Melamed and Villas Bôas, In press). Figure 7 shows that the increased solubility of Hg^0 due to the *Aldrich* humic acid was reverted in the presence of Ca. The authors pointed out the necessity of verifying the effect of this amendment, and the possible development of this technology in the presence of natural organic acids. Interestingly, the data in Figure 7 indicate that Ca prevents the dissolution of Hg^0 rather than a competitive complexation mechanism.

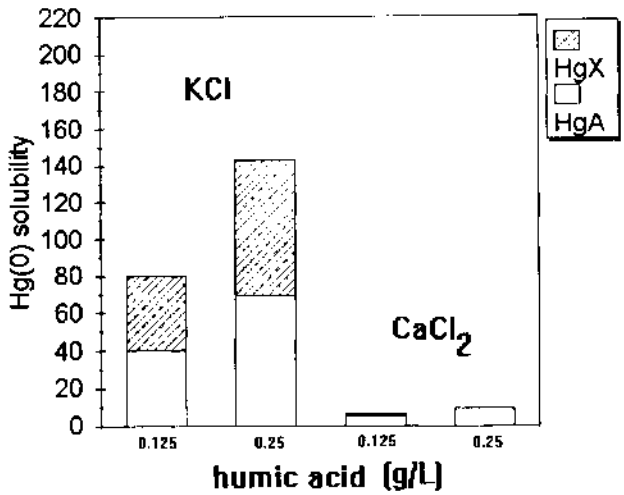


Figure 7. Effect of Ca-humic acid interaction on Hg^0 solubility.

5. Conclusions

The following conclusions were arrived at:

1. Legal issues are still pending of solution for the "garimpo" to develop as a sustainable activity.
2. Avoiding the use of mercury at the gravimetric separation stage is possible without diminishing gold recovery.
3. Introduction of retorts in the amalgamation process improved it and was well accepted by the garimpeiros (small scale miners).
4. Installing of fume hoods in gold shops proved to be very efficient in decreasing the overall mercury content in the air.
5. Confinement of processed material in ponds originating from the amalgamation stage, was adopted.
6. Recovery of mercury, via electro-oxidation seems to be a clean alternative in small-scale operations.
7. Projects as described in this article are as successful as long as solutions depend on consent, since in remote regions laws are impossible to enforce.

6. References

LACERDA, L.D. and SALOMONS, W. - Mercúrio na Amazônia: Uma Bomba Relógio Química? STA/CETEM/CNPq, Rio de Janeiro, 1992.

BARRETO, M.L - Uma Abordagem Crítica da Legislação Garimpeira - SED/CETEM/CNPq, Rio de Janeiro, 1993.

SILVA, A. P.; ALBUQUERQUE, G.S. and BARRETO, M.L. - The "Garimpo" Problem in the Amazon Region, Chap. 21, p. 281 in Chemistry of the Amazon, ACS Symposium Series 588, edited by SEIDL, P.R; GOTTLIEB, O.R. and KAPLAN, M.A., Washington, DC, 1995.

AKAGI, H.; MALM, O. and BRANCHES, F.J.P - Human Exposure to Mercury Due to Gold Mining the Amazon, Brazil - A Review, Environmental Sciences, 4, 3, p. 199-211, Tokyo, 1996.

VILLAS BÔAS, R.C. - The Mercury Problem in the Amazon Due to Gold Extraction, Journal of Geochemical Exploration, 58, p. 217-222, Elsevier, Netherlands, 1997.

VEIGA, M.M. et al. - Poconé: Um Campo de Estudo do Impacto Ambiental do Garimpo. STA/CETEM/CNPq, n. 1, 113 pp., Rio de Janeiro, 1991.

RODRIGUES FILHO, S. and MADDOCK, J.E.L - Mercury Pollution in Two Gold Mining Areas of the Brazilian Amazon, Journal of Geochemical Exploration, 58., p. 231, Elsevier, Netherlands; 1997.

FARID, L.H. et al. - Diagnóstico Preliminar dos Impactos Ambientais Gerados por Garimpos de Ouro em Alta Floresta/MT: Estudo de Caso. STA/CETEM/CNPq, n. 2 , Rio de Janeiro, 1992.

BRAGA, P.F.A. and ARAUJO, RV.V. de - Atividade Garimpeira de Ouro na Amazônia Brasileira; Amalgamação e Queima de Mercúrio. SDT/DNPM nº 2, Brasília, 1995.

ARAUJO, R.V.V. de and SANTOS, R.L. dos - Atividade Garimpeira de Ouro na Amazônia Brasileira; Queima de Mercúrio nas Casas Compradoras de Ouro; SDT/DNPM, n. 3, Brasília, 1995.

MELAMED, R. et al. - Mechanisms of Physico-Chemical Interaction of Mercury with River Sediments from Gell Mining Region in Brazil: Relative Mobility of Mercury Species, Journal of Geochemical Exploration, vol. 88, p. 119-174, Netherlands, 1997.

NOBRE, C. - Climatalogia e Dispersão de Mercúrio na Região de Alta Floresta, in: Avaliação do Impacto Sócio-Ambiental da Atividade Garimpeira, Causado por Emissões de Mercúrio na Atmosfera, Anexo 2, Aspectos Relacionados ao Transporte Atmosférico, às Taxas de Desposição dc Mercúrio e aos Biomonitores de Hg na Atmosfera; editado por Roberto Coelho, CETEM/PADCT/CNPq, Rio de Janeiro, 1996.

HOFFMANN, R. - Winning gold. Am. Sci., 82: 15-17, 1994.

WILKEN, R. - Heavy Metals Program, Analytical Support. Task Force Joint Meeting. GKSS-CETEM, 31 pp, 1991.

SOBRAL, L.G.S. and SANTOS, R.L.C. - Electroleaching Process for Remediation of Mercury Contamined Soils. In: W. J. van den Brink and F. Arendt (Editors), Contaminated Soil'95, pp. 1337-1347, 1995.

ANALYTICAL QUALITY CONTROL FOR THE DETERMINATION OF ORGANOMERCURY COMPOUNDS IN ENVIRONMENTAL SAMPLES

Milena Horvat
Department of Environmental Studies, Jožef Stefan Institute, Jamova 39, Slovenia

Abstract

A good quality control/quality assurance programme should be implemented in all environmental or health related studies on mercury and its organic compounds, particularly, for monomethylmercury (MeHg) which is the most toxic mercury compound. This can be achieved initially by analyzing suitable certified reference materials (CRMs), which are available from various producers such as the National Institute od Standards and Technology (NIST) from USA, National Institute of Environmental Studies (NIES), National Research Council of Canada (NRCC), Standards, Measurements and Testing programme (SM&T) of the European Commission, and the International Atomic Energy Agency (IAEA). It is well understood that these materials are not suffiecient, as most of them are of the marine origin. Therefore, many other actions should be undertaken to achieve, improve and/or maintain quality of data, including participation in interlaboratory studies, proficiency testing and production of laboratory reference materials. A review of these actions has shown that MeHg compounds detrmination in samples such as soil, sediment and water is rather difficult and is also method dependent. In addition, it has been shown that some of the most frequently employed analytical methods are subject to spurious MeHg formation in the presence of high concentrations of inorganic mercury and organic matter. These findings have put a number of previous data on MeHg in question and consequently prompt actions were undertaken by a number of well experienced laboratories. The paper also presents recent measures undertaken by the producers of reference materials to resolve these issues and check on the reliability of MeHg in already availbale CRMs.

Key words: mercury, methylmercury, reference materials, certified reference materials, interlaboratory tests, quality assurance/quality control

Introduction

In the environment mercury exists in different physical and chemical forms, with a wide range of properties. Conversions between these different forms provide the basis for mercury's complex distribution pattern, for local and global cycles, and for its biological enrichment and effects. The most important chemical forms are elemental mercury, divalent mercury, monomethylmercury (MeHg), and dimethylmercury (DMM). During recent years new analytical techniques have become available that have contributed significantly to the understanding of mercury chemistry in a natural system. In particular, these include ultra sensitive and specific analytical equipment and contamination-free methodologies. These improvements eventually allow for the determination of total and major species of mercury to be determined in air, water, sediments and biota. Methods are classified according to the isolation techniques and detection systems. They are selected depending on the nature of the sample and in particular the concentration levels of mercury. Frequently applied detection techniques are cold vapour atomic absorption spectrophotometry (CV AAS), more sensitive atomic fluorescence spectrophotometry (CV AFS), and various types of emission spectrophotometry. Speciation of mercury compounds also requires specific separation prior to detection, most frequently by gas chromatography (GC) and high performance liquid chromatography (HPLC). Methods for measurement of total and MeHg in biological and some

other environmental samples are well developed. However, this is not the case for samples with extremely low mercury concentrations, such as in water and air. The key elements for obtaining accurate measurements are connected with contamination-free sampling, sample storage and handling. Techniques for speciation of mercury species in water have recently been improved; however, methods for speciation of gaseous mercury compounds are difficult and are still under development. An overview of analytical methods for determination of total mercury and its species in various biological and environmental samples and the needs for future development are reviewed (Horvat, 1996; Horvat and Schroeder, 1995; Schroeder, 1995). The present paper addresses the question of reliability of analytical data. It is obvious that good quality control/quality assurance (QA/QC) programmes should be implemented in all mercury related studies, so that the values are comparable on a regional and global scale.

There are various means to achieve good quality data in analytical laboratories. Representative samples need to be properly collected and stored, prior to analysis, and all laboratory work conducted under a good QA programme. This can efficiently be achieved through skilled, well-trained, experienced and motivated staff. Protocols for sampling and sample storage should be well developed in order to prevent contamination and /or losses of Hg. In addition, interconversion of various Hg species during sample handling should also be prevented. Samples should be processed under very clean laboratory conditions and the appropriate quality labware and reagents (Hg-free) should be employed. The analysts should only use an analytical procedure on a routine basis after it has been validated for the range of concentrations and matrices to be dealt with the measurement programme using the relevant reference materials (RMs) and certified reference materials (CRMs). In the absence of these materials, there are some other strategies that can be adopted by the analysts, which are briefly described in the present paper. Other important measures include the quality assurance manual, training of personnel, a good managerial structure of the laboratory, the use of validated methods, the application of statistical control principles (e.g. control charts), and the external quality control measures (e.g. interlaboratory tests).

In the limited scope of this paper attention will be paid mainly to the role RMs, certified reference materials CRMs and interlaboratory testing in the determination of total mercury and organomercury compounds. These are necessary as an integral part of measures to ensure quality assurance of chemical analyses as prescribed by the Good Laboratory Practice, ISO 25 and EN 45 000 Series (CEN/CENELEC, 1989; ISO/IEC, 1990). They also represent the most necessary tool for a good quality control. Other QA aspects (e.g. strategy, management, etc.) are not a subject of this paper, but can simply be adopted from other open literature (Wegscheider, 1994).

Reference Materials and Certified Reference Materials

Reference Materials (RMs)

According to ISO (ISO/IEC, 1984) RM is a material or substance one or more properties of which are sufficiently well established to be used for the calibration of an apparatus, assessment of the measuring method, or for assigning values to materials. RMs can be (a) pure solutions for testing methods, (b) materials intended to test steps of an analytical procedure, and (c) laboratory reference materials having a composition which is close as feasible to the matrix to be analyzed by the users and which have known content. Such samples may be produced in the laboratory to verify the long-term reproducibility of analyses performed routinely and may be used for the evaluation of the performance of laboratories in intercomparison exercises. One of the very important prerequisite is to prepare representative RMs which implies correspondence of total and MeHg concentrations in the RM to those found in real, typical and uncontaminated samples of the same matrix. Such materials are, in particular, important for the determination of Hg in matrices for which CRMs are not available. An example of such a material is presented below. A reference value was determined

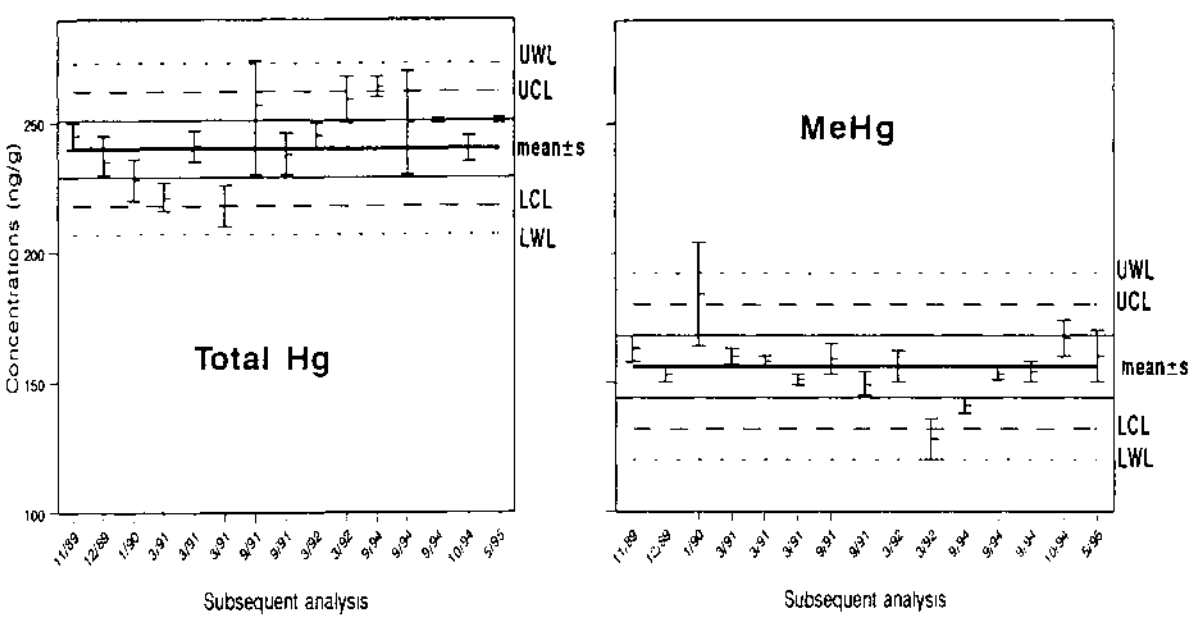
by the use of several independent methods (Table 1) (Horvat et al., 1988, 1990, 1992; Horvat, 1991). The same material was then successfully used for the statistical control schemes as shown in quality control charts (Figure 1). The RM was analyzed with 10-20 unknown samples and the results were able to detect the non-random fluctuations in the real analyses as it pose the same or similar problems to the analytical chemist as the unknown sample. The use of RMs is a very cost effective quality control measure, and should become an every day practice in laboratories engaged in routine analyses. This is also supported by the fact that limited number of RMs certified for MeHg is available on the market, and if all the laboratories would depend only on the available CRMs the stocks would quickly be exhausted.

Table 1. Determination of reference value in Human hair RM by using various independent analytical methods

Detection	Analytical method Separation	Total Hg* (mg/kg, DW)	MeHg* (mg/kg as Hg , DW)
CV AAS	distillation (Horvat et al., 1988)		147 ± 16
	ion-exchange (May and Stoeppler, 1987)		165 ± 13
GC-ECD	extraction (Horvat et al., 1990)		156 ± 8
	distillation (Horvat et al., 1988)		152 ± 13
	volatilization (Zelenko and Kosta, 1973)		161 ± 10
Hg total	CV AAS (Horvat et al., 1991)	241 ± 12	
	RNAA (Kosta and Byrne, 1969)	238 ± 10	
Reference value		***240 ± 11***	***156 ± 12***

Results are expressed as the mean ± standard deviation (±1 sigma).

Figure 1. Quality control charts for the determination of total and MeHg in human hair RM



Mean = arithmetic mean of the analyses; s = standard deviation; UWL = upper warning limit, mean + 2s; UCL = upper control limit, mean + 3s; LCL = lower control limit, mean – 3s; LWL 0 lower warning limit, mean – 2s.

There are also some other strategies that can be adopted to assign a consensus values to the sample of interest. Data for MeHg in RM with non-certified values for MeHg may be found from the literature, but should be treated with caution, particularly if the assessment of the reliability of data is not clearly evidenced from the original paper. Personal knowledge of the person and the technique may be helpful in assigning a consensus value. Another way is to organize a small scale collaborative study with laboratory that is skilled in Hg speciation work (Byrne, 1992). Such a methodology is regularly employed by a number of laboratories. It has even been suggested that RMs that have already been certified for total Hg should be recertified for MeHg, especially if they are still available at larger stocks (Horvat et al, 1993a, b).

Certified Reference Materials

Certified Reference Material (CRM) is a reference material one or more of whose property values are certified by a technically valid procedures, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body (ISO/IEC, 1984).

There are a number of CRMs available for total mercury in biological, sediment, and water samples (IAEA, 1995, 1996). Unfortunately, there are only a few biological samples and two sediment samples certified for MeHg compounds. The list of currently available CRMs is presented in Table 2. To our knowledge some new materials are in preparation and are listed in Table 3.

Table 2. Currently available reference materials certified for MeHg compounds

Producer[1]	CRM Code No.	Matrix	Certified Value[2]	
			MeHg $mg.kg^{-1}$ as Hg, DW	Total-Hg $mg.kg^{-1}$, DW
NRCC	DOLT-1	Dogfish Liver	0.080±0.011	0.225±0.037
NRCC	DOLT-2[3]	Dogfish Liver	0.693±0.053	1.99±0.10
NRCC	DORM-1	Dogfish Muscle	0.731±0.060	0.798±0.074
NRCC	DORM-2[3]	Dogfish Muscle	4.47±0.032	4.64±0.26
NRCC	TORT-1	Lobster Hepatopancreas	0.121±0.014	0.330±0.006
NRCC	LUTS-1	Non Defatted Lobster Hepatopancreas	0.0093±0.0006[4]	0.016±0.0022[4]
NIST	SRM 1974a	Mussel homogenate	0.0772±0.0038	0.176±0.013
	SRM 1974a		0.00882±0.00044[4]	0.0201±0.0015[4]
NIST	SRM 2974	Mussel homogenate	0.0772±0.0038	0.176±0.020
NIST	SRM 2976	Mussel homogenate	0.0277±0.0020	0.0610±0.0035
NIES	NIES No. 13	Human Hair	3.8±0.4	4.42±0.20
BCR	CRM 436	Tuna fish	2.82±0.15	2.85±0.16
BCR	CRM 464	Tuna fish	5.12±0.16	5.24±0.10
BCR	CRM 580	Estuarine Sediment	0.0702±0.0034	132±3
IAEA-MEL	IAEA-350	Tuna Fish Homogenate	3.65±0.35	4.68±0.28
IAEA-MEL	IAEA-356	Polluted Marine Sediment	0.0054±0.00089	7.62±0.65
IAEA-MEL	IAEA-142	Mussel Homogenate	0.047±0.004	0.126±0.007
IAEA	IAEA-085	Human Hair, Spiked	22.9 (21.9-23.9)[5]	23.2 (22.4-24.0)[5]
IAEA	IAEA-086	Human Hair	0.258 (0.236-0.279)[5]	0.573 (0.534-0.612)[5]

(1) NRCC - National Research Council Canada; IAEA-MEL International Atomic Energy Agency - Marine Environ. Laboratory; BCR - Institute for Reference Materials and Measurement, Commission of the European Communities; NIST - National Institute of Standards and Technology, USA, NIES – National Institute of Environmental Sciences, Japan

(2) Certified value ± 95% confidence interval (DW - dry weight) and/or uncertainty

(3) Replacement for previous DOLT-1 and DORM-1,

(4) Wet mass basis

(5) Mean value (95% Confidence interval)

Figure 2. Currently available CRMs certified for total Hg, MeHg, and percentage of MeHg.

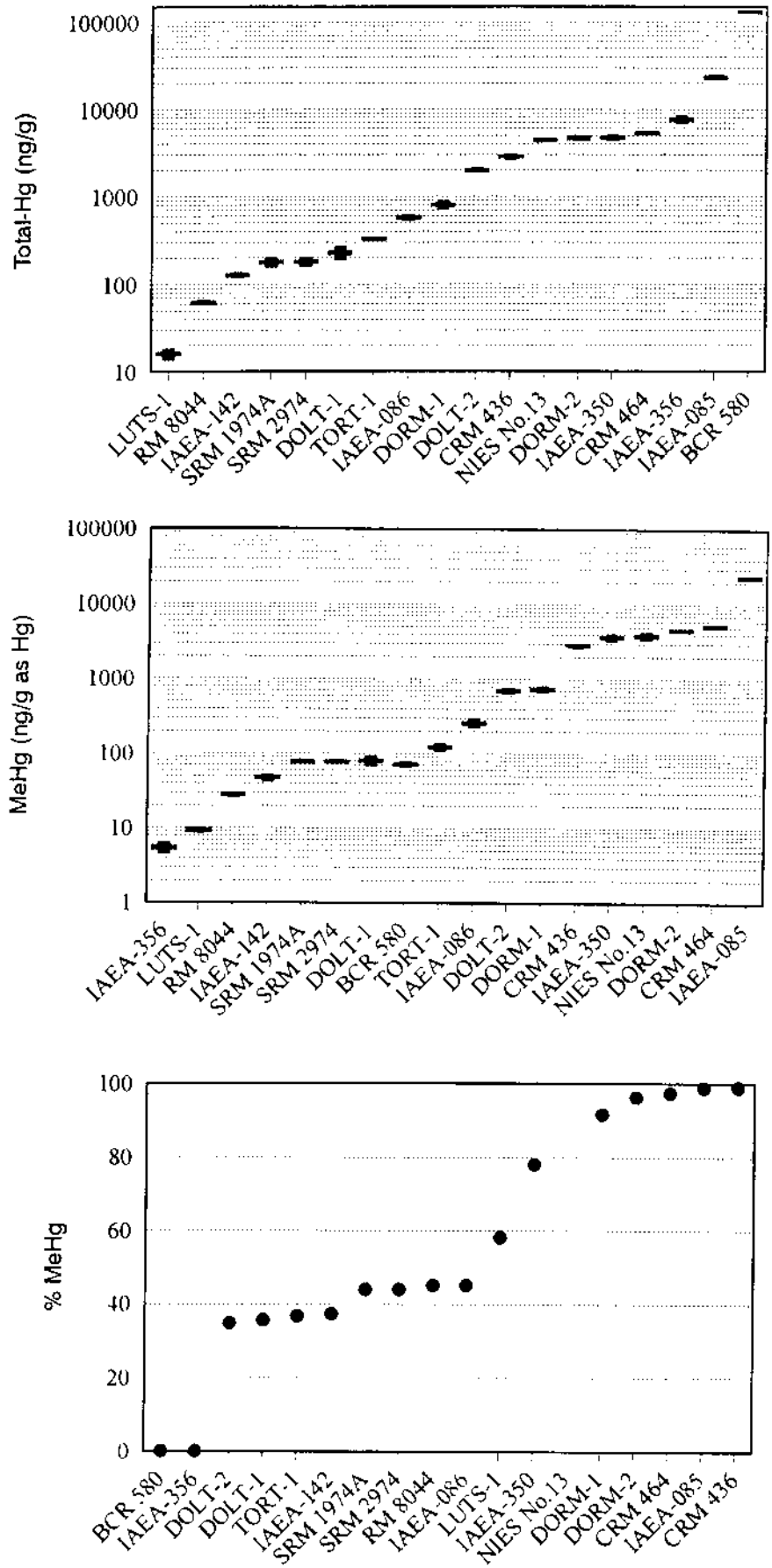


Table 2. Some current on-going and planned intercomparison exercises for certification of MeHg

Producer[(1)]	CRM Code No.	Matrix	Expected concentration	
			MeHg as Hg $mg.kg^{-1}$, DW	Total-Hg $mg.kg^{-1}$, DW
NIST	RM 8046 – GESREM III	Mussel Homogenate	<0.1	< 0.2
NIST	SRM 1566b	Oyster tissue	<0.1	< 0.2
IAEA-MEL	IAEA-140	Sea Plant Homogenate	<0.010	<0.1
IAEA-MEL	IAEA-?	Fish homogenate		

Most of the RMs certified for MeHg were prepared by intercomparison of the results obtained by various analytical procedures employed by different laboratories (Donais et al. 1997; Horvat et al. 1994a, 1996; Mee et al., 1992; Yoshinaga et al., 1997; Quevauviller et al., 1997, Quevauviller, 1997; Stone et al., 1995).

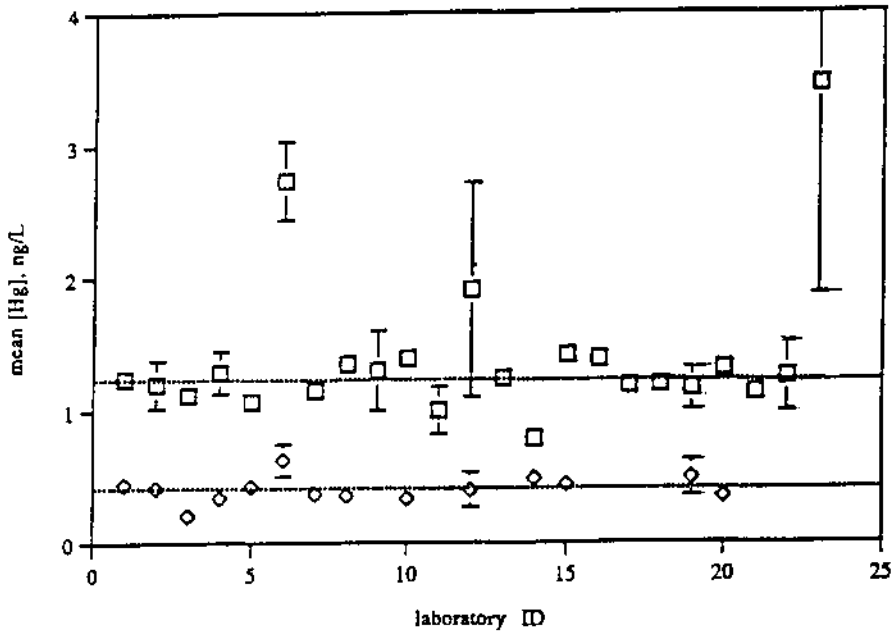
According to the recommendations in ISO Guide 33 on the use of CRMs (ISO/REMCO, 1996) the CRMs should be of similar composition and concentration levels to samples analyzed to ensure the accuracy of the results. Evidently, most of these materials are of the marine origin and are not sufficient to satisfy the requirements of many laboratories conducting research in terrestrial ecosystems and freshwater environments. The concentration levels of total and MeHg in currently available CRMs are presented in Figure 2, showing that a wide range of concentration levels is covered. Unfortunately, the use of CRMs represents only one aspect of the QA/QC programme and can only cover a limited number of biological and environmental samples.

Interlaboratory studies

There are three major types of interlaboratory studies, respectively focusing on the performance of analytical methods, the performance of laboratories and on the assignment of a most probable value of a quantity to the material with a stated uncertainty. In the case of mercury interlaboratory tests are, in particular, important where the CRMs are not available and the laboratory RM are difficult to prepare. For example, concentration levels of mercury in air and water are extremely low, and even highly sophisticated equipment can not guarantee accurate measurements. The reliability of the results depends on the overall procedure including sampling, storage, and laboratory handling. One way to check the accuracy of the results is to participate in field intercomparison exercises or by comparison of the results obtained by various methodologies.

Recently, such exercises have been organized for determination of total and methylmercury compounds in very low, natural lake water (Bloom et al, 1995). The results obtained were encouraging, showing that more than 80% of the participating laboratories obtained results for total and methylmercury within 20% of the consensus value (Figure 3). This demonstrates the comparability of the data sets being generated by diverse groups around the world. Such exercises are now regularly organized by the Frontier Geosciences, Seattle, USA under the MIP programme. In the future, intercomparison exercises should also include field sampling. Some attempts in this direction have already been done (Lepine and Chamberland, 1995). One of the important observation of the above intercomparisons is that no fundamentally different methods for mercury analyses were used, which means that the relatively narrow confidence intervals still remains unverified as to a true value. An important goal for the near future is therefore to develop fundamentally different methods for low-level total Hg and mercury speciation to stringently test the methods in common use today.

Figure 3. Mean results (typically n-3 bottles) as a function of submitting laboratory. Squares are total Hg, diamonds are MeHg. Error bars represent 1 s of the reported mean bottle values. Dashed lines indicate the consensus values (Bloom et al., 1995).



Field intercomparison exercises have also been conducted for the determination of total gaseous mercury and particulate-phase mercury in the atmosphere (Schroeder et al., 1994). The results obtained were not as satisfactory as for the above water intercomparison runs. Reasonable agreements for total gaseous mercury were obtained, however, frequently measurement of artifacts and inconsistent results were observed. Results for particulate phase mercury were unsatisfactory. It is clear that much more efforts are needed to obtain reliable data of mercury in the atmosphere in order to compare data on a global and regional scales. It was also concluded that measurement methods which are known to perform reliably and behave consistently in relatively clean air masses, may not display the same level of reliability and consistency in urban environments or industrial settings. It has been emphasized that any given methodology for sampling and analysis of Hg present in the atmosphere must always be validated carefully in any new or unusual surroundings.

As regards other biological and environmental samples the situation is far more favorable. For example, in the framework of the Analytical Quality Control Services (AQCS) of the International Atomic Energy Agency regional and world-wide interlaboratory studies are organized on a regular basis since 1973. Primarily these exercises are organized to intercompare analytical performance of laboratories involved in regional and world-wide programmes and when possible to assign reference values of those analytes where the data sets meet the certification criteria. In addition, these exercises are also designed to test the intercomparability of different instrumental techniques and to evaluate effectiveness of different procedures for sample digestion. The impact of these exercises was very important for the improvement of analytical protocols for the determination of total Hg and other trace and major elements and radionuclides. In addition, a large number of reference materials was produced (IAEA 1995, 1996).

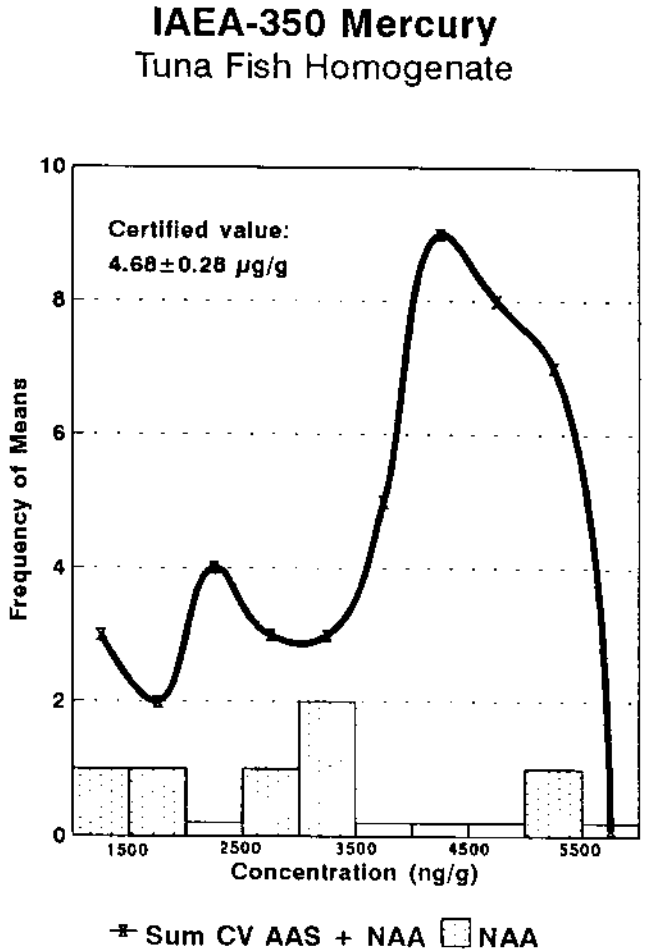
For example, Figure 4a shows the performance of various laboratories for determination of total mercury in the Intercomparison exercise IAEA-350, Trace elements in tuna fish homogenate. In order to examine the relative accuracy of different instrumental techniques, the data for total Hg were divided into concentration range intervals of 500ng/g and plotted as histograms. Results obtained by various laboratories and techniques revealed a skew of the data toward lower concentrations. Most probably this is due to incomplete digestion resulting from many commonly used techniques for the release of mercury from biological tissues. It should also be noted that most of mercury in this sample is in the form of MeHg which needs to be completely destroyed in order to measure total mercury by the cold vapour AAS. On the other hand stronger digestion may result in losses of mercury due to volatilization. Closer look at the data and the results of expert laboratories have confirmed that data grouped around the higher values are more accurate, as also obtained by the reference method based on radiochemical neutron activation (Mee et al., 1992).

On the other hand Figure 4b shows an example for total Hg determination in SD-M-2/TM, Deep Sea Sediment. Total mercury concentration is much lower than in IAEA-350 and most of the mercury is in inorganic form. It should be stressed that total mercury can easily be leached from sediment samples by acids, particularly those poor in organic matter, and even a very mild digestion guarantee quantitative release of Hg from bound sites. A skew toward higher concentrations, in this case, is related to contamination problems at such low mercury concentrations (Mee and Oregioni, 1991).

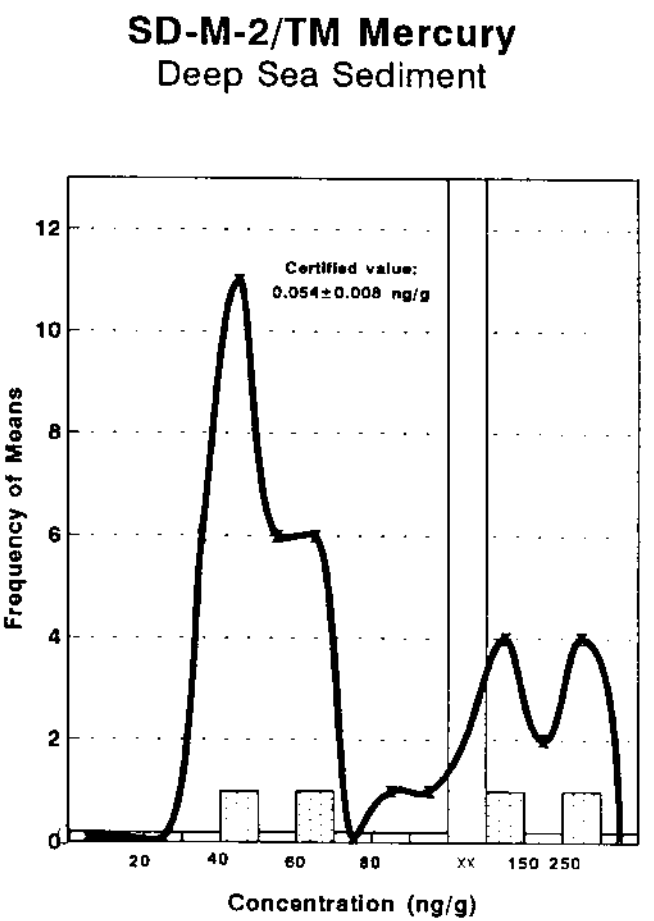
Results of these two examples agree well with other similar studies conducted by the European Commission programmes (Quevauviller, 1997) and clearly show that major problems for determination of total mercury are related to contamination and/or losses due to incomplete digestion and/or volatilization.

Another example of an interlaboratory study for the determination of methylmercury was conducted on the IAEA-356, Polluted Marine Sediment (Horvat et al., 1994a, b). The major objective in this exercise was to intercompare the performance of different methods and to produce a reference material certified for total and MeHg. Seven well-experienced laboratories participated using various isolation procedures (extraction, distillation, acid leaching, and alkaline digestion) and detection systems (CV AAS, CV AFS, GC-ECD and HPLC with CVAFS detection). The laboratories method means are plotted in ascending concentrations in Figure 4. Although it appears that results are well grouped, close examination of the results obtained by various methods have shown some significant differences. The results obtained by acid leaching followed by solvent extraction (Lab/meth. Code 1C and 6) are lower than other reported results. These can be explained in two ways: (1) acid leaching alone can not release MeHg from the sediment quantitatively (Horvat et al., 1995) or (2) distillation and alkaline digestion result in positive error due to spurious formation of MeHg during the isolation steps (Hintelmann et al 1997; Bloom et al. 1997). The sample was initially certified for MeHg taking all the results into account (95% conf. Interval from 5.07 to 5.84 ng/g). However, two years later it was shown that the certified value is too high due to artifact formation of MeHg during distillation and/alkaline digestion and the proposed range is 4.8 - 5.2 ng/g (Bloom et al. 1997). Further studies are in progress to resolve these problems, which will not only contribute to better and more accurate certified values but would also contribute significantly to method development and optimization in mercury speciation work on samples such as sediments, soils and contaminated water samples. This also includes the application of more sophisticated equipment such ICP-MS which permits the use of stable isotopes in measurement of monomethylmercury (CH_3Hg^+) by the isotope dilution method. In addition, this also allows the detection of species transformation in the environmental studies (Hintelmann et al. 1997; Hintelmann and Evans, 1997).

Figure 4. Comparison of the results for total mercury in two IAEA intercomparison samples

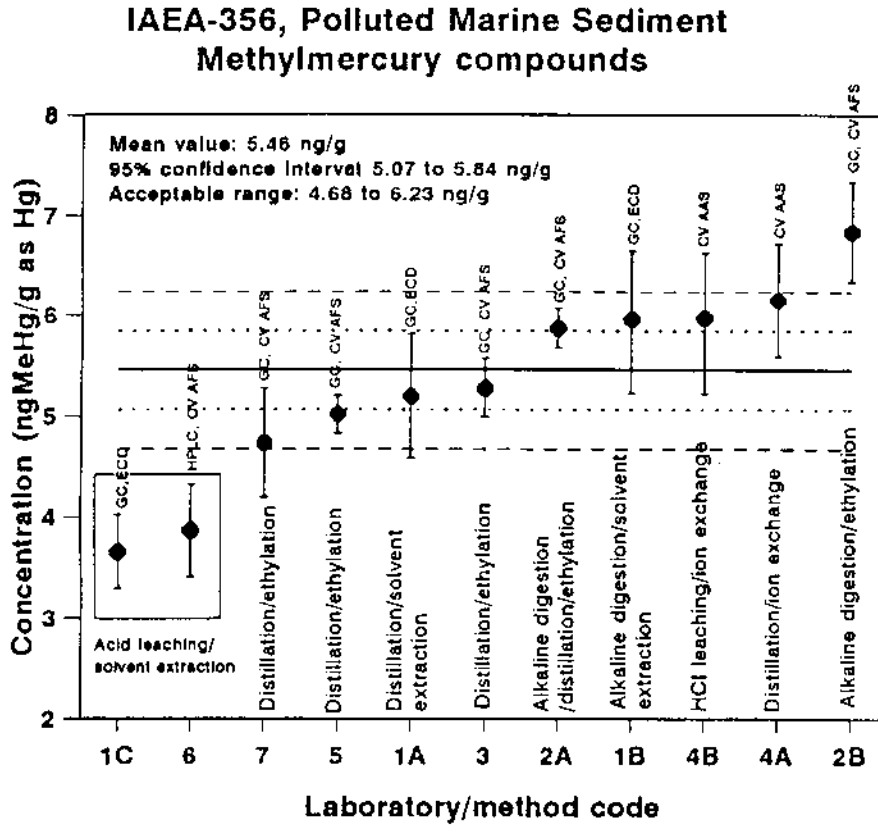


Frequency intervals are 500 ng/g.



Frequency intervals are 10 ng/g and 50 ng/g
Note scale change at XX.

Figure 5. Performance of various laboratories using different analytical methods for the determination of MeHg compounds in RM, Polluted Marine Sediment (Horvat et al., 1994a)



The laboratory method mean values for MeHg (calculated as Hg) plotted in ascending concentration on the y-axis and their respective laboratory method codes noted along the x axis. The respective standards deviations (SD) of the means are shown as error bars. Shaded area represents the 95% confidence interval.

Conclusions

In conclusion it is important to emphasize that globally-linked mercury research projects must include strong data quality assurance component. Apart from the regular use of appropriate reference materials the organization of interlaboratory tests on a regular basis (at least once every two years) is the minimum requirement. However, there are two problems that need to be addressed. The first is connected to the question of the reliability of data for organomercury determination in "difficult" samples (such as sediments, soils and organic rich water samples) as some recent studies show that the results are method dependent. This is very important for the assignement of consensus values in RMs and intercomparison samples for proficiency testing programmes and, in particular, for the production of CRMs. The second problem is connected to the reliability of data in samples for which reference materials can not be prepared (low level Hg in water samples and in air) and the only way to obtain comparable analytical results is the organization of field interlaboratory tests. Unfortunately, these are difficult and expensive to implement and the reliability of results is, therefore still dependent on the critical evaluation by analysts performing the analyses.

References

Bloom, N.S., Horvat, M. and Watras, C.J. (1995) Results of the international aqueous mercury speciation intercomparison exercise, *Water, Air, Soil Pollution*, **80**, 1257-1268.

Bloom, N.S., Colman, J.A., and Barber, L. (1997) Artifact formation of methyl mercury during aqueous distillation and alternative techniques for the extraction of methylmercury from environmental samples. *Fresenius J. Anal. Chem.*, **358**, 371-377.

Byrne, A.R. (1992) Some consideration regarding reference materials and their role in environmental monitoring, *Analyst*, **117**, 251-258.

CEN/CENELEC (1989) European Standard 45 001, "General criteria for the operation of testing laboratories", Brussels, Belgium.

Donais M.K., Saraswati R, Mackey E., Vangel M.G., Levenson M.S., Mandić V., Azemard S., Horvat M., Burow M., Emons H., Ostapczuk P. and Wise S.A.(1996) Certification of three mussel tissue Standard Reference Materials (SRMs) for MeHg and total mercury content, *Fresenius Z. Anal Chem.* **358**, 424-430.

Hintelmann, H., Falter, R., Ilgen, G., and Evans, R.D. (1997) Determination of artifactual formation of monomethylmercury (CH3Hg+) in environmental samples using stable Hg2+ isotopes with ICP-MS detection: calculation of contents applying species specific isotope addition. *Fresenius J. Anal. Chem.*, **358**, 363-370.

Hintelmann, H. and Evans, R.D. (1997) Application of stable isotopes in environmental tracer studies - Measurement of monomethylmercury (CH_3Hg^+) by isotope dilution ICP-MS and detection of species transformation. *Fres. J. Anal. Chem.* **358**, 378-385.

Horvat, M., May, K., Stoeppler, M., and Byrne, A.R. (1988) Comparative studies for methylmercury determination in biological and environmental samples, *Appl. Organomet. Chem.* **2**, 515-524.

Horvat, M., Byrne, A.R., and May, K. (1990) A modified method for the determination of methylmercury by gas chromatography, *Talanta*, **37**, 207-212.

Horvat, M. (1991) Determination of methylmercury in biological certified reference materials. *Wat. Air Soil Pollut.* **56**, 95-102.

Horvat M., Lupšina, V., Pihlar, B. (1991) Determination of total mercury in coal fly ash by gold amalgamation cold vapour atomic absorption spectrometry, *Anal. Chim. Acta*, **243**, 71-79.

Horvat, M. and Byrne, A.R. (1992) Preliminary study of the effects of some physical parameters on the stability of methylmercury in biological samples, *Analyst*, **117**, 665-668.

Horvat, M, Liang, L. and Bloom N.S.(1993a), Comparison of distillation with other current isolation methods for the determination of methyl mercury compounds in low level environmental samples, Part 1: Sediments, *Anal. Chim. Acta*, **281(1)**, 135-152.

Horvat, M., Liang, L. and Bloom, N.S. (1993b) Comparison of distillation with other current isolation methods for the determination of methyl mercury compounds in low level environmental samples, Part 2: Water, *Anal. Chim. Acta*, **282(1)**, 153-168.

Horvat, M., Mandić, V., Liang, L., Bloom, N.S, Padberg, S., Lee. Y.-H., Hintelmann, H., and Benoit, J. (1994a) Certification of methylmercury compounds concentration in marine sediment reference material IAEA-356, *Appl. Organomet. Chem.*, **8**, 533-540.

Horvat, M. Mee, L.D., and Oregioni, B. (1994b) Worldwide and regional intercomparison exercise for the determination of trace elements in polluted marine sediment IAEA-356. Report IAEA/AL/080.

Horvat, M. and Schroeder, W.H. (1995) "Determination of organic compounds. *In* "Encyclopedia of Analytical Science", pp. 3059-3070. Academic Press Limited.

Horvat, M. (1996) Mercury analyses and speciation. *In* " Global and Regional Mercury Cycles: Sources, Fluxes and Mass Balances" (W. Baeyens *et al.* Eds), pp 1-31. Kluwer Academic Publishers, The Netherlands.

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

IAEA-TECDOC-854, Survey of reference materials, Volume 1: Biological and environmental reference materials for trace elements, nuclides and microcontaminants, A report prepared by the IAEA and UNEP, December 1995, Vienna.

IAEA-TECDOC-880, Survey of reference materials, Volume 2: Biological and environmental reference materials for trace elements, nuclides and microcontaminants, A report prepared by the IAEA and UNEP, May 1996, Vienna.

ISO/REMCO Guide 33, Uses of certified reference materials, 1st Ed. 1989, rev. 1996

ISO/IEC Guide 25 (1990) "General requirements for the technical competence of testing laboratories", Third Edition, Geneva.

ISO/IEC Guide 43 (1984) "Development and operation of Laboratory Proficiency Testing", Geneva.

Kosta, L. and Byrne, A.R. (1969) Activation analyses for mercury in biological samples at nanogram levels, *Talanta* **16**, 1297 - 1302.

Lepine, L. and Chamberland, A. (1995) Filed sampling and analytical intercomparison for mercury and methylmercury determination in natural waters, *Wat. Air. Soil Pollut.* **80**, 1247-1256.

Mee, L.D. and Oregioni, B. (1991) IAEA, Worldwide intercomparison of trace element measurement in marine sediment SD-M-2/TM. Report IAEA/AL/053.

Mee, L.D., Oregioni, B., Horvat, M. (1992) Worldwide and regional intercomparison exercise for the determination of trace elements in tuna fish homogenate sample IAEA-350. Report IAEA/AL/052.

Quevauviller, Ph. Fortunati, G.U., Filippelli, M., Muntau, H. (1997) The certification of the contents (mass fractions) of total mercury and methyl mercury in estuarine sediment, CRM 580. EU BCR Information, EUR 17658 EN.

Quevauviller, Ph. (1997) Overview of SM&T (BCR) activities for the quality control of mercury determination in the environment. *Fres. J. Anal. Chem.*, **358**, 419-423.

Schroeder, W.H. (1995) "Mercury: Inorganic (and total) determination". *In* "Encyclopedia of Analytical Science", pp. 3050-3059. Academic Press Limited.

Stone, S.F., Backhouse, F.W., Byrne, A.R., Gangadharan, S., Horvat, M., Kratzer, K., Parr, R.M. Schladot, J.D., and Zeisler, R. (1995) Production of hair intercomparison material for use in population monitoring programmes for mercury and methylmercury exposure, *Fresenius Z. Anal. Chem.*, 351, 184-187.

Zelenko, V. and Kosta, L. (1973) A new method for the isolation of methylmercury from biological tissues and its determination at the parts-per milliard level by gas chromatography. *Talanta*, **20**, 115-123.

Wegscheider, W. (1994) "Proper sampling: a precondition for accurate analyses" *In.* Accreditation and quality assurance in Analytical laboratory (H. Gunzler ed.) Springer-Verlag, Berlin, Heidelberg, New York.

Yoshinaga, Y., Morita, M., and Okamoto, K. (1997) New human hair certified reference material for methylmercury and trace elements, *Fres. J. Anal. Chem.* **357**, 279-283.

IAEA PROGRAMMES ON HUMAN EXPOSURE TO MERCURY POLLUTION

R.M. Parr
International Atomic Energy Agency
Vienna, Austria

Abstract

During the past 40 years the International Atomic Energy Agency (IAEA) has been seeking to enlarge the contribution of atomic energy to peace, health and prosperity throughout the world. Among programmes in the area of human health, several activities have been supported which have made use of nuclear methods of analysis (in particular, neutron activation analysis) to study human exposure to mercury pollution. In this contribution, information is presented on several Co-ordinated Research Projects (CRPs) in which mercury has been one of the measurands of interest. The most relevant of these was concerned with the assessment of human exposure to mercury using hair as a bio-indicator. The measurands of interest included both total mercury and methyl mercury, and population groups were identified in several countries with evidence of excessive exposure. A major component of the IAEA's work has to do with analytical quality control, including the preparation and certification of analytical reference materials (RMs). With respect to mercury, two human hair RMs have recently been prepared which are certified for total- and methyl mercury at two different levels, "normal" and elevated. The IAEA also maintains a database of RMs from all producers, which presently lists more than 190 different RMs for mercury (including 11 for methyl mercury). Future activities are expected to include a new CRP on isotope studies of the biogeochemistry of mercury in polluted environments, and the preparation of new biological and environmental reference materials for mercury and methyl mercury.

Key Words Human exposure assessment, mercury, methyl mercury, quality control, hair

Introduction

The International Atomic Energy Agency (IAEA), which came into being in 1957, is an autonomous intergovernmental organization within the United Nations family. Together with its member states (presently 127 in number) the IAEA is called upon, by its statute, "to accelerate and enlarge the contribution of atomic energy to peace, health and prosperity throughout the world". The mention of "health" in this statute has, for many years, provided the justification for implementing a programme on applications of nuclear analytical techniques in health-related environmental monitoring and research. Within this area, a variety of topics have been supported which make use of nuclear and nuclear-related analytical techniques, such as neutron activation analysis (NAA), energy-dispersive X-ray fluorescence (ED-XRF), particle-induced X-ray emission (PIXE), and, more recently, inductively-coupled plasma mass spectrometry (ICP-MS).

The main beneficiaries of the IAEA's programmes are institutes and individual scientists in all member states, but particularly in developing countries. The main means by which the IAEA provides assistance to its counterparts in developing countries are (1) co-ordinated research, and (2) technical co-operation. Some relevant statistics (for 1997) are given below.

Co-ordinated research

- 144 Co-ordinated Research Projects (CRPs)
- 2180 Research Contracts
- 610 Research Agreements

- 99 Research Co-ordination Meetings (RCMs)
- Current annual budget: US$ 7.7 million

Technical co-operation

- ~ 1000 projects (including training courses)
- Current annual budget: US$ 74 million

Co-ordinated Research

As far as mercury is concerned, most of the Agency's support in recent years has been provided within the framework of co-ordinated research. A list of recent, current and possible future Co-ordinated Research Projects (CRPs) involving applications of nuclear analytical techniques, in which mercury is one of the measurands of interest, is given table 1.

The CRP which has had the greatest relevance to the topic of this NIMD '97 Forum is one which was completed in 1995 on the assessment of environmental exposure to mercury in selected human populations as studied by nuclear and other techniques. Participating countries were Brazil, Chile, China, the Czech Republic, Hungary, India, Italy, Malaysia, Slovenia and Vietnam. In this CRP, interest centred on using human hair as a bioindicator of exposure to inorganic and methyl mercury. WHO has tentatively set an action level of 5 μg/g of total mercury in hair; i.e. if levels of 5 μg/g or higher are observed then this suggests that further studies, including measurements of methyl mercury, are advised. Preliminary findings from this CRP already indicate that this level is exceeded in some populations in Brazil, Chile, China and Malaysia. Particularly high levels (average values in excess of 20 μg/g) were observed in some native Indian populations in the Amazon region of Brazil, and these findings are currently being followed up by more extensive studies supported under an IAEA Technical Co-operation Project. Another important outcome of this CRP was the development of new human hair reference materials for mercury analysis. These are described in the next section.

Also listed in table 1 is a proposed new CRP on isotope studies of the biogeochemistry of mercury in polluted environments. The idea for this CRP arose directly out of discussions held during the NIMD '97 Forum and, in the meantime, the CRP has been approved for inclusion in the Agency's programme starting in 1999. Its purpose will be to study the processes by which inorganic mercury is converted to methyl mercury in areas polluted as a result of gold mining and other industrial processes, and to assess the risk to populations in developing countries exposed to methyl mercury in their diets.

Analytical Quality Control Services

Since the early 1960s the IAEA has been operating a programme of Analytical Quality Control Services (AQCS) concerned mainly with intercomparison and reference materials for radionuclides, trace elements, pesticide residues and other organic microcontaminants. Most of the materials included in the present programme were developed in support of the kinds of activities described in the previous paragraphs. Through this AQCS programme the IAEA has established itself as a world-wide centre for analytical quality assurance, which assists its Member States to maintain and improve the quality of the analytical data obtained in their laboratories. Since the 1960s, more than 150 world-wide intercomparison runs have been conducted, and more than 70 reference materials have been certified. The present address list for the biennial catalogue comprises about 6,500 addresses, of which about 30% are in developing countries.

Of particular interest for human exposure assessment are two new human hair reference materials, which contain mercury and methyl mercury at two different levels. The set of materials is designated

Table 1: Recent, current and future CRPs in which mercury has been one of the measurands of interest

Years	No.(a)	Title
1984-1989	14	The significance of hair mineral analysis as a means for assessing internal body burdens of environmental pollutants
1984-1990	14	Human daily dietary intakes of nutritionally important trace elements as measured by nuclear and other techniques
1985-1990	11	Nuclear techniques for toxic elements in foodstuffs (in part of the Asia & Pacific Region)
1987-1992	20	Use of nuclear and nuclear-related techniques in the study of environmental pollution associated with solid wastes
1987-1992	10	Nuclear analytical techniques for the analysis of trace elements in agroindustrial products and foodstuffs (Latin America Region)
1990-1995	10	Assessment of environmental exposure to mercury in selected human populations as studied by nuclear and other techniques
1992-1997	9	Reference materials for micro-analytical nuclear techniques
1992-1997	19	Applied research on air pollution using nuclear-related analytical techniques
1995-1999	10	Ingestion and Organ Content of Trace Elements of Importance in Radiological Protection: Reference Asian Man Project
1996-2000	11	Assessment of levels and health-effects of airborne particulate matter in mining, metal refining and metal working industries using nuclear and related analytical techniques
1997-2001	12	Validation and application of plants as biomonitors of trace element atmospheric pollution, analyzed by nuclear and related techniques
1999-2002	-	Isotope studies of the biogeochemistry of mercury in polluted environments
2000-2003	-	Regional reference materials for environmental studies with traceability to SI units

(a) No. of participating countries

Table 2: Reference values for mercury and other trace elements in IAEA human hair reference materials

Measurand	Unit	Mean	95% Confidence Interval
IAEA-085 HUMAN HAIR, HIGH LEVEL			
Recommended Values			
Total Hg	(mg/kg)	23.2[a]	22.4 - 24.0
MeHg	(mg/kg, MeHg as Hg)	22.9	21.9 - 23.9
Fe	(mg/kg)	79.3	71.0 - 87.8
Zn	(mg/kg)	163	156 - 170
Information values			
Ca	(mg/kg)	930	847 - 1010
Cu	(mg/kg)	17	15.7 - 17.8
Mg	(mg/kg)	140	127 - 153
Mn	(mg/kg)	8.8	8.4 - 9.2
Sc	(mg/kg)	0.009	0.0084 - 0.0100
Se	(mg/kg)	1.1	0.96 - 1.17
IAEA-086 HUMAN HAIR, LOW LEVEL			
Recommended values			
Total Hg	(mg/kg)	0.573[a]	0.534 - 0.612
MeHg	(mg/kg, MeHg as Hg)	0.258	0.236 - 0.279
Fe	(mg/kg)	123	110 - 136
Zn	(mg/kg)	167	159 - 174
Information values			
Ca	(mg/kg)	1120	1000 - 1240
Cu	(mg/kg)	17.6	16.6 - 18.5
Mg	(mg/kg)	180	156 - 197
Mn	(mg/kg)	9.6	8.9 - 10.3
Sc	(mg/kg)	0.014	0.013 - 0.016
Se	(mg/kg)	1.0	0.80 - 1.20

IAEA-087, and consists of IAEA-085, which represents hair with an elevated level of methyl mercury, and IAEA-086, which contains a low level of methyl mercury.

Prior to the development of these IAEA materials, there were no certified hair reference materials for methyl mercury. A human hair material from the National Institute of Environmental Studies in Japan, NIES CRM No. 13 [1], was under development concurrently with the IAEA materials, and it was planned that the mercury and methyl mercury concentrations in the two IAEA materials would complement this material, as well as two already available certified reference materials, BCR 397 and GBW 07601, certified for total mercury. These reference materials, including the new IAEA and NIES reference materials, would encompass the working range of measurements in human hair for mercury and methyl mercury. The IAEA materials would additionally give recommended and information values for other trace elements that can be utilized for quality assurance purposes.

The starting material for IAEA-085 and IAEA-086 was ten kilograms of human hair obtained from India. This was divided into two parts, the first to provide a natural, low level, sample, and the second, with the aid of spiking, to provide a high level sample. To convert the hair into homogeneous materials, cryogenic milling was utilized. Seventy percent of the final material passed through a 0.075 mm sieve. Full details of the sample preparation methods have been described [2]. An international intercomparison exercise was then carried out to obtain analytical data on the two materials. Sets of data were obtained from 68 institutes in 40 countries, with experienced as well as less experienced laboratories contributing data on a voluntary basis. The evaluation of the intercomparison data followed the acceptance criteria used in previous intercomparisons organized by the IAEA.

The reference values for the two IAEA reference materials were established on the basis of results submitted by the participants in the intercomparison exercise. The reference and information values listed in table 2 represent overall mean values (excluding data that were detected and rejected as outliers) that were calculated on the basis of at least ten laboratory means. For the total mercury and methyl mercury values, the results submitted by invited expert laboratories were used in addition to those from the intercomparison means to establish the reference values.

These are not the only IAEA reference materials that have been certified for total mercury and/or methyl mercury. The complete list of currently available IAEA reference materials for these measurands is given in table 3.

Survey of available reference materials

The IAEA also maintains a database of analytical reference materials for trace elements, nuclides and organic contaminants in biological, environmental and related matrices. All known international producers (not only the IAEA) are included in the database. The purpose is to help analysts to select reference materials for QA/QC purposes that match as closely as possible, with respect to matrix type and concentrations of the measurands of interest, the "real" samples that are to be measured. An earlier version of the database was published by the Agency in two technical reports [3], and future updates are planned to be made available over the internet. A list of all materials currently in the database for which methyl mercury concentrations are specified is given in table 4, and a complete list of all materials for which total mercury is specified is reproduced in annex 1.

The future

Interest in mercury as a global pollutant may, if anything, be expected to increase in future years. Since nuclear and isotopic techniques have many important applications in studying this problem, it is to be expected that mercury pollution will continue to be an important topic in the IAEA's programmes on environmental health. Particular areas of emphasis in the near future are expected to include (1) a

Table 3: Complete list of currently available IAEA reference materials for mercury and methyl mercury *

Name	Code	Measurand	mg/kg	cert. code	U(%)
Whey Powder	IAEA-155	Hg	0.0026	C	44
Polluted Marine Sediment	IAEA-356	Hg, methyl-	0.0055	C	7
Hay Powder	IAEA-V-10	Hg	0.013	C	27
Mussel Homogenate	IAEA-142	Hg, methyl	0.047	C	7
Cotton Cellulose	IAEA-V- 9	Hg	0.06	C	33
Mussel Homogenate	IAEA-142	Hg	0.126	C	6
Lake Sediment	IAEA-SL-1	Hg	0.13	N	
Lichen	IAEA-336	Hg	0.2	C	16
Human Hair	IAEA-086	Hg, methyl-	0.258	C	8
Human Hair	IAEA-086	Hg	0.573	C	7
Polluted Marine Sediment	IAEA-356	Hg	7.62	C	8
Human Hair	IAEA-085	Hg, methyl-	22.9	C	4
Human Hair	IAEA-085	Hg	23.2	C	4

Table 4: List of reference materials for methyl mercury in the IAEA database *

Name	Code	mg/kg	cert. code	U (%)
Polluted Marine Sediment	IAEA-356	0.0055	C	7
Mussel Tissue	NIST-SRM-2976	0.0277	C	7
Mussel Homogenate	IAEA-142	0.047	C	9
Non Defatted Lobster Hepatopancreas	NRCC-LUTS-1	0.063	C	6
Mussel Tissue (Mytilus Edulis)	NIST-SRM-2974	0.0772	C	5
Mussel Tissue (Mytilus Edulis)	NIST-SRM-1974a	0.0772	C	5
Lobster Hepatopancreas	NRCC-TORT-2	0.152	C	9
Human Hair	IAEA-086	0.258	C	8
Cod Muscle	BCR-CRM-422	0.43	N	
Dogfish Liver	NRCC-DOLT-2	0.693	C	8
Total and Methylmercury in Tuna Fish	BCR-CRM-463	3.04	C	5
Human Hair	NIES-CRM-13	3.8	C	10
Dogfish Muscle	NRCC-DORM-2	4.47	C	7
Total and Methylmercury in Tuna Fish	BCR-CRM 464	5.5	C	3
Human Hair	IAEA-085	22.9	C	4

* Certification code: C=certified, N=not certified or information value
U (%) = the percentage uncertainty in the certified value (usually representing the 95% confidence interval).

new CRP on isotope studies of the biogeochemistry of mercury in polluted environments, and (2) the development of new biological and environmental reference materials for methyl mercury, particularly for the terrestrial and fresh-water environment.

Acknowledgments

Some of the information presented in this report is drawn partially from the programmes of other present and former colleagues in the IAEA. The author would like to acknowledge in particular his collaboration with S. Stone, and R. Zeisler, and assistance from A. Bleise in preparing some of the tables in this report. In addition he would like to thank all the scientists in many different countries who have participated in the Coordinated Research Projects and other activities referred to here.

References

1. Yoshinaga J., Morita M., Okamoto K., (1997) New human hair certified reference material for methyl mercury and trace elements. *Fresenius J. Anal. Chem.* **357**, 279-283
2. Stone S.F., Backhaus F.W., Byrne A.R., Gangadahran S., Horvat M., Kratzer K., Parr R.M., Schladot J.D., Zeisler R., (1995) Production of hair intercomparison materials for use in populatio nmonitoring programmes for mercury ad methyl mercury exposure. *Fresenius J. Anal. Chem.* **352**, 184-187
3. INTERNATIONAL ATOMIC ENERGY AGENCY, (1995 & 1996) Survey of Reference Materials, *Vol. 1*: Biological and Environmental Reference Materials for Trace Elements, Nuclides and Microcontaminants, IAEA-TECDOC-854, December 1995; *Vol. 2*: Environmentally Related Reference Materials for Trace Elements, Nuclides and Microcontaminants, IAEA-TECDOC-880, May 1996.

Complete list of reference materials for total mercury in the IAEA database [3]. The materials are listed in order of increasing mercury concentration. The fourth column indicates the certification code (C=certified, N=not certified, or information value). The fifth column indicates the percentage uncertainty in the certified value (usually representing the 95% confidence interval).

Name	Material Code	mg/kg	cert. code	U(%)
Microcrystalline Cellulose	NIST-RM 8416	0.0002	N	
Inorg. Const. in Bovine Serum	NIST-SRM 1598	0.0002	N	
Non-fat Milk Powder	NIST-SRM 1549	0.0003	C	67
Durum Wheat Flour	NIST-RM 8436	0.0004	C	50
Non Fat Milk Powder	GBW 08509	0.0005	N	
Wheat Flour	NIST-SRM 1567a	0.0005	N	
Milk Powder	ARC/CL-MP	< 0.001	N	
Wheat Flour	ARC/CL-WF	< 0.001	N	
Wholemeal Flour	BCR-CRM 189	0.001	N	
Corn Starch	NIST-RM 8432	0.0011	C	64
Wheat Gluten	NIST-RM 8418	0.0019	C	32
Brown Bread	BCR-CRM 191	0.002	N	
Soft Winter Wheat Flour	NIST-RM 8438	0.002	N	
Toxic Metals in Urine (Normal)	NIST-SRM 2670 "n"	0.002 *	N	
Mercury in Urine. Low level	NIST-SRM 2672a "l"	0.002 *	N	
Rye Bread Flour	CSRM 12-2-05	0.0024	N	32
Bovine Muscle	BCR-CRM 184	0.0026	C	23
Whey Powder	IAEA-155	0.0026	C	44
Wheat Bread Flour	CSRM 12-2-04	0.0027	N	44
Rice Flour	NIES-CRM-10B	0.003	N	
Corn Bran	NIST-RM 8433	0.003	C	33
Bovine Liver	NIST-SRM 1577b	0.003	N	
Tea	GBW 08505	0.004	N	
Rice Flour	NIES-CRM-10A	0.004	N	
Whole Egg Powder	NIST-RM 8415	0.004	C	75
Hard Red Spring Wheat Flour	NIST-RM 8437	0.004	N	
Rice Flour	NIES-CRM-10C	0.005	N	
Bovine Muscle Powder	NIST-RM 8414	0.005	C	60
Rice Flour	NIST-SRM 1568a	0.0058	C	9
Olivine Basalt	GBW 07105	0.0064	N	

Name	Material Code	mg/kg	cert. code	U(%)
Total Diet	ARC/CL-HDP	0.0066	C	55
Sandstone	GBW 07106	0.0084	N	
Spiked Skim Milk Powder (low level)	BCR-CRM 150	0.0094	C	18
Cabbage Leaves	AMM-CL-1	0.01	N	
Mercury in Water	GBW 08603	0.01	C	
Tea	GBW 07605	0.013	N	
Hay Powder	IAEA-V-10	0.013	C	27
Chestnut Soil	GBW 07402	0.015	C	13
Soil	GBW 07409	0.015	C	
Carbonate Rock	GBW 07108	0.016	C	6
Loess	GBW 07408	0.0166	C	10
Soil	CANMET-SO-3	0.017	C	41
Stream Sediment	GBW 07301	0.018	C	22
Xizang (Tibet) Soil	GBW 08302	0.018	N	
Rye Grass	BCR-CRM 281	0.0205	C	9
Green Algae (C. Kessleri)	CSRM 12-2-02	0.0239	N	23
Poplar Leaves	GBW 07604	0.026	C	12
Coal Fly Ash	CSRM-ECO-12-1-04	0.028	N	
Lucerne P-Alfalfa	CSRM 12-2-03	0.0282	N	31
Soil	CANMET-SO-4	0.03	C	20
Spinach Leaves	NIST-SRM 1570a	0.03	C	10
Peach Leaves	NIST-SRM 1547	0.031	C	23
Podzolitic Soil	GBW 07401	0.032	C	9
Tomato Leaves	NIST-SRM 1573a	0.034	C	12
Mercury in Rice	GBW 08508	0.038	C	
Coal Fly Ash	GBW 08401	0.039	N	
Steam Coal	BCR-CRM 182	0.04	C	18
Clay Loam	CMI-CRM-7003"s2"	0.04	N	22
Stream Sediment	GBW 07302	0.04	C	25
Estuarine Sediment	NIST-SRM 1646a	0.04	N	
Witbank Coal	SABS-SARM-18	0.04	N	
Light sandy soil, elevated level	CMI-CRM-7002"s2"	0.041	N	24
Stream Sediment	GBW 07308	0.042	C	12
Oriental Tobacco Leaves	ICHTJ-CTA-OTL-1	0.043	N	
Bovine Liver	BCR-CRM 185	0.044	C	7
Stream Sediment	GBW 07304	0.044	C	20
Apple Leaves	NIST-SRM 1515	0.044	C	9

Name	Material Code	mg/kg	cert. code	U(%)
Stream Sediment	GBW 07306	0.045	C	18
Light sandy soil, elevated level	CMI-CRM-7002 "s1"	0.046	C	11
Peach Leaves	GBW 08501	0.046	C	26
Offshore Marine Sediment	GBW 07314	0.048	C	
Sea Lettuce (U. Lactuca)	BCR-CRM 279	0.05	N	
Stream Sediment	GBW 07303	0.05	N	
Light sandy soil	CMI-CRM-7001 "s2"	0.052	N	23
Stream Sediment	GBW 07307	0.053	C	30
Clay Loam	CMI-CRM-7003 "s1"	0.054	C	15
Natural Moroccan Phosphate Rock	BCR-CRM 032	0.055	C	20
Stream Sediment	GBW 07312	0.056	C	7
Calcareous Loam Soil	BCR-CRM 141 "t"	0.0568	C	8
Light sandy soil	CMI-CRM-7001 "s1"	0.059	N	24
Yellow-brown Soil	GBW 07403	0.06	C	5
Cotton Cellulose	IAEA-V- 9	0.06	C	33
Tennessee River Sediment	NIST-RM 8406	0.06	N	
Laterite	GBW 07407	0.061	C	7
Oyster Tissue	NIST-SRM 1566a	0.0642	C	10
Soil	GBW 07410	0.066	C	
Light Sandy Soil	BCR-CRM 142R "t"	0.067	C	16
Mussel	GBW 08571	0.067	C	12
Stream Sediment	GBW 07311	0.072	C	8
Yellow-red Soil	GBW 07406	0.072	C	7
Orthic Luvisols Soil	CSRM 12-1-08	0.0785	C	13
Light sandy soil, elevated levels	CMI-CRM-7002 "s"	0.08	N	20
Soil	CANMET-SO-2	0.082	C	11
Stream Sediment	GBW 07309	0.083	C	7
Light sandy soil	CMI-CRM-7001 "s"	0.085	N	24
Light sandy soil	CMI-CRM-7001 "t"	0.087	C	7
Rendzina Soil	CSRM 12-1-09	0.0874	C	16
Light sandy soil, elevated levels	CMI-CRM-7002 "t"	0.09	C	13
Marine Sediment	NRCC-MESS-2	0.092	C	10
Clay Loam	CMI-CRM-7003 "s"	0.093	N	25
Clay Loam, elevated level	CMI-CRM-7004"s2"	0.094	C	15
Clay Loam	CMI-CRM-7003 "t"	0.096	C	15
Stream Sediment	GBW 07305	0.1	C	20
Spiked Skim Milk Powder (high level)	BCR-CRM 151	0.101	C	10

Name	Material Code	mg/kg	cert. code	U(%)
Toxic Metals in Urine (Elevated)	NIST-SRM 2670 "e"	0.105 *	C	8
Mercury in Urine. High level	NIST-SRM 2672a "h"	0.105 *	C	8
Non Defatted Lobster Hepatopancreas	NRCC-LUTS-1	0.112	C	13
Sudbury Sediment	NWRI-SUD-1	0.113	N	51
Gas Coal	BCR-CRM 180	0.123	C	6
Marine Sediment	NRCC-BCSS-1	0.129	C	9
Lake Sediment	IAEA-SL-1	0.13	N	
Coking Coal	BCR-CRM 181	0.138	C	8
Coal Fly Ash	NIST-SRM 1633b	0.141	C	13
Soil	GBW 07411	0.15	C	
Pine Needles	NIST-SRM 1575	0.15	C	33
Clay Loam, elevated level	CMI-CRM-7004 "s1"	0.16	N	19
Eutric Cambisols Soil	CSRM 12-1-07	0.171	C	9
Mussel Tissue	BCR-CRM 278	0.188	C	4
Municipal Incinerator ash	EPA-SRS203-225	0.2	C	
Lichen	IAEA-336	0.2	C	16
Sulfur in Coal, 1%	NIST-SRM 2692a	0.2	N	
OFS Coal	SABS-SARM-19	0.2	N	
Prawn	GBW 08572	0.201	C	2
Clay Loam, elevated level	CMI-CRM-7004 "s"	0.21	N	19
River Sediment	GBW 08301	0.22	C	18
Clay Loam, elevated level	CMI-CRM-7004 "t"	0.223	C	7
Aquatic Plant (P. riparioides)	BCR-CRM 061	0.23	C	9
Sasolburg Coal	SABS-SARM-20	0.25	C	18
Lobster Hepatopancreas	NRCC-TORT-2	0.27	C	22
Plankton	BCR-CRM 414	0.276	C	7
Olive Leaves (O. europaea)	BCR-CRM 062	0.28	C	7
Stream Sediment	GBW 07310	0.28	C	6
Yellow-red Soil	GBW 07405	0.294	C	6
Humber River Sediment	NWRI-HR-1	0.328	N	45
Aquatic Plant (L. major)	BCR-CRM 060	0.34	C	12
Steel Plant Flue Dust	CSRM-OK-12-1-05	0.349	N	25
Blend Coal	BCR-CRM 040	0.35	C	17
Human Hair	GBW 07601	0.36	C	14
Bovine Liver	CZIM-LIVER	0.37	C	5
Toronto Harbour Sediment	NWRI-TH-1	0.415	N	28
Lichen	BCR-CRM 482	0.48	C	4

Name	Material Code	mg/kg	cert. code	U(%)
Organics in Marine Sediment	NIST-SRM 1941a "t"	0.5	N	40
Cod Muscle	BCR-CRM 422	0.559	C	3
Human Hair	IAEA-086	0.573	C	7
Limy-yellow Soil	GBW 07404	0.59	C	6
Great Lakes Sediment	NWRI-TH-2	0.651	N	39
Zinc Ore (blende)	BCR-CRM 109	0.96	C	12
Plating Sludge No.1	EPA-CRM009-100	1	N	
River Sediment	BCR-CRM 320 "t"	1.03	C	12
Lake Ontario Sediment	NWRI-WQB-1	1.09	C	14
Sewage Sludge amended Soil (Aqua regia soluble)	BCR-CRM 143R "s"	1.1	N	
Sewage Sludge amended Soil	BCR-CRM 143R "t"	1.1	C	6
Pond Sediment	NIES-CRM-02	1.3	N	
San Joaquin Soil	NIST-SRM 2709	1.4	C	6
Buffalo River Sediment	NIST-SRM 2704	1.44	C	5
Mercury in Water	NIST-SRM 1641c	1.47 *	C	3
Estuarine Sediment	BCR-CRM 277 "t"	1.77	C	3
Pig Kidney	BCR-CRM 186	1.97	C	2
Dogfish Liver	NRCC-DOLT-2	1.99	C	5
Dust	EPA-CRM014-050	2	N	
Municipal Waste Incinerator Ash	EPA-SRS019-50	2	N	
Sewage Sludge	BCR-CRM 145R "t"	2.01	C	11
Fly Ash	BCR-CRM 038	2.1	C	7
Polluted Farmland Soil	GBW 08303	2.15	C	6
Human Hair	GBW 09101	2.16	C	10
Great Lakes Sediment	NWRI-WQB-3	2.75	C	10
Total and Methylmercury in Tuna Fish	BCR-CRM 463	2.85	C	6
Diatomaceous Earth Cake	EPA-CRM004-100	3	N	
Sewage Sludge (domestic origin), Aqua regia soluble	BCR-CRM 144R "s"	3.11	C	6
Sewage Sludge (domestic origin)	BCR-CRM 144R "t"	3.14	C	7
Human Hair	NIES-CRM-13	4.42	C	4
Marine Sediment	NRCC-PACS-1	4.57	C	4
Dogfish Muscle	NRCC-DORM-2	4.64	C	6
Contaminated water filter media	EPA-CRM002-100	5	N	
Dry Soil	EPA-CRM003-50	5	C	
Total and Methylmercury in Tuna Fish	BCR-CRM 464	5.24	C	2

Name	Material Code	mg/kg	cert. code	U(%)
Montana II Soil	NIST-SRM 2711	6.25	C	3
Polluted Marine Sediment	IAEA-356	7.62	C	8
Sewage Sludge	BCR-CRM 146 "t"	9.49	C	8
Plating Sludge No.3	EPA-CRM011-100	10	N	
Zinc Ore (blende)	BCR-CRM 108	10.9	C	6
Human Hair	BCR-CRM 397	12.3	C	4
Industrial Incinerator Ash	EPA-CRM-012-100	20	N	
Human Hair	IAEA-085	23.2	C	5
Paint chips	EPA-CRM013-050	24	N	
City Waste Incineration Ash	BCR-CRM 176	31.4	C	4
Montana I Soil	NIST-SRM 2710	32.6	C	6
Tennessee River Sediment	NIST-RM 8407	50	C	4
Copper Plant Flue Dust	CSRM-KHK-12-1-06	52.4	C	12
Zinc Ore (blende)	BCR-CRM 110	148.4	C	2
Non-Ferrous Dust	CANMET-PD-1	389	C	5

* = mg/litre

PERSPECTIVES ON ATMOSPHERIC MERCURY AND ITS FATE IN AQUATIC ECOSYSTEMS.

By

Donald B. Porcella
Electric Power Research Institute
Palo Alto CA USA 94304
Tel: 650/938-4775, fax: - -1069
Email: dporcell@epri.com

ABSTRACT

Mercury (Hg) is a neurotoxin to humans and wildlife with a quantitatively important gas phase that makes its atmospheric cycle an important part of exposure pathways to target organisms. The important atmospheric forms are elemental Hg (Hg°), inorganic gas phase Hg ($HgII_{gs}$), and inorganic particulate Hg (HgIIp). Each form has differing behavior depending on its chemical and physical properties. Broad-scale estimates of Hg inputs to the atmosphere suggest that annual anthropogenic fluxes to the atmosphere as Hg° are about 40 percent of the total global cycle, oceanic evasion is about 40 percent, and (by difference) the remaining input to the global cycle (20 percent) is evasion from terrestrial sources, largely via soils and vegetation. Recent data on cycling of Hg through forested ecosystems show that the cycle of evasion and deposition are strongly controlled by season, particularly factors of light and temperature. The evasion from oceans and land consists of re-emissions of previously deposited Hg as well as releases from geologic sources. Additional anthropogenic inputs to the atmosphere as HgII are assumed to be about the same quantity as the anthropogenic Hg° flux, but generally are not a large part of the global cycle, depositing locally and regionally for the most part. Regional deposition is composed of differing proportions of the global background and inputs from anthropogenic sources in different geographic areas. Historic anthropogenic emissions from industrial activities are not well quantified. However, the patterns of historic deposition indicate peak deposition during the mid-1960's in the USA, a value coincident with a peak in industrial consumption of Hg.

Temporal and spatial patterns of emissions and deposition of Hg are important, but have little relation to risk to health unless there is conversion of HgII to MMHg (monomethyl-Hg), the most toxic form of Hg. At present in developed countries, almost all Hg exposure to humans and wildlife arises from consumption of fish that contain measurable levels of MMHg. The effects of this low level of exposure are not easily observed. In any case, model analyses of exposure and effects show that low level Hg exposure will not be easily controlled, and that changes in deposition are not rapidly reflected in changes in fish Hg. Understanding the reasons for this slow rate of change in fish Hg remains a scientific puzzle, yet one amenable to manipulative field experiments.

Introduction

A great amount of peer-reviewed information is now available about mercury cycling, much of which was first presented in international conferences held in Gavle Sweden (Lindqvist et al. 1991b), Monterey CA USA (Watras and Huckabee, 1994), Whistler BC Canada (Porcella et al.

1995), and more recently in Hamburg Germany in 1996 (see Wheatley and Wyzga 1997; Wilken et al. 1996). Unfortunately, this information has not been readily available to all persons with concerns about managing mercury. Consequently, this paper summarizes some of the most recent results on North American emissions, how they relate to Hg as a global pollutant, the role of global Hg cycling, perspective on historic uses and deposition of Hg, and the conversion of HgII forms to MMHg.

Hg is considered a global pollutant for two major reasons:

- Anthropogenic emissions of mercury are widespread: gold mining, industrial and commercial activities, and emissions from combustion when mercury is present as a trace contaminant in fuels and ores.

- Atmospheric cycling on a global basis: Hg has an important atmospheric phase due to its strong vapor phase -- Hg^0 and fine particulate cycling -- HgIIp. The atmosphere distributes much of this mercury globally.

Generally, scientists have found with trace-metal-clean sampling and analytical techniques, that background Hg levels are 1-2 ng $\cdot m^{-3}$ in air, 0.1-2 ng $\cdot l^{-1}$ in surface waters, 5-25 ng $\cdot l^{-1}$ in rainwater, and less than 0.1 $\mu g \cdot g^{-1}$ in sediments remote from natural Hg minerals and wastewater sources. Values in excess of these concentrations generally indicate local influences (both natural and anthropogenic) or samples that have been contaminated through failure to use clean sampling and analysis techniques. Sample contamination is an important problem in the analysis of air and water samples; Hg contamination is less of a problem for soils, sediments and biota, and older reported values for these media are generally more meaningful. Since 1990, most investigators have used the methods developed by Fitzgerald and co-workers to obtain meaningful results (e.g., Gill and Fitzgerald 1987).

Hg from anthropogenic and natural sources has an important atmospheric phase that contributes to the total risk to organisms. After deposition from the atmosphere primarily as different phases of HgII, the bioavailable inorganic HgII can be methylated, producing the most toxic form of Hg, MMHg (CH_3Hg^+). This Hg can be bioaccumulated and represent a risk to health of fish-eating humans and wildlife. Assessment of this risk depends on accurate estimates of exposure, which in areas without geologic or direct discharge sources, comes from atmospheric deposition. Exposure estimates require an understanding of the myriad sources to the atmosphere, transformation and transport of the different chemical species, and the factors responsible for deposition (Pai et al. 1997; EPMAP 1994).

Global Cycles of Mercury

Although local and regional deposition of HgII occur (Seigneur et al. 1994), here we consider the global atmosphere as the system of interest, and this cycle is driven by Hg(0). Slemr (1985) and Fitzgerald (1986) provided the first constraints on the global atmospheric cycle. Fitzgerald (1986) estimated that the 'close-to-steady-state' annual Hg inputs to the atmosphere were 5000-6000 Mg/yr with about one-third coming from marine evasion, one-third anthropogenic, and one-third (by difference) from land sources (Tab. 1 modified from Porcella 1994). With a one-year

atmospheric residence time, this annual input is balanced by deposition at an average global deposition rate of about 10 ug Hg/m^2yr. As a rule, wet and dry deposition occur after oxidation of Hg(0) to HgII; for example, Hg(0) is weakly soluble in water, and oxidation is needed to dissolve Hg in precipitation. The atmospheric inputs include re-emission of previously deposited Hg of

Table 1
Simple Global Mass Balance of Mercury

Atmospheric pool -	5000 Mg
Anthropogenic sources	2000 to 4000 Mg/year
Local and regional -	1000 to 2000 Mg/year
Global -	1000 to 2000 Mg/year
Marine sources* -	2000 Mg/year
Terrestrial sources* -	1000 to 2000 Mg/year

Hg Global Atmospheric Residence Time is about one year.

* Includes re-emissions and natural sources.

both anthropogenic and natural origin. In an analysis of the global mass balance of mercury, Mason et al. (1994) assumed that global anthropogenic mercury emissions totaled 4000 Mg/yr, of which 2000 entered the global cycle and 2000 deposited locally and regionally. More recently, Pirrone et al. (1996) updated earlier calculations by Nriagu and Pacyna (1988) and estimated the total anthropogenic contribution as 2000 Mg/yr, and we can assume an even split between global and the regional/local components. These results suggest an even greater evasion from terrestrial systems of 2000 Mg/yr.

Much of the background emission is assumed to be in the form of elemental Hg (Hg(0)), that evades from the ocean surface as well as from water surfaces, soil, minerals, and vegetation located on land surfaces (Lindberg 1997; Lindberg et al. 1998). Forest fires and other high temperature 'natural' emission sources likely emit at least partially oxidized Hg as particulate and gas-phase forms of Hg (Fitzgerald 1986). The so-called background emission includes Hg that previously deposited from both natural and anthropogenic sources (EPMAP 1994). The soil and the oceans provide large Hg reservoirs which serve as deposition receptors, and then later act as a source to the atmosphere. Apparently, these reservoirs are not necessarily sinks for mercury, although there may be internal sink/sources like deep ocean mixing.

Municipal-industrial, combustion, mining, and natural sources contribute Hg to the atmosphere and other environments around the globe. Estimated North American emissions are shown in Tab. 2, with data obtained from a variety of estimates in the US (USEPA, 1994; Midwest Research Institute 1997; Pai et al. 1997; Pirrone et al. 1996; Porcella et al. 1996) and Canada (noted in Porcella et al. 1996). Mexico emisssions are extrapolated as described in Porcella et al. (1996). Power generation emissions are about 10 percent less than USEPA, because recent and quite accurate data on actual emissions were used (Chu and Porcella 1995). Although these emissions estimates give a rough guide to on-going anthropogenic sources, speciation results are needed to assess the impacts of these emissions on surface waters. Generally, most assessments assume a 50:50 split between Hg(0) and HgII, but more accurate estimates are needed for accurate risk analysis.

As a way to constrain the simple mass balance tabulated in Tab. 1, Hudson et al. (1995) developed a global mercury cycling model (G-MCM) calibrated to historic atmospheric deposition and historic mercury emissions to estimate natural sources and re-emissions. As a starting hypothesis, the G-MCM utilized some of the data in Tab. 1, i. e., the total cycle of 5000 Mg/yr and anthropogenic emissions of Hg(0) of 2000 Mg/yr. Using the historic anthropogenic mercury emissions to the

Table 2
Estimated North American Anthropogenic Sources of Mercury Emissions to the Atmosphere

CATEGORY	CANADA	MEXICO	USA
POWER GENERATION	6.0	2.8*	42.5
INDUSTRIAL/COMMERCIAL COAL	6.8	1.5*	21.5
RESIDENTIAL COAL	-*	0.1*	1.5
MUNICIPAL WASTE COMBUSTION	1.4	15.5*	26.0
MEDICAL WASTE COMBUSTION	0.3	3.8*	14.5
MANUFACTURING/SMELTING	32.0	55.7*	18.2
MISCELLANEOUS	-*	2.6*	29.4
TOTAL	46.5	82.0	153.6

*Extrapolated - see text. Dash (-) indicates value included in number above

atmosphere -- largely Spanish precious metal extraction in the Americas (Nriagu 1994) -- and incorporating deep ocean circulation, Hudson et al. (1995) estimated different natural and re-emitted anthropogenic Hg emissions than did Mason et al. (1994) in their first mass balance (Tab. 3). Thus, the ratio of anthropogenic to natural emissions was 2.1 for the MFM hypothesis (Mason et al. 1994) while the G-MCM gave a ratio of 1.3. The effectiveness of control of anthropogenic

Table 3
Comparison of two different model estimates of global mercury cycling
(MFM is Mason et al. 1994; G-MCM is Hudson et al. 1995).

Sources	MFM	G-MCM
Natural	1600	2200
Anthropogenic		
– Current	2000	2000
– Re-emitted Anthr.	1400	500
– Re-emitted Mining	-	300
Totals	5000	5000
Anthropogenic/natural	2.1	1.3

*Metric tons/year = million g/year.

sources would be substantially less in the assessment provided by Hudson et al. (1995). The ratios reflecting current anthropogenic emissions into the global atmosphere to total background would be

the same for both approaches, i. e., about 0.7. To more accurately assess control effectiveness, we need more accurate data on global anthropogenic Hg emissions (as shown in Tab. 2) plus an assessment of natural emissions. For example, if Pirrone et al. (1996) are correct, then the effectiveness of controls would be even less.

Historic Deposition Estimates

Within the US (Fig. 1) and other industrialized countries (e.g., Lindqvist et al. 1991a), the industrial use of Hg has decreased substantially. Industrial Hg consumption in the US has decreased by 85 percent from its peak during 1964-68. One would expect that the Hg content of organisms would show a similar decrease. However, no data other than deposition archives (lake sediment and peat cores) show a sharp decline, and there are few archives and data sets that shed light on the pattern of Hg deposition or, even more important, the Hg content of organisms.

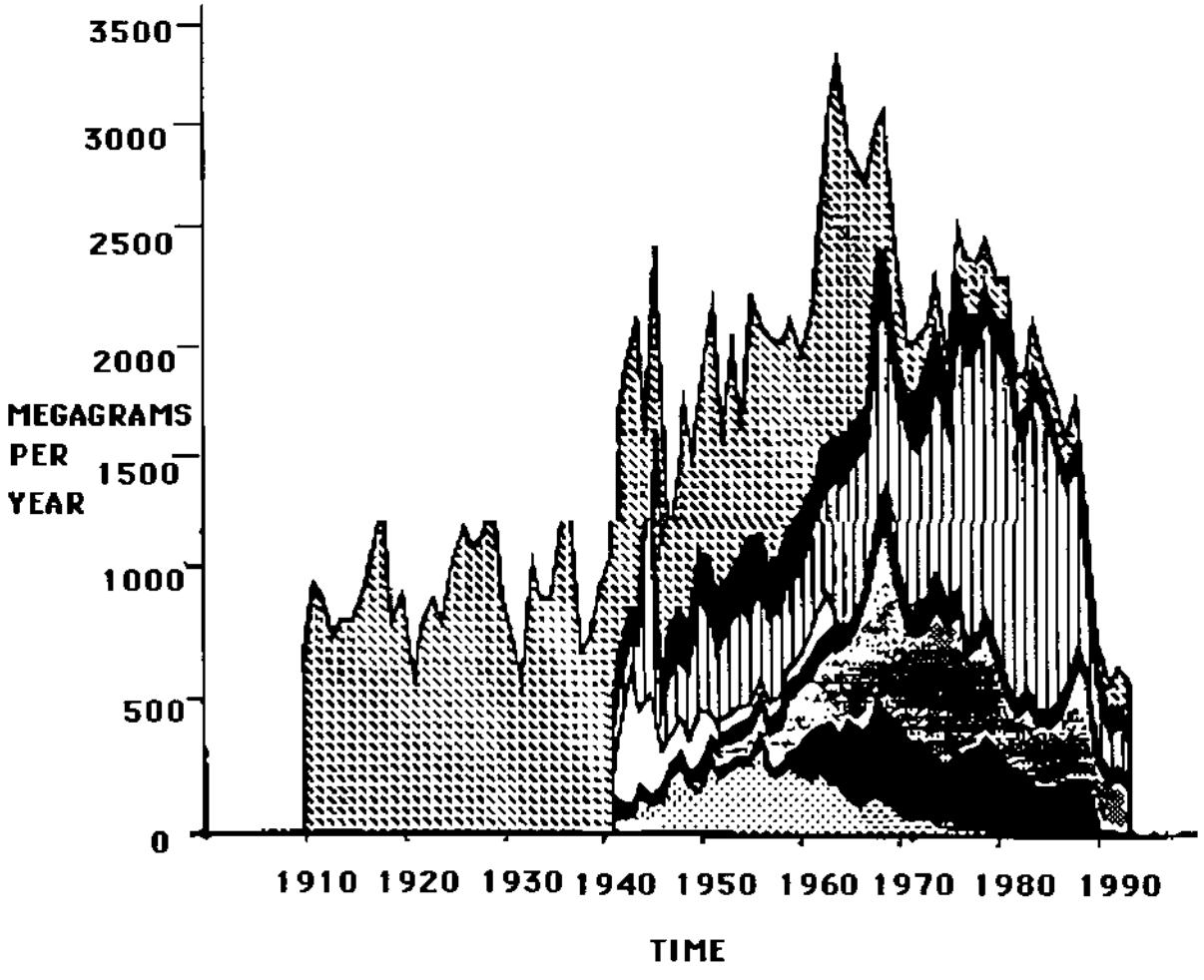


Figure 1. Total US industrial Hg consumption (figure based on Engstrom and Swain 1997).

Recently, Benoit et al. (1994; also, see Zillioux et al. 1993) showed for the first time that Hg deposition peaked in the 1950-1970's in peat cores collected in Minnesota (Fig. 2). Similarly, the data in Swain et al. (1992) are consistent with the concept that Hg deposition peaked during the same period; Engstrom and Swain (1997) showed this to be the case in eastern lakes in Minnesota. However, Engstrom and Swain (1997) showed data for similar cores in Alaska with a continued increase and no peak. These results suggest that the global background is increasing (Alaskan cores) while regional sources have decreased (Minnesota).

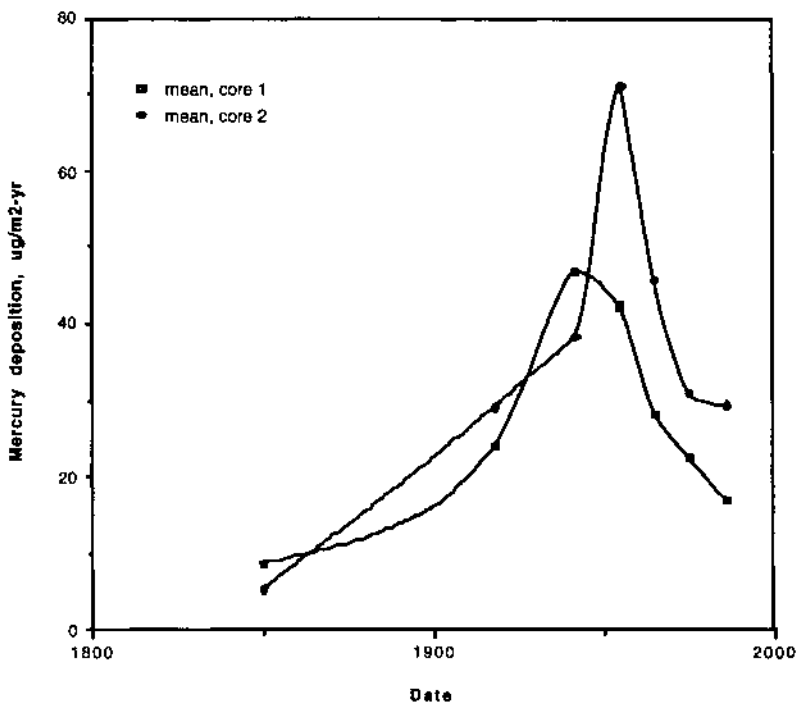


Figure 2. Patterns of Hg deposition measure in two peat cores from northeren Minnesota, USA (data from Benoit et al. 1994).

Historic Patterns of Hg in Biota

Methylation: Except in cases where Hg point source discharges are substantial and at Hg contaminated sites, organisms are exposed to chronic, low-level Hg concentrations. Because very little Hg(0) dissolves in water, HgII comprises almost all the aqueous Hg and is present in two major forms: inorganic HgII and methylated HgII (MMHg). In lakes and wetlands (Fig. 3), methylation produces MMHg in a process associated with microorganisms (largely, sulfate-reducing bacteria); enzymes in other bacteria can remove the methyl group in a process called demethylation. Usually demethylators also reduce the Hg to elemental Hg(0). These reactions have been described by Hudson et al. 1994 (also, see Gilmour et al. 1992, Winfrey and Rudd 1990). The rate of production depends on several important factors such as sulfate concentrations, dissolved organic carbon (DOC), and other water quality variables (Hudson et al. 1994). Although

some MMHg deposits from the air (e.g., Bloom and Watras 1989; Fitzgerald et al. 1991) -- probably largely of marine origin (Prestbo, E. Personal Communication) -- net methylation in the aquatic environment is by far the main source of the Hg accumulated in biota. Fish appear to bind

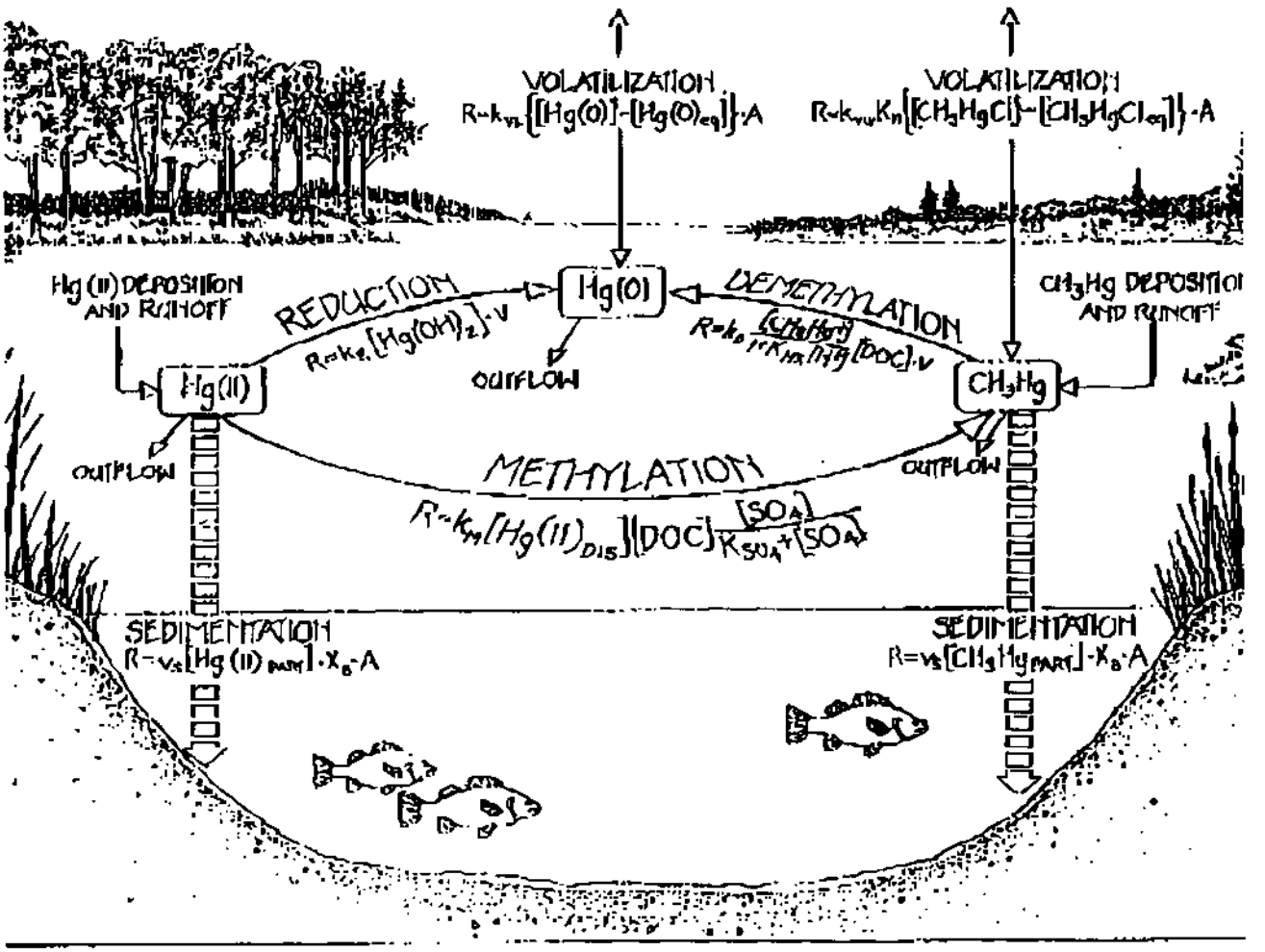


Figure 3. Rate processes and reactions leading to the cycling of chemical forms of Hg in aquatic ecosystems, and the methylation and demethylation of HgII (from Hudson et al. 1994).

MMHg strongly, and its biological halflife is on the order of 2 years (Wiener and Spry 1996). It is through fish consumption that Hg exposure in humans and in fish-eating birds and mammals takes place. Therefore, the key to understanding risk from Hg is the linkage of Hg inputs to fish accumulation -- a link dominated by methylation. This link should lead to related responses between changes in inputs and biotic Hg concentrations -- almost exclusively as MMHg (Bloom 1992; Watras and Bloom 1992).

Some Results. Biotic archives are rare. Newton et al. (1993) analyzed collected bird livers in Britain over a 25 year period showing a strong decrease in Hg concentration over time (Fig. 4). This decrease coincides with the decline in industrial use described in several areas (Lindqvist et al. 1991a; Engstrom and Swain 1997). Species with an aquatic-based food web (grey heron) showed a stronger decrease than the terrestrial based species (sparrowhawk and kestrel). The terrestrial food chain was likely contaminated by MMHg-treated seed grain; however, the grey heron would likely obtain MMHg according to the processes outlined in the previous paragraph.

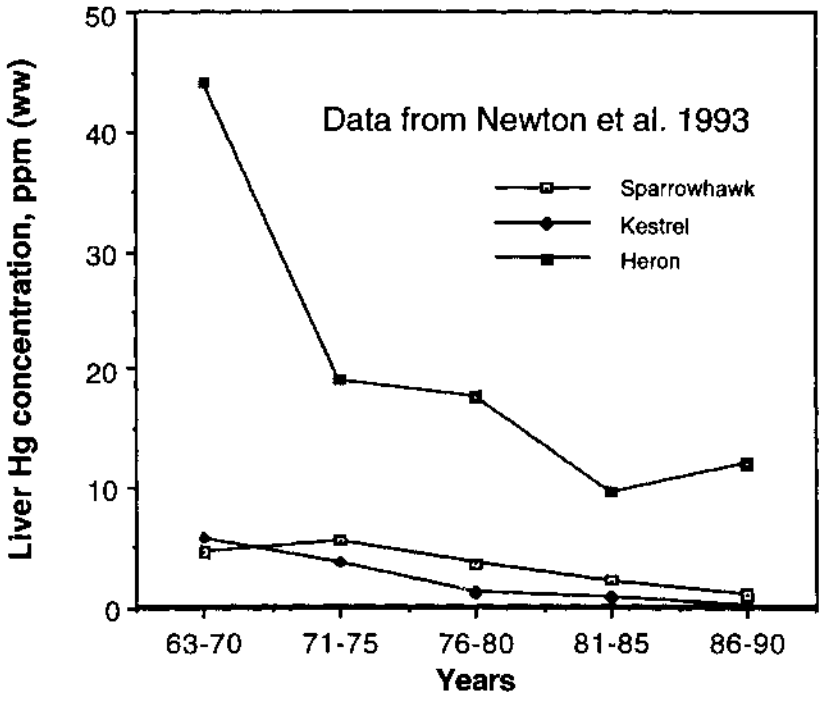


Figure 4. Decreases in bird liver Hg from the United Kingdom (data from Newton et al. 1993).

The temporal decrease in Hg content for aquatic species is well described for the English-Wabigoon River system in Canada, which received a Hg point source discharge from a chlor-alkali plant (Parks and Hamilton 1987). Sharp decreases in Hg content in two predaceous fish and a crayfish, show that cessation of the point source caused a reduction in biotic Hg (Fig. 5). However, Hg levels have not decreased below values reported in sites remote from point sources, indicating a continued supply from sediments or from global background. Other time series data for fish exist, but are of short duration. Swain (E. B., Personal Communication) suggests that Minnesota fish Hg varies in a manner consistent with the patterns described in Benoit et al. (1994).

We used the Mercury Cycling Model (Hudson et al. 1994) to assess various scenarios of environmental mercury processes to explain some of the relationships that are shown in Fig. 4 and

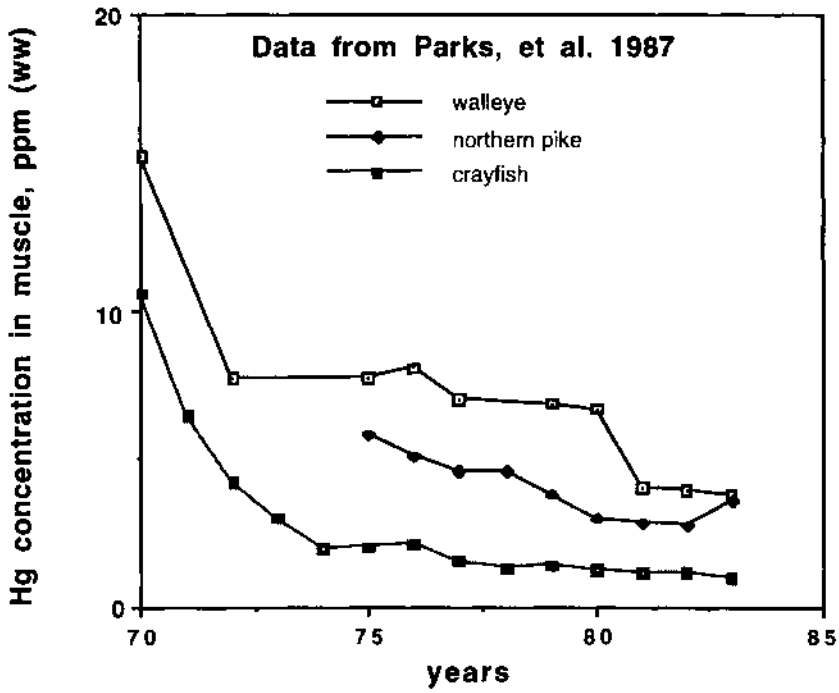


Figure 5. Declines in fish and crayfish Hg after cessation of Hg chloralkali waste discharges to the English-Wabigoon River system, Canada (data from Parks et al. 1987).

Fig. 5. The base case is for a small (10 hectare), shallow (less than 10 m maximum depth), seepage lake in Northern Wisconsin (see Watras et al. 1994), and shows a typical annual cycle for the weighted average fish-eating predatory fish. The cycle reflects seasonal changes in fish biomass and in the net production of MMHg. After changes in appropriate process inputs (particulate concentration in water, demethylation rate, and deposition rate), the MCM simulation shows how fish Hg changes incomparison with the base case. Note the time lags in fish response vary between the different processes.

It is clear from Fig. 6, that environmental factors have more impact on MMHg formation than does loading of Hg. In almost all cases -- except point-source discharge -- environmental influence factors appear to have a greater effect on fish Hg accumulation than does loading (e. g., see Grieb et al. 1990). Several scenarios show that methylation is not strongly sensitive to loading, as assessed by relative changes in fish Hg. Large changes in demethlation rates (unlikely to happen) and large increases in particle concentration (10 times, like adding a shovelful of clay to a small seepage lake) cause a halving of fish MMHg concentrations. For elimination of a single atmospheric source like a power plant where one would expect a maximum of about 5 percent change in deposition, one obtains such a small change that it is difficult to discern a difference from the base case.

Needed Measurements. These results emphasize the lack of data on biotic Hg and reinforce the need to establish careful time series of Hg accumulation in biological populations. Small numbers of museum samples make it difficult to obtain this time series data for biota. As a first step, accurate historic patterns of Hg deposition obtained from a range of geographic sites could provide a picture of how Hg exposures have changed. To evaluate patterns of historic Hg deposition, peat and lake

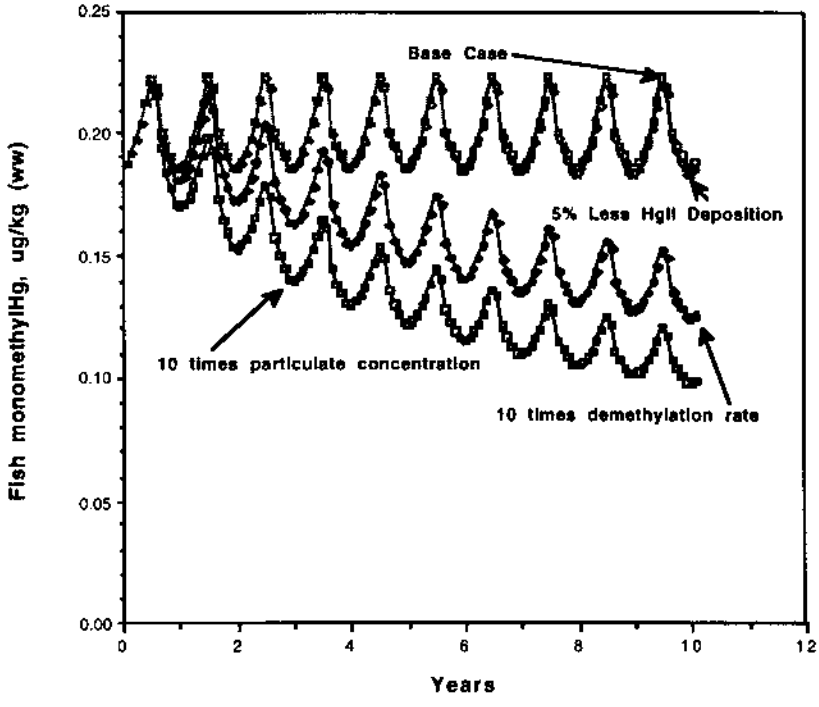


Figure 6. Different process scenarios produce markedly different temporal patterns in fish MMHg concentrations (based on Porcella 1994; and Hudson et al. 1994).

sediment cores -- properly obtained -- could provide an invaluable retrospective look at Hg deposition. A working group of scientific experts on the collection and analysis of Hg in lake, peat and other archive cores prepared a protocol that will make it easier for scientists around the globe to obtain, analyze, and interpret these patterns of Hg deposition (Protocol, 1996). The protocol is available to any scientist with an interest in making historic deposition measurements. A meeting to discuss results of globally spread-out coring operations will be held in 1999, around the time of the Fifth International Conference on Mercury as a Global Pollutant (Rio de Janeiro, May 1999).

References

Benoit, J. M., W. F. Fitzgerald, and A. W. H. Damman. 1994. "Historical Atmospheric Mercury Deposition in the Mid-Continental United States as Recorded in an Ombrotrophic Peat Bog." In Mercury as a Global Pollutant. Edited by C. J. Watras and J. W. Huckabee. Ann Arbor: Lewis Publishers, pp. 187-202.

Bloom, N. S. 1992. On the Chemical Form of Mercury in Edible Fish and Marine Invertebrate Tissue. Can. J. Fish. Aquat. Sci. 49:1010-1017.

Bloom, N. S. and C. J. Watras. 1989. Observations of methylmercury in precipitation. Sci. Tot. Environ. 87/88:199-207.

Constantinou, E., M. Gerath, D. Mitchell, C. Seigneur, and L. Levin. 1995. Mercury from Power Plants: Environmental Cycling and Health Effects. Water Air Soil Pollut. 80:325-335.

Chu, P. and D. B. Porcella. 1995. Mercury stack emissions from U. S. electric utility power plants. Water, Air, Soil Pollut. 80:135-144.

Engstrom, D. R. and E. B. Swain. 1997. Recent declines in atmospheric mercury deposition in the Upper Midwest. Env. Sci. Tech. 31:960-967.

EPMAP (Expert Panel on Mercury Atmospheric Processes). 1994. Mercury Atmospheric Processes: A synthesis report. Workshop Porceedings. EPRI, Palo Alto, CA. EPRI/TR-104214. 24pp.

Fitzgerald, W. F. 1986. "Cycling of Mercury Between the Atmosphere and Oceans." In The Role of Air-Sea Exchange in Geochemical Cycling. NATO Advanced Science Institutes Series. Edited by P. Buat-Menard. Dordrecht, Netherlands: Reidel, pp. 363-408.

Fitzgerald, W. F., R. P. Mason, and G. M. Vandal. 1991. Atmospheric Cycling and Air-Water Exchange of Mercury Over Mid-Continental Lacustrine Regions. Water Air Soil Pollut. 56: 745-768.

Gill, G. A. and W. F. Fitzgerald. 1987. Picomolar Mercury Measurements in Seawater and Other Materials Using Stannous Chloride Reduction and Two-Stage Gold Amalgamation with Gas Phase Detection. Marine Chem. 20:227-243.

Gilmour, C. C., E. A. Henry, and R. Mitchell. 1992. Sulfate Stimulation of Mercury Methylation in Freshwater Sediments. Environ. Sci. Technol. 26:2281-2287.

Grieb, T. M., C. T. Driscoll, S. P. Gloss, C. L. Schofield, G. L. Bowie, and D. B. Porcella. 1990. Factors Affecting Mercury Accumulation in Fish in the Upper Michigan Peninsula. Environ. Toxicol. Chem. 9:919-930.

Hudson, R. J. M., S. A. Gherini, C. J. Watras, and D. B. Porcella. 1994. "A Mechanistic Model of the Biogeochemical Cycle of Mercury in Lakes." In Mercury as a Global Pollutant. Edited by C. J. Watras and J. W. Huckabee. Ann Arbor: Lewis Publishers. Ann Arbor, MI. pp. 473-523.

Hudson, R. J. M., S. A. Gherini, W. F. Fitzgerald, and D. B. Porcella. 1995. Anthropogenic influences on the global mercury cycle: a model-based analysis. Water, Air, Soil Pollut. 80:265-272.

Lindberg, S. E. 1996. Forests and the global biogeochemical cycle of mercury: The importance of understanding air/vegetation exchange processes. In W. Baeyens et al. (eds.), Global and Regional Mercury Cycles: Sources, Fluxes, and Mass Balances. Kluwer Academic Publ. Netherlands. pp. 359-380.

Lindberg, S. E., P. J. Hanson, T. P. Meyers, and K-W Kim. 1998. Air/surface exchange of mercury vapor over forests: The need for a reassessment of continental biogenic emissions. Atmos. Environ. In press.

Lindqvist, O., K. Johansson, M. Åstrup, A. Andersson, L. Bringmark, G. Hovsenius, Å. Iverfeldt, M. Mieli, and B. Timm. 1991a. Mercury in the Swedish Environment—Recent Research on Causes, Consequences and Corrective Methods. Water Air Soil Pollut. 55:i-261.

Lindqvist, O. (ed.) 1991b. Mercury as an Environmental Pollutant. Water Air Soil Pollut. 56:3-847.

Mason, R. P., W. F. Fitzgerald, and F. M. M. Morel. 1994. The Biogeochemical Cycling of Elemental Mercury: Anthropogenic Influences. Geochim. Cosmochim. Acta. 58:3191-3198.

Midwest Research Institute. 1997. Draft Report to USEPA. Locating and Estimating Hg Atmospherric Sources. EPA-RTP, NC, USA.

Newton, I., I. Wyllie, and A. Asher. 1993. Long-Term Trends in Organochlorine and Mercury Residues in Some Predatory Birds in Britain. Environ. Pollut. 79:143-151.

Nriagu, J. O. 1994. Mercury Pollution From the Past Mining of Gold and Silver in the Americas. Sci. Total Environ. 154:1-8.

Nriagu, J. O. and J. M. Pacyna. 1988. Quantitative Assessment of Worldwide Contamination of Air, Water and Soils by Trace Metals. Nature 333:134-139.

Pai, P., S. Heisler, and A. Joshi. 1997. An Emissions Inventory for Regional Atmospheric Modeling of Mercury. Water Air Soil Pollut. In press.

Parks, J. W. and A. L. Hamilton. 1987. Accelerating Recovery of the Mercury-contaminated Wabigoon-English River System. Hydrobiol. 149:159-188.

Pirrone, N., G. J. Keeler, and J. O. Nriagu. 1996. Regional Differences in Worldwide Emissions of Mercury to the Atmosphere. Atmos. Environ. 30:2981-2987.

Porcella, D. B. 1994. "Mercury in the Environment: Biogeochemistry." In Mercury as a Global Pollutant. Watras. Edited by C. J.Watras and J. W. Huckabee. Ann Arbor: Lewis Publishers, pp. 1-19.

Porcella, D. B., J. W. Huckabee, and B. Wheatley (Eds.). 1995. Mercury as a Global Pollutant. Water, Air, Soil Pollut. 80: 1-1336.

Porcella, D. B., P. Chu, and M. A. Allan. 1996. Inventory of North American Hg Emissions to the Atmosphere. In Global and Regional Mercury Cycles: Sources, Fluxes and Mass Balances. Baeyens et al. (eds.). Kluwer Acad. Publ. pp. 179-190.

Protocol. 1996. Protocol for Estimating Historic Atmospheric Mercury Deposition. EPRI TR-106768. Electric Power Research Institute. Palo Alto, CA. 49 pp.

Seigneur, C., J. Wrobel, and E. Constantinou. 1994. A Chemical Kinetic Mechanism for Atmospheric Mercury. *Environ. Sci. Technol.* 28:1589-1597.

Slemr, F., W. Seiler, and G. Schuster. 1985. Distribution, Speciation, and Budget of Atmospheric Mercury. J. Atmos. Chem. 3:407-434.

Swain, E. B., D. R. Engstrom, M. E. Brigham, T. A. Henning, and P. L. Brezonik. 1992. Increasing Rates of Atmospheric Mercury Deposition in Midcontinental North America. Science. 257:784-787.

USEPA. 1994. Mercury Study Report to Congress (draft: 12/12/94). OAQPS/ORD. Vol. 2-7. Research Triangle Park, NC 27711.

Watras, C. J. and J. W. Huckabee. 1994. Mercury as a Global Pollutant. Ann Arbor: Lewis Publishers. Ann Arbor, MI. 727 p.

Watras, C. J. and N. S. Bloom. 1992. Mercury and Methylmercury in Individual Zooplankton: Implications for Bioaccumulation. Limnol. Oceanogr. 37:1313-1318.

Watras C. J., N. S. Bloom, R. J. M. Hudson, S. A. Gherini, R. Munson, S. A. Klaas, K. A. Morrison, J. Hurley, J. G. Wiener, W. F. Fitzgerald, R. Mason, G. Vandal, D. Powell, R. Rada, L. Rislove, M. Winfrey, J. Elder, D. Krabbenhoft, A. W. Andren, C. Babiarz, D. B. Porcella and J. W. Huckabee. 1994. Sources and fates of mercury and methylmercury in remote temperate lakes. In "Mercury as a Global Pollutant". Watras, C. J. and J. W. Huckabee, Eds. Lewis Publishers. pp. 153-157.

Wheatley, B. and R. Wyzga. (eds.) 1997. Mercury as a global pollutant: human health issues. Water Air Soil Pollut. 97(1,2):

Wiener, J. G. and D. J. Spry. 1996. Toxicological significance of mercury in freshwater fish. In "Environmental Contaminants In Wildlife: Interpreting Tissue Concentrations." (W. N. Beyer, G. H. Heinz and A. W. Redmon-Norwood, eds.). Spec. Publ. Society Env. Tox. Chem. Lewis Publishers, Boca Raton, FL, USA. pp. 297-339.

Wilken, R.-D., S. L. Lindberg, M. Horvat, G. Petersen, D. Porcella, W. Schroeder, J. R. Wisniewski, J. Wisniewski, B. Wheatley, M. Wheatley, R. Wyzga. 1996. Fourth International Conference on Mercury as a Global Pollutant. Conference Summary Report. GKSS-FG:GmbH, Germany and ORNL, USA. Contact: Ralf Ebinghaus, Fax:49-4152-872366. 18 p.

Winfrey, M. R. and J. W. M. Rudd. 1990. Environmental Factors Affecting the Formation of Methylmercury in Low-pH Lakes. Environ. Toxicol. Chem. 9:853-869.

Zillioux, E. J., D. B. Porcella, and J. M. Benoit. 1993. Mercury Cycling and Effects in Freshwater Wetland Ecosystems. Environ. Toxic. Chem. 2: 2245-2264.

THE GENETICS OF MERCURY RESISTANCE IN PSEUDOMONAS K-62

HIDEMITSU PAN-HOU AND MASAKO KIYONO

Faculty of Pharmaceutical Sciences, Setsunan University
45-1, Nagaotogecho, Hirakata, Osaka 573-01, Japan

Abstract The mercurial resistance of Pseudomonas K-62 was found to be conferred by two plasmids of 26-kb and 68-kb. By cloning and sequencing the genes determining mercury resistance on the 26-kb plasmid, two separate gene clusters were found in a 7.8-kb DNA sequence. One of these includes 6 open reading frames, five of which were identified as merR, merT, merP, merA and merB in order and the other was found to consist of merR, merB and merD. The genes encoded proteins with regulatory, transport and enzymatic functions.

Key words Mercurial resistance, Plasmid-determined mer-operon, Pseudomonas K-62.

Introduction

Mercury compounds are distributed widely across the earth and are still one of the most important environmental pollutants at global level. Mercury is toxic to all living organisms because of their high affinity for sulfhydryl groups in protein. Indeed many functional biomolecules have critical thiol groups and are inhibited by mercury compounds in vitro. However, bacteria have evolved mechanisms of resistance to mercurials, and play a major role in the global cycling of mercury in the natural environment. Bacterial resistance to mercurials is probably always determined by enzymatic degradation and/or reduction of mercuric ion to the less toxic and volatile mercury. To understand the environmental microbiology of toxic metals and to use bacterial processes for bioremediation in the future, we must understand the biochemical activities and their genetic determinants.

Today, the best biochemically characterized system for mercury resistance is that of Pseudomonas K-62 strain, the first mercury resistant strain studied which was isolated from phenylmercury polluted soil. The mercury resistance shown by this strain has been reported to be based on the enzymatic degradation of organic mercury by the lyase enzymes and then reduction of the yielded

mercuric ion by the reductase to less toxic and volatile mercury as shown in Fig. 1. The biochemical mechanism and the properties of both enzymes concerned with the mercury resistance have been extensively studied about 25 years ago, however the gene locus still remains unknown.

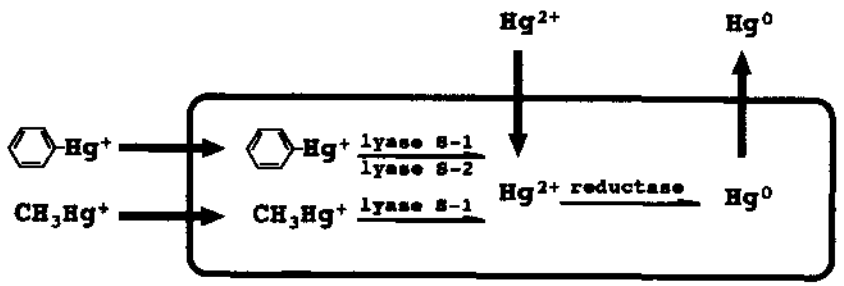


Fig. 1 The reductive decomposition of organomercury compounds in mercury-resistant *Pseudomonas* K-62 (Tezuka and Tonomura , 1976)

In the present study, we demonstrated for the first time that the mercurials resistance of Pseudomonas K-62 is conferred by two plasmids of 26 and 68-kb. In addition, by cloning the resistance determinants on the 26-kb plasmid which was designated plasmid pMR26, we found that pMR26 contains two inducible functional mer operons.

Materials and Methods

Bacteria and regents Pseudomonas K-62 strain with broad-spectrum mercury resistance was kindly supplied by Professor K. Tonomura (Fukuyama University). The mer gene probes Tn501merR, and pDU1358 merA and merB were kindly donated by Dr. B. H. Olson (University of California). Restriction enzymes and DNA ligation kit were obtained from Takara Shuzo (Kyoto, Japan). Radioactive mercury compounds were purchased from Amersham (Bucks, UK). All the other chemicals were the highest purity available from regular commercial sources.

DNA manipulations Plasmids of Pseudomonas K-62 were prepared by the method of Sasakawa et al. (1986). Endonuclease digests were carried out as recommended by the suppliers. Southern blotting, Standard molecular cloning, transformation, electrophoresis and construct of mutants were done according to the method described by Sambrook et al. (1989). Nucleotide sequence was determined by

the dideoxy chain termination DNA sequencing method described by Sanger et al. (1977).

Mercury volatilization Bacteria were grown to mid-exponential phase and then induced with mercury. The washed cells were resuspended in the original volume of fresh medium containing 100 µg/ml ampicillin, 100 µM EDTA and 5 µM radioactive mercury compounds. After incubation at 37°C, aliquots were removed periodically and the amount of radioactivity was measured. A qualitative detection of non-radioactive mercury volatilization by the cells was done according to the X-ray method described by Nakamura and Nakahara (1988).

Mercury uptake The late-log phase induced bacterial cells were suspended in L-broth containing 100 µg/ml chloramphenicol, 100 µM EDTA and 5 µM radioactive mercury compounds. After incubation at 37°C aliquots (0.5 ml) were removed periodically and filtered on a Whatman GF/B glass microfiber filter, and the filters were washed three times with 5 ml of L-broth. The radioactivity on the filter was counted.

Mercurial resistance Bacterial resistance to mercury compounds was determined on Petri dishes or in liquid medium according to the method of Foster et al. (1979) and Pan-Hou et al. (1981) respectively.

Results and Discussion

Plasmids were extracted from Pseudomonas K-62 and analyzed by electrophoresis. Six plasmids with molecular sizes of 8.5, 26, 31, 56, 68 and 82 -kb were identified in this soil strain. After treatment of this strain with ethidium bromide, several plasmid deficient mutants were isolated. As shown in Table 1, plasmid deficient mutants 26 and 68, which lacked either the 26 or 68-kb plasmid respectively, showed a higher sensitivity to the mercury compounds than its parent. Mutant strain TY, which was cured of the 26-kb plasmid and the 68-kb plasmid was more sensitive to the mercurials than mutant 26 or 68. These results demonstrate that the mercurial resistance of Pseudomonas K-62 is plasmid-mediated and that both the 26 and 68-kb plasmids are required for full expression of mercurial resistance in Pseudomonas K-62.

Southern blot analysis showed that among the six plasmids only the 26-kb plasmid which was later designated pMR26, hybridized

Table 1 Mercurial resistance of *Pseudomonas* K-62 and its plasmid-deficient mutants

	Plasmid (kb)						Mercurial resistance (ppm)*	
	82	68	56	31	26	8.5	Hg^{2+}	$C_6H_5Hg^+$
Wild	+	+	+	+	+	+	600	60
Mutant 31	+	+	+	-	+	+	600	60
Mutant 26	+	+	+	+	-	+	150	10
Mutant 68	+	-	+	+	+	+	50	20
Mutant TY	+	-	+	+	-	+	<10	<5

*Mercurial resistance was expressed by the minimum concentration of mercurials which inhibited the growth after 24 h incubation.

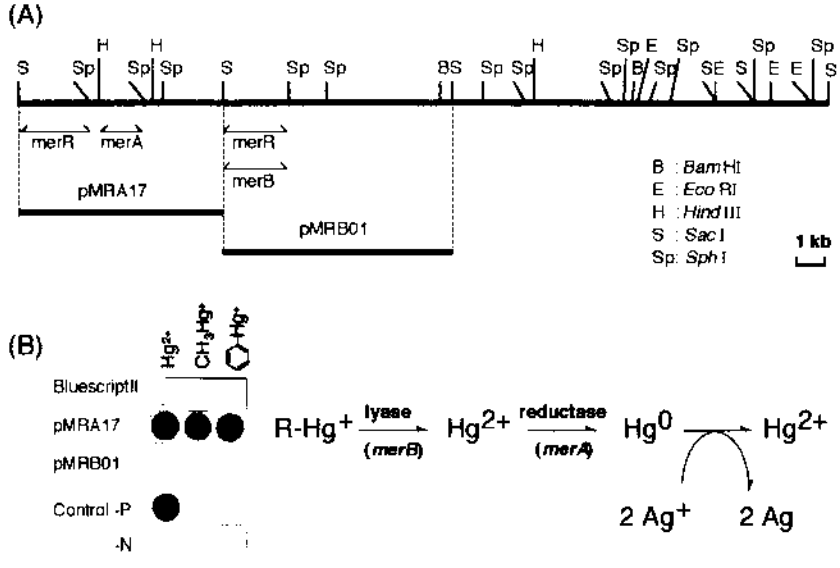
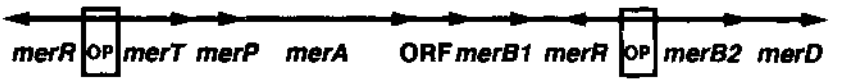


Fig. 2 Endonucleases restriction map of 26-kb plasmid (pMR26) isolated from *Pseudomonas* K-62 (A), & Volatilization of mercury from various mercurials detected by the X-ray film method (B)

Control-P (positive control) was obtained by the chemical reduction of Hg^{2+} with acidic $SnCl_2$. Control-N (negative control) was done in the absence of bacteria.

with the known mer gene probes, Tn501 merR and pDU1358 merA and merB under conditions of both low and high stringencies. It is not clear why the 68-kb plasmid which had the same activity as pMR26 to volatilize mercury from the mercury compounds, did not hybridize with the known mer gene probes (Data not shown). One possibility is that the mer genes residing on the 68-kb plasmid are significantly less homologous with the known mer gene probes. The predicted merA encoding mercuric reductase and merB encoding organomercurial lyase enzyme of the pMR26 were mapped on the 6.6- and 7.4-kb SacI fragments, respectively, both of which contained the predicted merR as shown in Fig. 2A. The two SacI fragments were then inserted into Bluescript II, a cloning vector and the resultant plasmids (pMRA17 and pMRB01) were then transformed into E. coli XL1-Blue. As shown in Fig. 2B, cell with the recombinant plasmid pMRA17, which did not hybridize with the merB probe was able to volatilize mercury not only from mercuric mercury but also from organic mercury. These results suggest that an unique merB gene which did not hybridize with the merB probe but with the same function may be resided in pMRA17, because organomercury can't directly reduce Ag^{+} emulsion to form a foggy on the film, it should be degraded by the lyase enzyme encoded by merB, and then reduced by the reductase encoded by merA. In contrast, cell with pMRB01, which contains the predicted merR and merB but lacks merA had no ability to volatilize mercury from both the mercury compounds.

Nucleotide sequence of a 7800 base pairs encompassing the whole mercurial resistance determinants on the pMR26 was analyzed. The 7800-bp sequence includes nine open reading frames (ORFs), five of which in pMRA17 were identified as merR, merT, merP, merA and merB1 and three of which in pMRB01 were identified as merR, merB2 and merD, respectively, by comparison with the DNA and amino acid sequences of previously sequenced mer operons as shown in Fig. 3 The remaining ORF located between merA and merB1, had no homology with the already known mer genes and seemed to be a new gene. In most cases, merB is linked to the other mer genes and immediately maps downstream from merA. However, the merB1 was mapped 770-bp downstream from merA following the unidentified ORF. Two inverted repeat-like elements upstream from the predicted merR were found in the DNA sequence. These observations suggested that the mer



merR : regulatory protein
merT : mercury transport protein
merP : mercury binding protein
merA : mercuric reductase
merB : organomercurial lyase
merD : downregulatory protein

Fig. 3 Organization of mercury resistance genes on plasmid pMR26 from *Pseudomonas* K-62

operon on pMR26 can could be part of a transposon-like structure (Data not shown). The arrangement of the mer genes determining mercury resistance in this isolated strain does not correspond to any other so far reported.

In general, mercurial resistance is known to be inducible and to be paralleled by the induction of the mercurial detoxifying enzymes, organomercurial lyase and mercuric reductase. Furukawa and Tonomura (1972) reported that the two Pseudomonas's lyases different to its mercuric reductase were expressed constitutively in the bacterium. By cloning the resistance determinants from this strain, we found that the mercurial resistance was mercury inducible phenotype (Kiyono et al. 1995a, b). To resolve this discrepancy, expression of the corresponding mer polypeptides in maxicells in the absence or presence of mercury induction was further studied. As shown in Fig. 4, at least six radioactive mer polypeptides with molecular masses of 60, 23.5, 20, 13, 9.2 and 7 KDa were identified in the maxicells with pMRA17, and one radioactive mer polypeptide with molecular masses of 23.5 KDa in in the maxicells with pMRB01 when the cells were preinduced by mercurials, but not in the cells without mercury induction. The molecular sizes are in reasonable agreement with the 59.9 (MerA), 22.9 (MerB1), 18.7 (uORF), 12.5 (MerT), 9.5 (MerP) and 23.1 (MerB2) KDa sizes that are predicted from translation of the DNA sequences. The 7 KDa polypeptide is believed to be the product of MerP after the removal of a signal peptide during export. These results clearly demonstrated that the two organomercurial

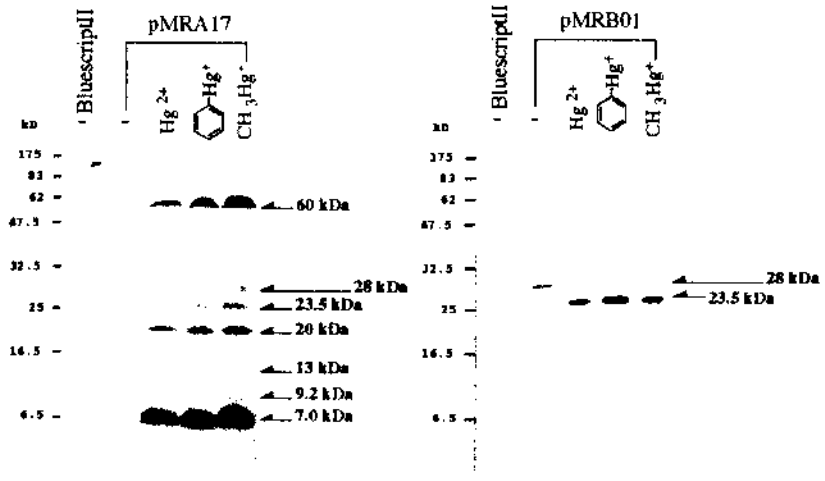


Fig. 4 Inducible synthesis of *mer* polypeptides in maxicells by mercurials

-; uninduced, Hg^{2+}; induced with 1μM Hg^{2+}, ⬡-Hg^{+}; induced with 1μM ⬡-Hg^{+}, CH_3Hg^{+}; induced with 1μM CH_3Hg^{+}. Molecular mass markers (in kD) are indicated at left.

lyase enzymes encoded by merB1 and merB2, respectively, as well as the mercuric reductase is mercury inducible. In addition, there was no difference in the number of the induced polypeptides among the three mercury inducers. These results imply that merR is a broad spectrum regulatory gene, because it can respond to both inorganic and organic mercury (Kiyono et al. 1997).

The protein encoded by the ORF located between merA and merB1. was found to be always coexpressed in maxicells under mercury induction as shown in Fig. 4. Deletion mutation in ORF region without affect its ability to volatilize mercury from inorganic and organic mercury (Data not shown). However, the deletion mutant cells become more sensitive to phenylmercury than the cell with pMRA17, but still retains its full resistance to mercuric ion and methylmercury (Data not shown). These results suggest that this ORF may involve in the phenylmercury resistance via a different mechanism. This new mer gene is then named merE. The precise function of this merE gene must wait further biochemical and genetic studies.

In most cases, the order, number and function of mer gene on both narrow spectrum and broad spectrum mer operons are basically the same. The merT and merP have been implicated in mercuric ion transport and been shown to be required for bacterial resistance

to mercuric mercury, because mercury must be transported into the cell before it can be reduced by the reductase encoded by merA. It seems reasonable to expect that transport of organic mercury into the cells is necessary for the lyase enzyme to act upon it. However, there is no information about the role of merT and merP in the transport of organomercury. To clarify this question, we construct a deletion plasmid, pMRD141 from pMRA17 by eliminating the genes corresponding to merB1 and merA and then compared their mercury resistance phenotypes. As expected, deletion of merB1 and merA genes indeed result in complete loss of its ability to volatilize mercury from the mercurials (Kiyono et al. 1995b). In addition, as shown in Fig. 5, deleted the two genes, not only result in loss of its resistance phenotype but also render the cells hypersensitive to mercuric ion and phenylmercury but not to methylmercury (Uno et al. 1997). It has been reported that expression of merT-merP in the absence of merA would render the host hypersensitive to mercuric ion based on hyperaccumulation of the mercuric mercury. The hypersensitivity to phenylmercury seems to be resulted from hyperaccumulation of phenylmercury

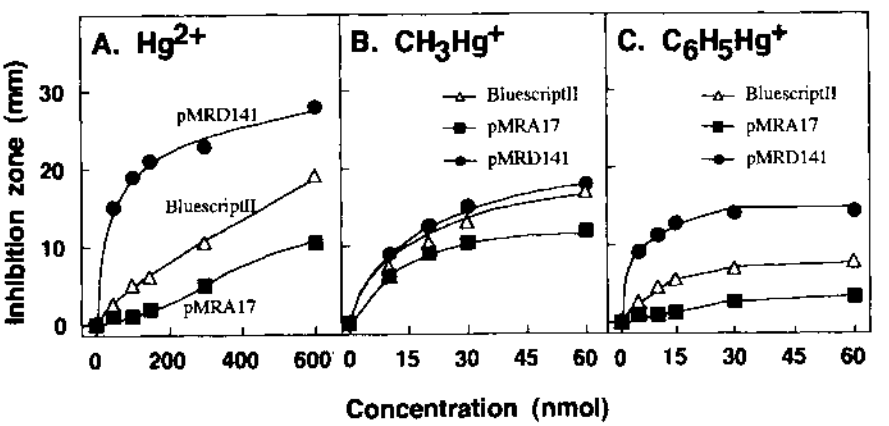


Fig. 5 Mercurials resistance

mediated by merT and merP in the absence of merB and merA. This finding promote us to study the mercury uptake by the deletion mutant. Cells with pMRD141 took up about 3 times more mercuric mercury and about 2 times more phenylmercury than the cells with cloning vector. However, no significant difference in the uptake of methylmercury was found between the two cells with pMRA17 or cloning vector, Bluescript II as shown in Fig. 6. These results

demonstrate that merT and merP are required not only for mercuric mercury transport but also for phenylmercury transport into the cells, but did not participate in the transport of methylmercury. we do not have enough information to discuss the transport of methylmercury at present, but it may be passively diffused into cells without any carriers. To our knowledge, this is the first report describing the role of merT and merP in the organomercury transport.

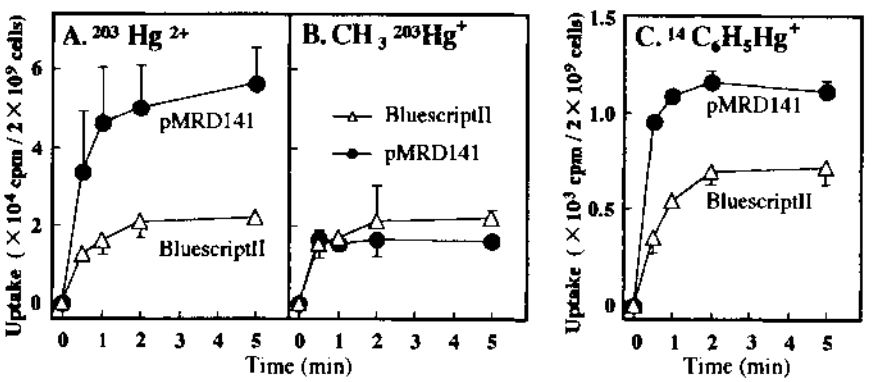


Fig. 6 Mercury uptake by *E. coli* XL1-Blue carrying pMRD141

Our results obtained above reveal that the mercury resistance operons of pMR26 consist of a cluster of linked mer genes which encoded polypeptides with regulatory, transport and enzymatic functions. Binding mercury outside the cell, carrying mercury across the cellular membranes and presenting mercury to the cytoplasmic organomercurial lyase and/or mercuric reductase are believed to be essential for this strain to express its mercurial resistance as summarized in Fig. 7.

Of the various bacteria so far reported, Pseudomonas K-62 is known to have a relatively high phenylmercury resistance. The higher resistance to phenylmercury may be achieved by the merE in addition to the two functional merB genes on the pMR26 and some unidentified genes on the 68-kb pair plasmid. I believe this is the first case of resistance system that at least more than three structural mer genes involve in the phenyl mercury resistance.

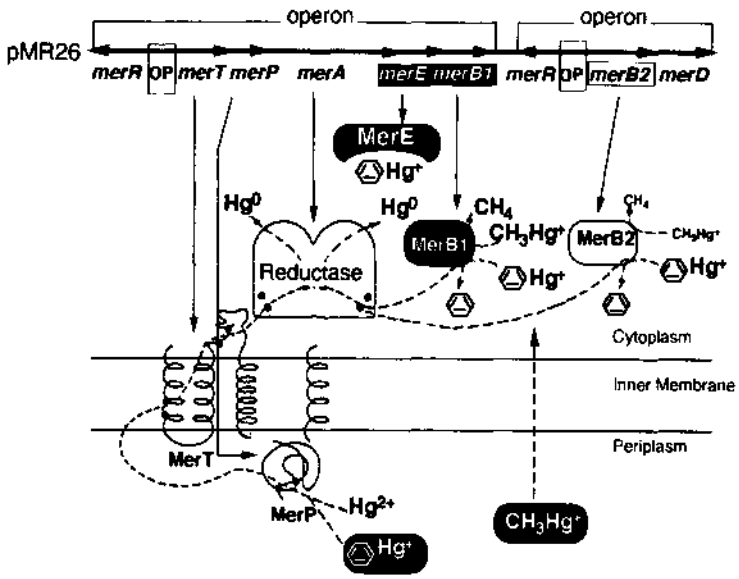


Fig. 7 The *mer* genes and products of representative organomercurial resistance system in *Pseudomonas* K-62

Acknowledgments

We are grateful to Professor K. Tonomura for the gift of the Pseudomonas K-62 strain, and thank Dr. B. H. Olson for generously providing the mer gene probes. This work was supported in part by a grant from Japan Public Health Association.

References

Foster, T. J., Nakahara, H., Weiss, A. A. and Silver, S. (1979) Transposon A generated mutations in the mercuric resistance genes of plasmid R100. J. Bacteriol., 140, 167-181.

Furukawa, K. and Tonomura, K. (1972) Induction of metallic mercury releasing enzyme in mercury resistant Pseudomonas. Agri. Biol. Chem., 36, 2441-2448.

Kiyono, M., Omura, T., Fujimori, H. and Pan-Hou, H. (1995a) Organomercurial resistance determinants in Pseudomonas K-62 are present on two plasmids. Arch. Microbiol., 163, 242-247.

Kiyono, M., Omura, T., Fujimori, H. and Pan-Hou, H. (1995b) Lack of involvement of merT and merP in methylmercury transport in mercury resistant Pseudomonas K-62. FEMS Microbiol. Lett., 128, 301-306.

Kiyono, M., Omura, T., Inuzuka, M., Fujimori, H. and Pan-Hou, H. (1997) Nucleotide sequence and expression of the organo-mercurial resistance determinants from Pseudomonas K-62 plasmid pMR26. Gene, 189, 151-157.

Nakamura, K. and Nakahara, H. (1988) Simplified X-ray method for detection of bacterial volatilization of mercury chloride by Escherichia coli. Appl. Environ. Microbiol., 54, 2871-2873.

Pan-Hou, H. S. K. and Imura, N. (1981) Role of hydrogen sulfide in mercury resistance determined by plasmid of Clostridium cochlearium T-2. Arch. Microbiol., 129, 49-52.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) Molecular cloning: a laboratory manual, 2 nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y.

Sanger, F., Nicklen, S. and Coulson, A. R. (1977) DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci., 74, 5463-5467.

Sasakawa, C., Kamata, K., Sakai, T., Murayama, S., Makino, S. and Yoshikawa, M. (1986) Molecular alteration of the 140-mega dalton plasmid associated with loss of virulence and Congo red binding activity in Shigella flexneri. Infect Immun., 51, 470-475.

Tezuka, T. and Tonomura, K. (1976) Purification and properties of an enzyme catalyzing the splitting of carbon-mercury linkages from mercury resistant Pseudomonas K-62 strain. J. Biochem., 80, 79-87.

Uno, Y., Kiyono, M., Tezuka, T. and Pan-Hou, H. (1997). Phenyl mercury transport mediated by merT-merP genes of Pseudomonas K-62 plasmid pMR26. Biol. Pharm. Bull., 20, 107-109.

Possibility of a measurement method of toxicity to the germ-line by methylmercury exposure.

Takashi Kuwana[1], Koichiro Hashimoto[2], and Mitsuru Naito[3]
1) Pathology Section, National Institute for Minamata Disease.
2) Laboratory of Developmental Biology, Meiji Institute of Health Science.
3) Genetic Engineering Laboratory, National Institute of Animal Industry.

Introduction

The need of the analysis that the heredity toxicity of methyl-mercury toward the fetus is examined in detail being pointed out in IPCS90. When we analyze the toxic effect of methylmercury to the developmental process of reproductive cells (germ-line cells; primordial germ cells, germ cells, spermatocytes, oocytes etc.), we could not help to expose methylmercury to the embryo via the mother's body. But, it is unacceptable to distinguish a secondary influence to come from the direct influence toward the germ-line cells and the influence on other organization in this case and to analyze it. In the present study, a focus was limited in the germ-line cells which is the direct source of the next generation, the following research was done by using the avian embryo to establish the method of bioassay on the direct toxic influence of methyl-mercury toward the germ-line cells.

Fig.1
Localization of Chick Primordial Germ Cells

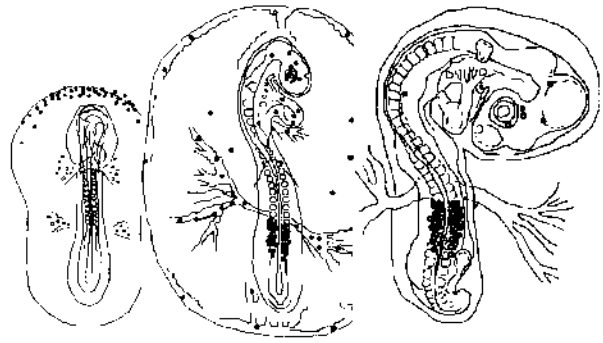
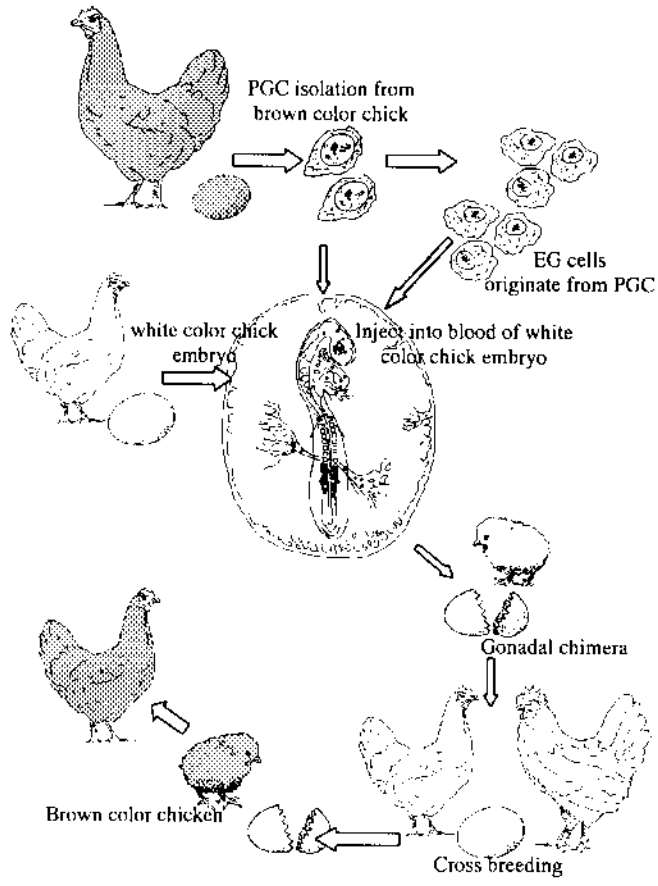


Fig.2
PGC Transplantation to Another Embryo



Avian primordial germ cells (PGCs), which are the original cells of future sperm and ova, migrate from the region far from the presumptive gonadal region in the out of the embryonic body proper to the future gonad as same as the mammals, and they differ to the functional gametes in the developing gonads. Only in avian, the PGCs migrate via the embryonic blood circulation in their migrating phase (Fig. 1). This features in their migration type will be a favorable point when we isolate the PGCs from the embryo or transplant them back into the embryo. We are already able to isolate the chick PGCs from the embryonic blood, and produce the germ-line chimera using the injection into

the blood vascular system of another chicken line embryo (Fig. 2).

Materials and Methods

pH measurement

Fertilized eggs of White Leghorn and Japanese quail were incubated at 38.5°C and 60% relative humidity in a forced air incubator (P-800, Showa Incubator Lab., Japan) to obtain embryos at various developmental stages (Hamburger and Hamilton, 1951). The pH levels of embryonic blood, yolk and albumen as the environment of the embryonic cells were measured at various developmental stages.

Embryonic blood samples of 2 µl were collected from the embryos at stages 12, 13, 14, 15, 16, 17, 18 and 25, and from 6- and 11-day embryos and at hatching. Yolks which presented just behind the embryo and thin albumen were collected from the eggs at stage 1 to hatching. All specimens were collected using glass micro-capillaries which were rinsed 3 times by double distilled water before use. After collecting from the eggs, approximately 2 µl of each specimen was immediately measured for pH by pH BOY-C1 (Shindengen, Japan).

Moreover, thin albumen and yolk just behind an embryonic disk from unincubated fertilized chick eggs were used for the pH measurement.

Source of cultured cells

Chick: Germinal crescent region at stage 3-4
Germinal crescent region at stage 3-5
Germinal crescent region at stage 4-8 (SPF embryo)
Whole embryo at stage 4-8 (SPF embryo)
Whole embryo at stage 13
Primordial germ cells (PGCs) from stage 15 embryo
Presumptive gonadal region and vascular area at stage 17
Whole embryo at stage 20
Developing gonads of stage 25 male embryo (SPF embryo)
Developing gonads of stage 25 female embryo (SPF embryo)
Whole male embryo at stage 25 (SPF embryo)
Whole female embryo at stage 25 (SPF embryo)
Developing gonads of day-4 embryo (stage 24)
Developing gonads of day-5 embryo (stage 27)
Developing gonads, heart and liver of day-7 embryo (stage 31)

Quail Germinal crescent region at stage 3-5 (stage 1-3 of Zacchei, 1961)
Whole embryonic body of stage 11 (L2 line)
Whole embryonic body of stage 11 (H2 line)
Presumptive gonadal region and Area vasculosa at stage 17 (stage 14 of Zacchei)
Developing gonads, heart and liver of day-7 embryo (stage 31; stage 22 of Zacchei)
Testis, heart and liver of hatching (male) (stage 46; stage 33 of Zacchei)

SPF eggs of White Leghorn were obtained from Nisseiken Co., Ltd. (Yamanashi, Japan). Fresh quail L2 and H2 inbred line eggs (the 47th generation) (Takahashi et al., 1984) were obtained from the animal center of the National Institute of Pollution.

Establishment of subculture conditions for embryonic cells in birds

Cells from chick and quail embryo were cultured with Kuwana's Avian-1 medium (KAv-1 medium); α-MEM (GIBCO BRL, USA) added with 1 mM D-glucose, 5×10^{-5} M 2-ME, and 10 mM EPPS (Wako Chem., Japan), containing 5% of FBS (JRH Biosciences, USA) and CS (JRH Biosciences, USA). Medium pH was adjusted by NaCO3 to the designated pH (8.0), similar to that of the embryonic blood in air condition.

Five ml of the KAv-1 medium in the plastic culture flask (No. 25102S, CORNING Co., Ltd., USA) was changed every two days, and the cells were subcultured when the cells proliferated so as to cover 90% of the culture area.

Viability of PGCs

Viability of PGCs was compared at two different pHs using KAv-1 medium. Chick PGCs were collected from embryonic blood at stage 13-15 according to Kuwana & Fujimoto (1984) using KAv-1 medium. Each 100 PGCs were cultured with KAv-1 medium at pH 7.4 or 8.4. The PGCs were cultured in these conditions for 4 h or 24 h, and the viabilities of the PGCs were confirmed under phase contrast microscopy by morphological criteria and absorption test of trypan blue.

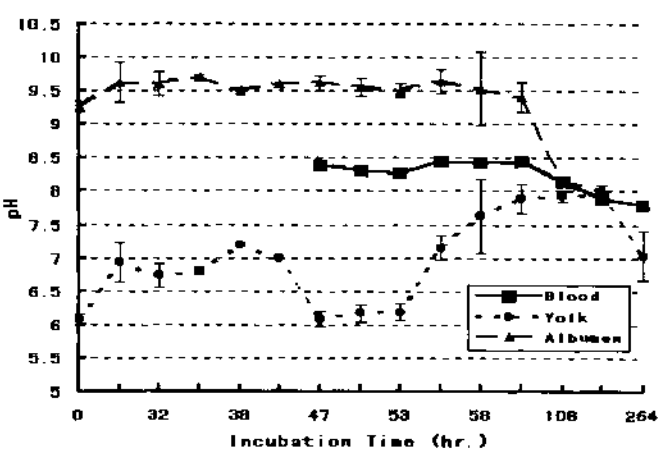
Results

a) Transition in pH as environment of embryonic cells in chick

1) Embryonic blood

Chick embryonic blood circulation begins at stage 10, and one can suck out 2 μl of blood only after stage 12. The transition in the blood pH was shown in Table 1 and Figure 1. The pH at stage 12 indicated 8.4 and then ranged 7.7 to 8.4 throughout the embryonic period. The blood pH at stages 12 to 18 ranged from 8.1 to 8.5 (average 8.3) as shown in Figure 3.

Fig. 3



2) Yolk

The pH of yolk just behind the embryo was measured after stage 1 till embryos were 6 days old (stage 25). The pH value of the yolk just behind the embryo indicated an acidic value till 35 h after incubation and then increased gradually till stage 25 (108 h of incubation time).

3) Albumen

Unincubated fertilized egg albumen had a pH of approximately 9.3, then decreased gradually.

Throughout stage 12 to 25, the pH of embryonic blood consistently ranged between that of the yolk and the albumen (Fig.3).

The transition in pH in quail also showed a result similar to that in chick (data not shown).

b) Establishment of subculture conditions for embryonic cells in birds

In the present study, the culture medium pH was adjusted to 8.0 in air. All the cells proliferated well in this culture condition, and 6 kinds of cells, which were tried to subculture for a long-term, were subcultured for over 3 months (20 passages) except liver and primordial germ cells. Finally, all the subcultured cells was stocked in liquid nitrogen at the respective number of passages shown in Table 2.

In preliminary experiments, we attempted to culture the chick cells of germinal crescent region at stage 3-5 with KAv-1 adjusted to pH 7.2 and KAv-1 adjusted to pH 8.0. At pH 8.0, the cells had been subcultured over 3 months as described above. In contrast, when the culture medium pH was adjusted to 7.2, all the same cells had died at the 10th and 14th passage (Fig. 2).

Fig.4

Species	*Embryonic stage*	*Tissues*	*No. of passages*
Chick	1	Whole blastdisk	1
	3-4	Germinal crescent region (GCR)*1	7
	3-5	GCR	7
	4-8	GCR*1	18
	4-8	Whole embryo	13
	5	Whole embryo*1	17
	11	Presumptive gonadal region*1	7
	12	Presumptive gonadal region*1	8
	13	Whole embryo	4
	15	Primordial germ cell	0
	16	Whole embryo*1	8
	17	Presumptive gonadal region	7
	17	Area vasculosa	7
	20	Whole embryo	4
	25	Male developing gonads*1	1
	25	Female developing gonads*1	1
	25	Male whole embryo*1	20
	25	Female whole embryo*1	24
	4th days embryo	Developing gonads	24
	5th days embryo	Developing gonads	6
	7th days embryo	Developing gonads	5
	7th days embryo	Heart	5
	7th days embryo	Liver	0
Quail	3-5	Germinal crescent region	9
	11	Whole embryo*2	18
	11	Whole embryo*3	25
	15	Whole embryo*2	10
	17	Presumptive gonadal region	8
	17	Area vasculosa	35
	7th days embryo	Developing gonads	12
	7th days embryo	Heart	16
	7th days embryo	Liver	0
	hatching (male)	Testis	3
	hatching (male)	Heart	2
	hatching (male)	Liver	0

*1 SPF embryo, *2 L2 line embryo, *3 H2 line embryo.

d) Viability of PGCs

When 100 PGCs were cultured at pH 7.4, their viability was 5.75% after 4 h and 3.75 % after 24 h respectively. When 100 PGCs were cultured at pH 8.0, their viability was 95% after 4 h and 18.25 % after 24 h, respectively (Table 1). In the present experiments, the PGCs were considered dead cells when their periphery was not demarcated, bubbling occurred on their surfaces or they were taken to be completely destroyed. Additionally, the viability of the PGCs was reconfirmed by the absorption test of trypan blue.

Table 1

	0 h	4 h	24 h
pH 7.4 (n=4)	100	5.75±0.96*	3.75±0.96*
pH 8.0 (n=4)	100	95±2.16*	18.25±3.30*

* mean ± standard deviation

Discussion

Firstly, we examined the conditions for isolation, cryo-preservation of the PGCs, and *in vitro* culture for a long-term. In the isolating method, the ratio of viability for 4 hr incubation increased to approximately 96% using the new culture medium. Furthermore, we established an avian embryonic culture medium (KAv-1 medium) as a result that the embryonic blood was analyzed in chick and quail embryo. It succeeded in establishing avian embryonic cell lines first in the world using KAv-1 medium. This means that the chick fibroblast cell lines were established which produce membrane bind type stem cell factor (SCF) that is essential for the avian PGC-culture. It is being carried out at present to develop the culture condition for the avian PGCs with those fibroblasts.

We already produced germ-line chimeras between two lines of chicken (White Leghorn and Rode Island Red lines). In the present study, we improved the method for production of germ-line chimera between White Leghorn and Barred Plymouth Rock lines. In this case, we obtained offspring derived from injected PGCs at the high efficiency by the back cross of the germ-line chimera. Moreover, chick PGCs isolated, cryo-preserved in the liquid nitrogen for 4 to 5 months and inject into another chick embryo. Though efficiency was low, transplantation was done well and we obtained offspring derived from injected PGCs.

Though an imperfect part is left, the possibility to decide toxic evaluation to the germ-line cells by the series of above embryo operation may be established.

References

FUJIMOTO, T., NINOMIYA, T., and UKESHIMA, A. (1976). Observations of the primordial germ cells in blood samples from the chick embryo. Develop. Biol. 49:278-282.

HAMBURGER, V., and HAMILTON, H.L. (1951). A series of normal stages in the development of the chick embryo. J. Morphol. 88:49-92.

KUWANA, T. (1993). Migration of avian primordial germ cells toward the gonadal anlage. Develop. Growth & Differ. 35:237-243.

KUWANA, T., and FUJIMOTO, T. (1984). Locomotion and scanning electron microscopic observations of primordial germ cells from the embryonic chick blood *in vitro*. Anat. Rec. 209:337-343.

KUWANA, T., HASHIMOTO, K., NAKANISHI, A., YASUDA, Y., TAJIMA, A., and NAITO, M. (1996). Long-term culture of avian embryonic cells *in vitro*. Int. J. Dev. Biol., 40: 1061-1064.

SWIFT, C.H. (1914). Origin and early history of the primordial germ cells in the chick. Am. J. Anat. 15:483-516.

ZACCHEI, A.M. (1961). Lo Sviluppo embionale della quaglia giapponese (Coturnix coturnix japonica T. S.). Arch ital. Anat. Embriol. 66:36-62 .

Postnatal effects of in utero exposure to methylmercury; with emphases on co-exposure to heat or selenium deficiency[1]

Hiroshi SATOH, Chiho WATANABE, Kun YIN and Hitomi INABA
Environmental Health Sciences, Tohoku University Graduate School of Medicine

Abstract

Pregnant mice were immersed in water at 37°C or 42°C for 10 minutes once or twice daily from day 12 through day 15 of gestation. Two hours after the heat exposure on day 12 of gestation, the maternal mice were injected s.c. with 5 mg Hg/kg body weight of MeHg (as chloride) or saline. Prenatal exposure to heat significantly induced inactivity in an open field test (OPF) in males and retarded walking ability in both males and females. There were some interactions between the effects of these two agents; the difference in walking ability caused by heat were more distinctive in the saline-treated groups than in the MeHg-treated groups in both sexes, the difference in locomotion in the OPF caused by MeHg in females was more distinctive in the normothermic group (37°C) than in the hyperthermic groups (42°C once or twice).

Female mice were fed on Se-sufficient diet (0.4 mg Se/kg of diet; Se+) or Se-deficient diet (0.02 or 0.05 mg Se/kg of diet; Se- or Se--) for 4 weeks before mating. A single dose of 5 mg Hg/kg body weight of MeHg on day 12 of gestation or 3 multiple doses of 3 mg Hg/kg body weight through days 12 to 14 of gestation were administered s.c. to mice. Prenatal Se deficiency decreased the body weight and the absolute brain weight, reduced walking ability and activity in the OPF, induced a preference for higher temperature. Prenatal MeHg administration affected litter size, decreased body weight, retarded walking ability, induced a preference for higher temperature. For the interactions between MeHg and Se deficiency, scores in the walking test reduced by MeHg in Se- and Se-- groups were greater than in Se+ groups.

In summary, the toxicity of prenatal exposure to MeHg on postnatal growth and behavior of mouse offspring can be modified by prenatal heat exposure or by maternal Se deficiency. Unexpectedly, MeHg and heat together obscured or lessened, rather than enhanced, the other's effects on various behaviors. On the other hand, MeHg and Se deficiency together enhanced each other's effects in behavioral functions. Though this study was done with mice, it is suggested that the risk to offspring might increase when pregnant women are exposed to MeHg under the condition of Se deficiency.

Introduction

The toxicity of methylmercury (MeHg) has been found to be enhanced by heat exposure

[1] Part of this paper is based on Yin et al. (1997).

in adult rats (Yamaguchi et al. 1984). Administration of selenium (Se) has been found to protect against body weight loss and mortality in Japanese quails and rats (Ganther et al. 1972), but Se deficiency enhanced the fetal toxicity of MeHg in mice (Nishikido et al. 1987). Ethanol potentiated the toxicity of MeHg in terms of neurological manifestation and mortality (Tamashiro and Arakaki 1986). Since MeHg is an environmental pollutant, its toxicity can be modified by various co-existing environmental factors.

Among these factors, we evaluate modifying effects of heat and Se on the MeHg toxicity during perinatal periods. Maternal brief hyperthermia is common during pregnancy, as it can be induced by fever, excessively hot baths and heat waves, and Se deficiency continually exists in Se-poor areas of the world.

Materials and Methods

Twenty-six pregnant ICR mice were purchased from Nihon SLC (Hamamatsu) for the experiment with heat co-exposure. The mice were randomly assigned to 3 groups. On days 12 through 15 of gestation, the mice were immersed for 10 min in 37°C water once daily (group N), in 42°C water once daily (group H), or in 42°C water twice daily (group HH). Two hours after the water bath immersion on day 12 of gestation, half of each group was injected s.c. with MeHg (as chloride) at a dose of 5 mg Hg/kg body weight (N+, H+ and HH+ groups), while the others were given the same volume of saline solution (N-, H- and HH- groups).

A Se-deficient diet based on Torula yeast was purchased from Oriental Yeast Co. Ltd. (Tokyo) for the Se-deficiency experiment. The levels of 0.05 or 0.4 mg Se/kg in the diets were prepared with adding sodium selenite to the Torula yeast diet. Se concentrations determined were less than 0.02, about 0.05 or about 0.4 mg Se/kg weight for the Se-deficient diets (Se--, Se-) or for the Se-sufficient diet (Se+), respectively. From 4 weeks of age, female mice were randomly assigned to 1 of the 3 groups fed on Se-sufficient and Se-deficient diets. At 8 weeks of age, 2 or 3 females were mated overnight with a male that had been fed a commercial stock diet. On day 12 of gestation, each group was further divided into 3 subgroups: One subgroup (Hg+ group) was administered s.c. a single dose of 5 mg Hg/kg body weight of MeHg (as chloride). Another (Hg++ group) was administered s.c. 3 multiple doses of 3 mg Hg/kg body weight of MeHg and the third (Hg- group) was administered the same volume of saline solution from days 12 to 14 of gestation.

All the pregnant mice were allowed to deliver spontaneously. On the morning of delivery, the litter size of each dam was recorded and each pup was checked for gross abnormalities. The pups were nursed by their natural dams until weaning. The pups were observed daily, and the emergence of developmental landmarks (pinna detachment, development of fur and eye opening) were recorded. On the pre-determined days, pups were randomly selected from each litter for evaluating the reflex and locomotor developments, open field test (OPF) and thermogradient test (Watanabe and Satoh 1994).

Tissue samples were taken from pups after behavioral examinations and from dams on day 21 after delivery. The removed tissues were kept at -80°C until the later assays. Tissue total Hg concentration was determined by atomic absorption spectrophotometry after the tissue samples were wet-ashed with a mixture of nitric acid, sulfuric acid, and perchloric acid (Akagi and Nishimura 1991). The accuracy of the assay was ascertained by including a reference material (CRM185 Bovine Liver, Community Bureau of Reference, Brussels, Belgium).

All the statistical calculations were performed by the General Linear Model procedure of the SYSTAT statistical package.

Results and Discussion

During gestation and lactation, none of the mothers in any of the groups died, nor did they show any overt neurological symptoms, such as abnormal gait.

Effects of methylmercury and heat on the offspring

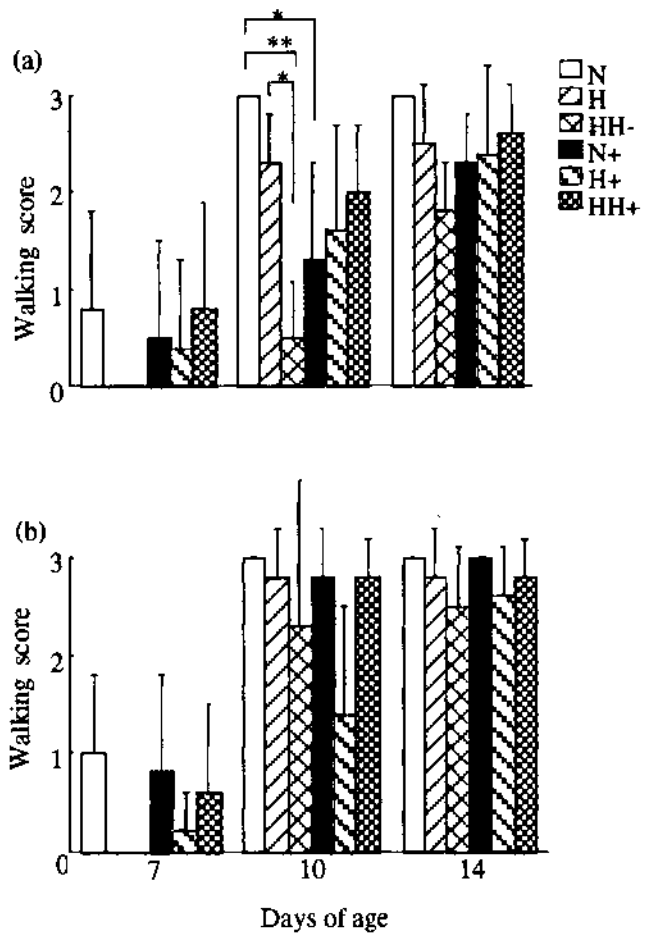


Fig. 1 Effect of exposure to heat and MeHg during pregnancy on the mean developmental scores of walking activity of male (a) and female (b) pups on days 7, 10 and 14 of age.

No effect of heat or MeHg was observed regarding birth weight, litter size, sex ratio, or the observed developmental landmarks. No significant difference among groups was found in the death rate or the body weight of pups from birth to 5 weeks.

As for neurobehavioral development of neonates, heat was significant on day 7 of age, and the interactions between MeHg and heat were significant on days 7, 10 and 14 of age on walking activity in both males (Fig. 1a) and females (Fig. 1b). By post-hoc comparisons, the differences between groups N- and N+, between groups N- and HH-, and between groups H- and HH- were

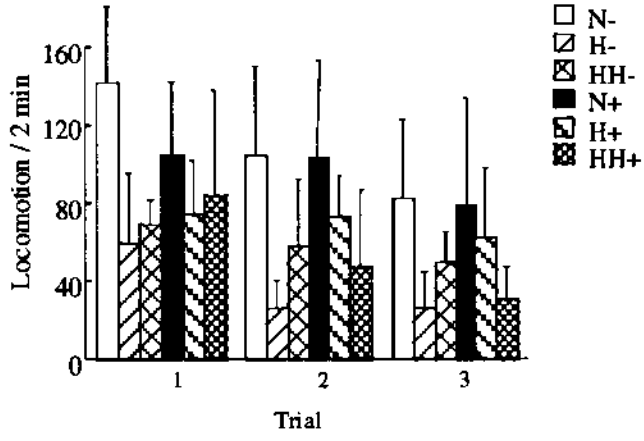


Fig. 2 Effect of exposure to heat and MeHg during pregnancy on the locomotion of male offspring in an open-field test at 5 weeks of age.

significant in males on day 10 of age, although no such effects of MeHg or heat were observed in females. In the righting reflex, negative geotaxis and pivoting tests, no significant effect of heat, MeHg or sex was observed in either trial.

Because there were significant interactions between sex and heat or sex and MeHg with regard to several OPF parameters, the OPF data for males and females were analyzed separately. For males, heat exerted a significant reducing effect on locomotion in all the trials (Fig. 2), while no significant effect of MeHg on locomotion was found. Heat also caused significantly prolonged latency in the second and third trials, and the difference in latency was more distinctive among the 3 saline-treated groups than among the 3 MeHg-treated groups in the second trial (Fig. 3).

For females, locomotion was significantly affected by MeHg in the second trial, where the difference in locomotion was more distinctive in the normothermic groups (N- and N+) than among the hyperthermic groups (H-, H+, HH- and HH+). On the second trial, the locomotion in group N+ was significantly lower than that in group N- by post-hoc comparison. No significant effect of heat or MeHg on latency was found. The other OPF indices were not statistically significant.

Effects of MeHg were significant on Hg concentrations in the brain and liver on days 3 and 21 of age, and in kidney on day 21 of age. From day 3 to day 21 of age, Hg concentrations remarkably decreased in the brain (by 97.8-98.0%) and liver (by 94.2-95.0%). On day 21 of age, however, Hg concentrations in the brain, liver and kidney in the MeHg-treated groups were still significantly higher than those in the saline-treated groups by post-hoc comparisons. The interaction between MeHg and heat on the brain Hg concentration was significant on

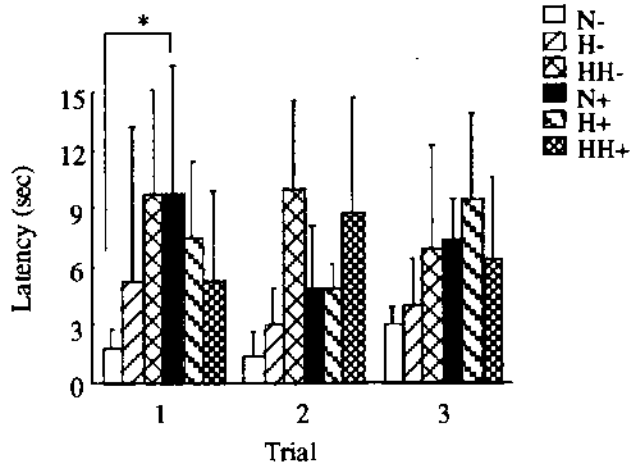


Fig. 3 Effect of exposure to heat and MeHg during pregnancy on the latency of male offspring in an open-field test at 5 weeks of age.

day 21 of age. No significant effect of heat was found on Hg concentrations in the liver on days 3 and 21 of age or in the kidney on day 21 of age. No sex differences were found in Hg concentrations in the brain or liver on either day of age, or in the kidney on day 21 of age.

Effects of methylmercury and selenium deficiency on the offspring

The survival rates of fetuses were significantly affected by MeHg or Se deficiency and

Table 1. *Effects of MeHg and dietary Se on the body weights of pups*

Group	N	Se (mg/kg)	MeHg (mg Hg/kg)	Body weight (g)		
				7 Days	14 Days	21 Days
Se+Hg -	6	0.4	0 x 3	4.5 ± 0.2	9.0 ± 0.4	14.9 ± 0.4
Se+Hg+	6	0.4	5 x 1	5.5 ± 1.0	9.1 ± 2.0	15.0 ± 2.8
Se+Hg++	6	0.4	3 x 3	4.4 ± 0.6	7.7 ± 0.7	12.1 ± 1.6
Se-Hg -	6	0.05	0 x 3	5.0 ± 0.8	8.9 ± 0.9	13.4 ± 1.8
Se-Hg+	4	0.05	5 x 1	5.3 ± 0.4	9.1 ± 0.7	13.0 ± 0.7
Se-Hg++	4	0.05	3 x 3	4.5 ± 0.4	8.3 ± 0.5	12.1 ± 0.9
Se--Hg -	6	0.02	0 x 3	5.0 ± 0.4	8.6 ± 0.4	12.3 ± 0.8
Se--Hg+	6	0.02	5 x 1	4.7 ± 0.8	8.3 ± 1.2	12.2 ± 2.0
Se--Hg++	7	0.02	3 x 3	4.9 ± 0.4	8.1 ± 1.0	11.4 ± 1.6

the litter size was significantly affected by MeHg. There was a significant interaction between MeHg and Se deficiency on the pups' body weight on day 7 of age. The body weights were significantly affected by MeHg on days 14 and 21 of age and by Se deficiency on day 21 of age (Table 1).

On day 21 of age, Se deficiency significantly affected the absolute brain weight, while it did not affect the relative brain weight (Table 2). The absolute brain weights were significantly lower in the 3 Se-- groups than in the Se+Hg- group (post-hoc comparisons). The reduction of the absolute brain weight appeared to be due to the reduction of the body weight in the Se-deficient groups, because no significant difference was found in the relative brain weight among groups.

In the righting reflex test, the 3 Hg+ and 3 Hg++ groups showed lower scores than those of the 3 Hg- groups, and MeHg was found to be a significant factor on day 4 of age. On day 7 of age, Se deficiency significantly affected the scores of the righting reflex test,

Table 2. *Effects of MeHg and dietary Se on the brain weights of 21-day-old mice*

Group	N	Se	MeHg	Brain weight	
		(mg/kg)	(mg Hg/kg)	Absolute (g)	Relative (%)
Se+Hg-	6	0.4	0 x 3	0.52 ± 0.02	3.5 ± 0.1
Se+Hg+	4	0.4	5 x 1	0.48 ± 0.02	3.7 ± 0.2
Se+Hg++	6	0.4	3 x 3	0.46 ± 0.02	3.9 ± 0.5
Se-Hg-	6	0.05	0 x 3	0.47 ± 0.04	3.6 ± 0.2
Se-Hg+	4	0.05	5 x 1	0.44 ± 0.05	3.5 ± 0.3
Se-Hg++	4	0.05	3 x 3	0.44 ± 0.05	3.7 ± 0.4
Se--Hg-	6	0.02	0 x 3	0.42 ± 0.03	3.5 ± 0.3
Se--Hg+	4	0.02	5 x 1	0.43 ± 0.07	3.7 ± 0.3
Se--Hg++	5	0.02	3 x 3	0.43 ± 0.03	3.8 ± 0.6

while no significant effect of MeHg was found. In the walking test, scores in the 3 Se- and 3 Se-- groups were lower than in the 3 Se+ groups, and Se deficiency was found to be significant factors on days 7, 10 and 12 of age (Figs. 4a, 4b and 4c, respectively). Although no significant interaction between MeHg and Se deficiency was found in the walking test, the effect of MeHg in the Se- and Se-- groups was stronger than in the Se+ groups. For example, in the walking test scores on day 7 of age (Fig. 4a), the scores for the Se-Hg+, Se--Hg+ and Se+Hg+ groups were reduced by 63%, 100% and 0%, respectively, compared to the scores of the respective Hg- groups, and the scores for the Se-Hg++, Se--Hg++ and Se+Hg++ groups were reduced by 100%, 100% and 40%, respectively.

As for behavior in the thermogradient on days 8 (Fig. 5a) and 14 (Fig. 5b) of age, all the mice showed a slow movement toward the warmer end from 0 to 5 min. Se-deficient mice showed a preference for warmer temperature than the other pups did from the initial 5 min to the end of this test (20 min) on days 8 and 14 of age. The preferred temperatures in Se--Hg- and Se--Hg++ groups were significantly higher than those in Se+Hg- group on day 8 of age (post-hoc comparisons). MeHg-treated mice also showed the same behavioral change as the Se-deficient mice did on day 14 of age.

Se deficiency decreased the locomotion in the open field test, and there was a significant interaction between MeHg and Se deficiency at 4 weeks of age. The locomotions in Se--Hg++ group were significantly lower than those in Se+Hg- and Se-Hg++ groups on the second trial, and in Se--Hg++ group were significantly lower than those in Se-Hg++ groups

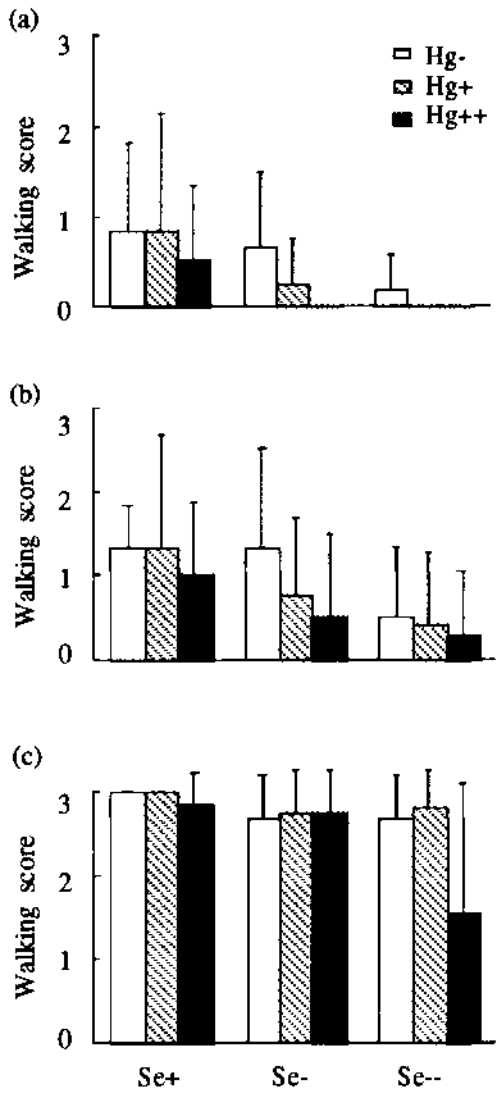


Fig. 4 Effect of prenatal MeHg and Se deficiency on the mean developmental scores of walking activity of pups on days 7 (a), 10 (b) and 12 (c) of age.

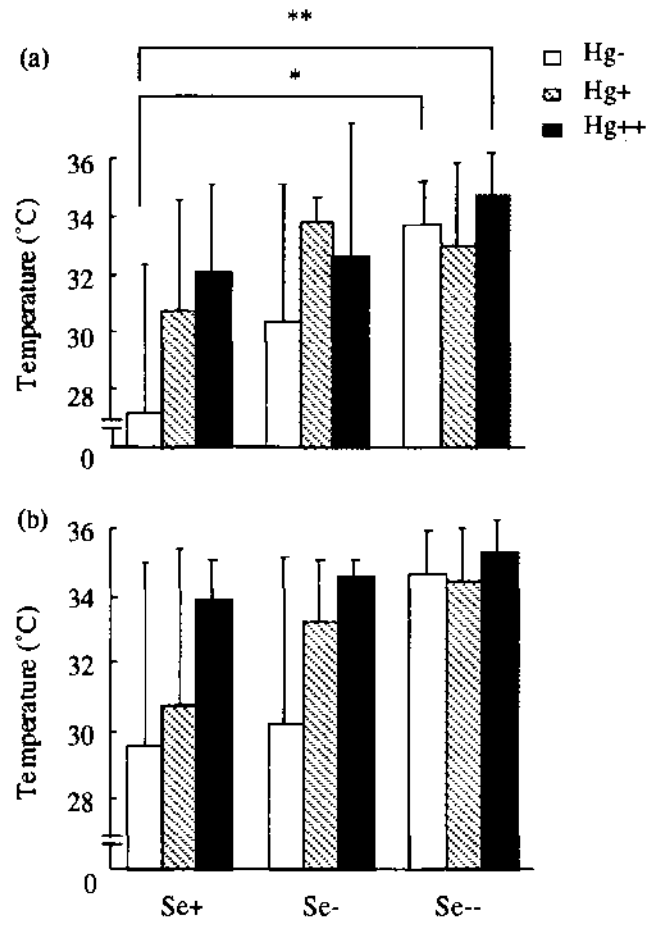


Fig. 5 Effect of prenatal MeHg and Se deficiency on the temperature preference on days 8 (a) and 14 (b) of age.

on the third trial (post-hoc comparisons). Two-way ANOVA revealed a significant effect of MeHg on the percentage of central area.

The brain Se concentrations were significantly affected by Se deficiency on days 3 and 21 of age. Total Hg concentrations in the brain were significantly increased by MeHg on days 3 and 21 of age, and remarkably decreased (by 96-98%) from day 3 to day 21 of age. Se deficiency did not affect brain Hg concentration on days 3 and 21 of age.

The results of the present study show that prenatal exposure to either MeHg or heat could induce adverse changes in postnatal behavior of mice. An unexpected result of this study was the way in which the behavioral effects of heat and MeHg interacted: i.e., no mutual augmentation between these two factors was recognized under the present experimental conditions. Instead, mutual suppressive effects were observed. These interactions are difficult to explain and thus should be a focus of further research. On the other hand, the major finding of the Se-deficiency experiment was that MeHg and Se

deficiency tended to augment each other's effects on postnatal behavior. Though this study was done with mice, these results may be applicable for evaluating the risk to pregnant women of exposure to MeHg in combination with heat or Se deficiency.

References

Akagi, H. & Nishimura, H. (1991). Speciation of mecury in the environment. In: Suzuki, T.; Imura, N.& Clarkson, T.W., Eds. Advances in mercury toxicology. New York, Plenum press. 53-76.

Ganther, H. E., Goudie, C., Sunde, M.L., Kopecky, M.J., Wagner, P., Sang-Hwan OH. & Hoekstra, W.G. (1972). Selenium: Relation to decreased toxicity of methylmercury added to diets containing tuna. Science **175**, 1122.

Nishikido, K., Furuyashiki, K., Naganuma, A., Suzuki, T. & Imura, N. (1987). Maternal selenium deficiency enhances the fetolethal toxicity of methyl mercury. Toxicol. Appl. Pharmacol. **88**, 322-328.

Tamashiro, H. & Arakaki, M. (1986). Effects of ethanol on methyl mercury toxicity in rats. J. Toxicol. Environ. Health **18**, 595-605.

Watanabe, C. & Satoh, H. (1994). Brain selenium status and behavioral development in selenium-deficient preweaning mice. Physiol. Behav. **56**, 927-932.

Yamaguchi, S., Shimojo, N., Sano, K., Kano, K., Hirota, Y. & Saisho, A. (1984). Effects of environmental temperatures on the toxicity of methylmercury in rats. Bull. Environ. Contam. Toxicol. **32**, 543-549.

Yin, K., Watanabe, C., Inaba, H. & Satoh, H. (1997) Growth and behavioral changes in mice prenatally exposed to methylmercury and heat. *Neurotoxicology and Teratology*, **19**, 1-7.

EFFECTS OF METHYLMERCURY ON GLUTATHIONE METABOLISMS

Akira Yasutake[1] and Kimiko Hirayama[2]

[1] *Biochemistry Section, National Institute for Minamata Disease, Minamata, Kumamoto 867 and* [2] *Kumamoto University College of Medical Science, 4-24-1 Kuhonji, Kumamoto 862, Japan*

ABSTRACT

Because of its high affinity to the sulfhydryl group, the in vivo fate of methylmercury (MeHg) is closely related to the glutathione (GSH) metabolism. Here, to examine the possible effects of MeHg on the GSH metabolism, C57BL female mice were challenged by this heavy metal at a marginal dose level to induce a slight renal dysfunction. Liver and blood GSH levels decreased by 16% and 20%, respectively, 24 h after MeHg (160 μ mol/kg) administration, whereas kidney and plasma levels drastically increased. The GSH half-lives obtained using L-buthionine-(S,R)-sulfoximine were shortened by 17% in the liver, but lengthened by 28% in the kidney. The accelerated secretion of GSH from the liver and/or blood cells might have caused the increased plasma levels of the tripeptide, which in turn could increase the supply of the constituent amino acids for GSH synthesis to the kidney. Furthermore, renal γ-glutamylcysteine synthetase activity, a rate-determining enzyme in GSH biosynthesis, was found to be enhanced in the MeHg-treated group. The marked increase in the renal GSH levels induced by MeHg could be due to the increased synthesis and the decreased efflux of the tripeptide in this tissue. The MeHg-induced alterations of GSH metabolism described here might reflect one of the defense mechanisms of bioorganisms against the challenge by MeHg.

Key words: Mouse - Methylmercury - Glutathione - γ-Glutamylcysteine synthetase - Renal failure

Abbreviations used: BSO, L-buthionine (S,R)-sulfoximine; CySH, cysteine; DTT, dithiothreitol; γ-GCS, γ-glutamylcysteine synthetase; GSH, glutathione; GSHS, glutathione synthetase; γ-GTP, γ-glutamyltranspeptidase; MeHg, methylmercury; PCA, perchloric acid; SBD-F, 4-fluoro-7-sulfobenzofurazan ammonium salt

INTRODUCTION

Glutathione (GSH) is the major cytosolic low molecular weight sulfhydryl compound which acts as a cellular reducing reagent and a protective reagent against numerous toxic substances including heavy metals. A series of animal experiments using methylmercury (MeHg) revealed that the fate of this heavy metal closely related with the inter- and intra-organ metabolism of GSH and related metabolites (Hirayama et al. 1987; 1990; 1991; Naganuma et al. 1988). Accordingly, the metabolic pathway for GSH has an important role in tissue distribution and elimination of MeHg.

Although GSH has a dominant role in the MeHg metabolism, toxic dose levels of MeHg are documented to reduce cellular GSH levels (Di Simplicio et al. 1990; Sarafian and Verity 1991). Nevertheless, information available concerning the effects of MeHg on the dynamic aspects of GSH is very scarce. Previously, the authors found that a single administration of MeHg (160 μmol/kg) effectively caused a slight acute renal dysfunction in female C57BL mice without lethal effect (Yasutake et al. 1990). In the present study, using this intoxication model, the effects of the organic Hg on the GSH metabolism were investigated focusing on the pathway from the liver to kidney. The tissue levels, synthetic activities and secretion rates of the tripeptide were also examined. The results were discussed from the standpoint of a possible biological defense mechanism against MeHg toxicity.

MATERIALS AND METHODS

Chemicals.

MeHg chloride and L-buthionine-(S,R)-sulfoximine (BSO) were purchased from Tokyo Kasei Co. (Tokyo) and Sigma Chemical Co. (St. Louis, Mo), respectively. GSH, ATP, β-NADPH, dithiothreitol (DTT), glycine and glutamic acid were purchased from Wako Pure Chemical Ind. Ltd. (Osaka). L-cysteine (CySH) was obtained from Nakarai Chemical Co. (Tokyo), and γ-glutamylcysteine was prepared by a partial hydrolysis of GSH using carboxypeptidase A (Nardi et al. 1990).

Effects of MeHg on tissue GSH levels.

Eight-week-old female C57BL/6N mice (CLEA Japan, Osaka) were orally administered MeHg chloride (160 μmol/kg), and distilled water was given to the control group. Twenty-four hours after administration, blood was collected by heart

puncture under ether anesthesia, then centrifuged at 4 ℃ to separate plasma. An aliquot of plasma was immediately mixed with the same volume of 5 % perchloric acid (PCA) containing 1 mM EDTA. The acidified plasma samples were washed with benzene to remove MeHg (Yasutake et al. 1991), then reacted with a fluorescence labeling reagent 4-fluoro-7-sulfobenzofurazan ammonium salt (SBD-F) prior to HPLC analyses of GSH and CySH according to Toyooka and Imai (1983). The labeled compounds were chromatographed using a Waters μ-Bondapack C_{18} column, and detected by Hitachi fluorophotometer 650. Brain, liver and one of the kidneys excised were immediately homogenized in ice-cold 50 mM L-serine-boric acid mixture. Aliquots of the homogenates were used in the determination of total GSH, protein and Hg levels. Total GSH levels were determined by the enzymatic recycling method reported by Tietze (1969) after dilution with 5 % PCA (1 mM EDTA). Protein levels were determined according to Lowry et al. (1951). Hg levels were measured by an oxygen-combustion gold amalgamation method (Jacobs et al. 1961) using a Rigaku mercury analyzer SP-3. Another kidney was homogenized in ice-cold 50 mM Tris-HCl (pH 8.0) and used in γ-glutamyltranspeptidase (γ-GTP) assay (Orlowski and Meister 1963).

Assay of GSH biosynthesis.

Activities of γ-glutamylcysteine synthetase (γ-GCS) and GSH synthetase (GSHS) in liver and kidney were determined according to Nardi et al. (1990) with a slight modification as follows. The tissue homogenates obtained from MeHg (160 μmol/kg)-pretreated and control mice were centrifuged at 12,000 rpm for 5 min. For γ-GCS assay, an incubation mixture (100 μl) containing 0.1 M Tris-HCl (pH 8.2), 6 mM ATP, 50 mM KCl, 6 mM DTT, 20 mM $MgCl_2$, 3 mM CySH, 15 mM L-glutamic acid and 50 mM serine-boric acid was preincubated for 15 min at 37 °C. The reaction was initiated by addition of the supernatant fraction (5 μl) of the tissue homogenate. The mixture was incubated at 37 °C for 10 min, then 5 % PCA containing 1 mM EDTA (100 μl) was added to terminate the reaction. The acidified mixture was centrifuged at 10,000 rpm for 3 min. The supernatant fraction was reacted with SBD-F (Toyooka & Imai 1983) and the production of γ-glutamylcysteine was quantified using HPLC as described above. GSHS activity was determined similarly as with the previous enzyme activity using a reaction mixture containing 3 mM γ-glutamylcysteine and 30 mM glycine instead of CySH and glutamic acid. The production of GSH was determined similarly.

GSH half-lives.

MeHg chloride (160 μmol/kg, PO) was administered to one of the two groups (20 each) of mice. Distilled water was administered to the other group. Twenty-four hours after administration, the mice were intravenously injected with BSO (2 mmol/kg) to inhibit γ-GCS, a rate-limiting enzyme in GSH biosynthesis. At 0, 20, 40, 60 and 90 min after the injection, mice (4 in each group) were sacrificed to excise liver and kidney. GSH half-lives in both tissues were determined as reported before (Hirayama et al. 1987).

Effect of MeHg treatment on GFR.

Two groups of mice (5 each) were treated by MeHg chloride or distilled water as above. Twenty-four hours after, a polypropylene tube (0.85 mm, ID) was inserted in the bladder under pentobarbital anesthesia, and the mice were then intravenously injected with ^{14}C-inulin (185 kBq/mg) at a dose level of 18.5 kBq/mouse. Radio-activities in urine collected for the following 1 hr were determined using an Aloka liquid scintillation system LSC-3500.

RESULTS

When female C57BL mice were administered a toxic dose of MeHg chloride (160 μmol/kg), the GSH levels did not change uniformly among various tissues (Table 1). In blood plasma, both the total and reduced form of GSH elevated by 58% and 86%, respectively, 24 hr after administration. Additionally, plasma CySH levels also increased by 52%, though total CySH (cystine plus CySH) remained unchanged. Since the increases were more marked in reduced forms of both thiols than in total values, the portions of the reduced forms in both plasma thiol compounds were higher in the MeHg-treated mice than in the control group. The total GSH levels in the kidney also increased by 109% after MeHg administration. On the other hand, liver and blood GSH levels decreased by 16 and 20%, respectively, whereas the brain level remained unchanged.

GSH is synthesized from its constituent amino acids catalyzed by the action of two enzymes, γ-GCS and GSHS. Both of these enzyme activities in the liver were unchanged 24 hr after MeHg treatment (Fig. 1). On the other hand, the γ-GCS activity in the kidney was significantly enhanced by MeHg administration, while that of GSHS was unaffected. If the tissue homogenates prepared from the control mice were incubated with the addition of MeHg at a level comparable to that of the MeHg-administered animals, the activities of both enzymes remained

unchanged (data not shown).

Table 1. Effects of MeHg on plasma thiols and tissue GSH levels in mice.[a]

Tissue	Control	MeHg-Treated
Plasma Thiols (μM)		
GSH (Total)	19.2 ± 2.1	30.4 ± 1.1**
GSH (Reduced)	7.42 ± 2.41	13.8 ± 1.3**
CySH (Total)	44.5 ± 7.5	46.5 ± 2.2
CySH (Reduced)	8.33 ± 0.88	12.7 ± 0.9**
Total GSH (mM)		
Whole Blood	0.597 ± 0.054	0.483 ± 0.029**
Kidney	3.27 ± 0.13	6.86 ± 0.26**
Liver	8.63 ± 0.92	7.21 ± 0.38*
Brain	1.67 ± 0.04	1.71 ± 0.06

[a] Mice were orally administered MeHg (160 μmol/kg) or saline. Twenty-four hours later, whole blood, plasma and tissue homogenates were deproteinized with 5% PCA containing 1 mM EDTA, then used in assays. Plasma thiol levels were determined by HPLC following fluorescent labeling using a SBD-F (Toyooka and Imai, 1983). Tissue GSH levels were determined according to Tietze (1969). Each value represents mean ± SD obtained from 5 mice.
Significant differences from control group were indicated by ** ($p < 0.01$) and * ($p < 0.05$).

Table 2. Effects of MeHg on half-lives of hepatic and renal GSH in mice.[a]

Mice	Liver	Kidney
Control	89 min	36 min
MeHg-treated	74 min	46 min

[a] Twenty-four hours after MeHg (160 μmol/kg) oral administration, the mice were injected with L-buthionine-(R,S)-sulfoximine (2 mmol/kg). At appropriate intervals, 4 mice in each time group were sacrificed, then tissue total GSH levels were determined according to Tietze (1969).

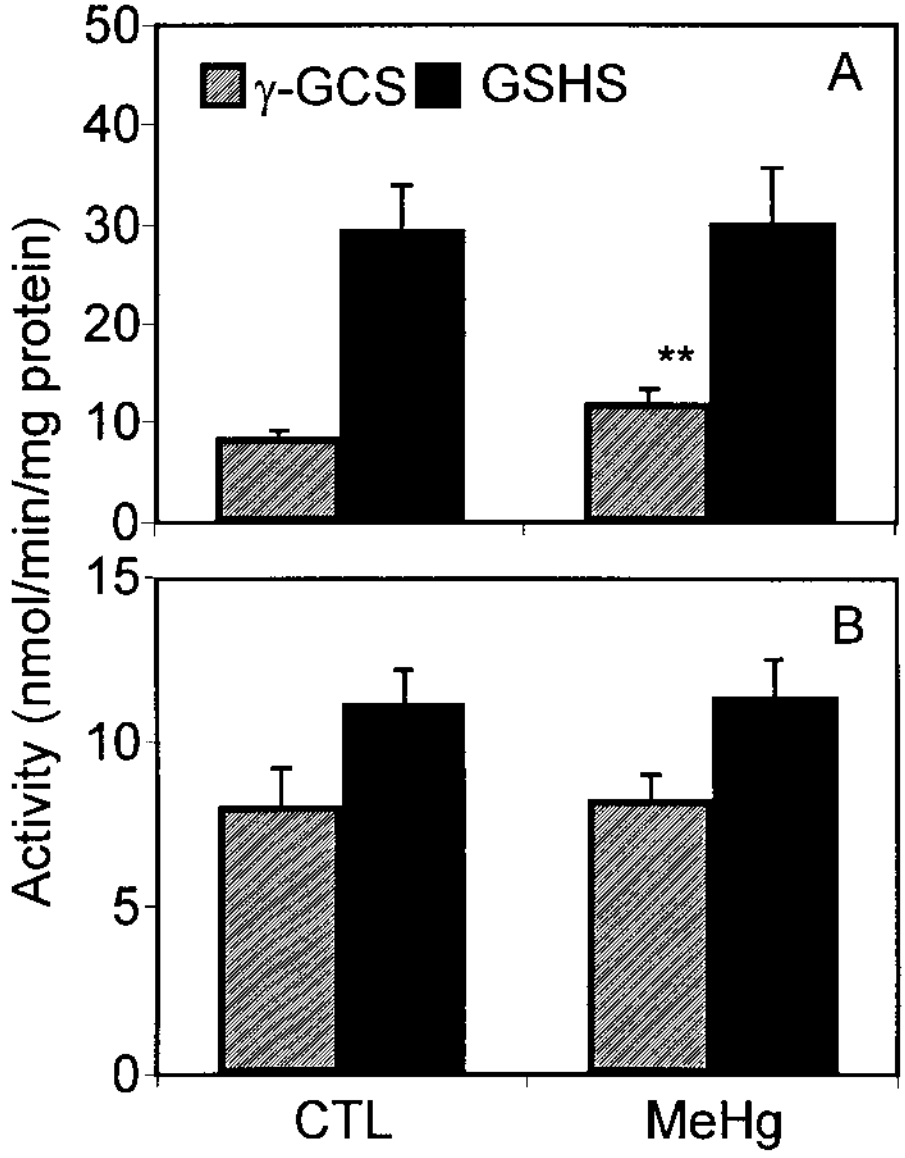


Fig. 1. Effects of MeHg on γ-GCS and GSHS activities in kidney and liver of mice. Twenty-four hours after MeHg chloride (160 μmol/kg) administration, kidney and liver were homogenized, and centrifuged at 12,000 rpm for 5 min. The enzyme activities in the supernatant fractions of kidney (A) and liver (B) homogenates were determined according to Nardi et al. (1992) using SBD-F to label the reaction product. Values represent mean ± SD obtained from 5 mice.
** Significantly different from control value ($p < 0.01$).

Half-lives of the hepatic GSH obtained using BSO (Hirayama et al. 1987) were 89 and 74 min in the control and MeHg-treated mice, respectively (Table 2). MeHg (160 μmol/kg) shortened the hepatic GSH half-life by 17% at 24 hr after the administration. Contrary to the liver, half-life in the kidney was lengthened from 36 min in the control to 46 min in MeHg-treated group (Table 2).

To know the rate by which the circulating GSH was filtered at the kidney, urinary elimination rates of ^{14}C-inulin were examined. Figure 2 showed the rates of the radio-labeled inulin excreted in urine for 1 hr following the injection. No significant difference was observed in the renal inulin filtration rates between control and MeHg-treated groups. On the other hand, the renal γ-GTP activity which had a dominant role in hydrolyzing the circulating GSH, was slightly (9%) inhibited by MeHg treatment (Fig. 3). This inhibition would not affect the plasma GSH level, because the kidney activity was much more than sufficient to process the circulating GSH under the physiological condition.

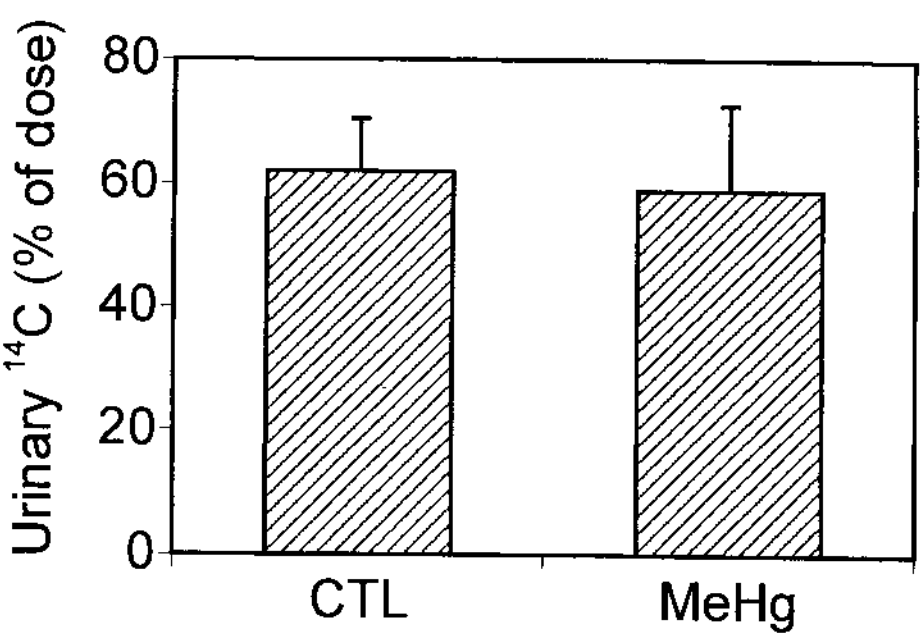


Fig. 2. Effects of MeHg on urinary elimination of ^{14}C-inulin in mice. At 24 hr after MeHg (160 μmol/kg) or saline administration, mice were intravenously injected with radioactive inulin (18.5 kBq/mouse). The radio-activities in urine collected for the following 1 hr were determined. Each value represents mean ± SD obtained from 5 mice.

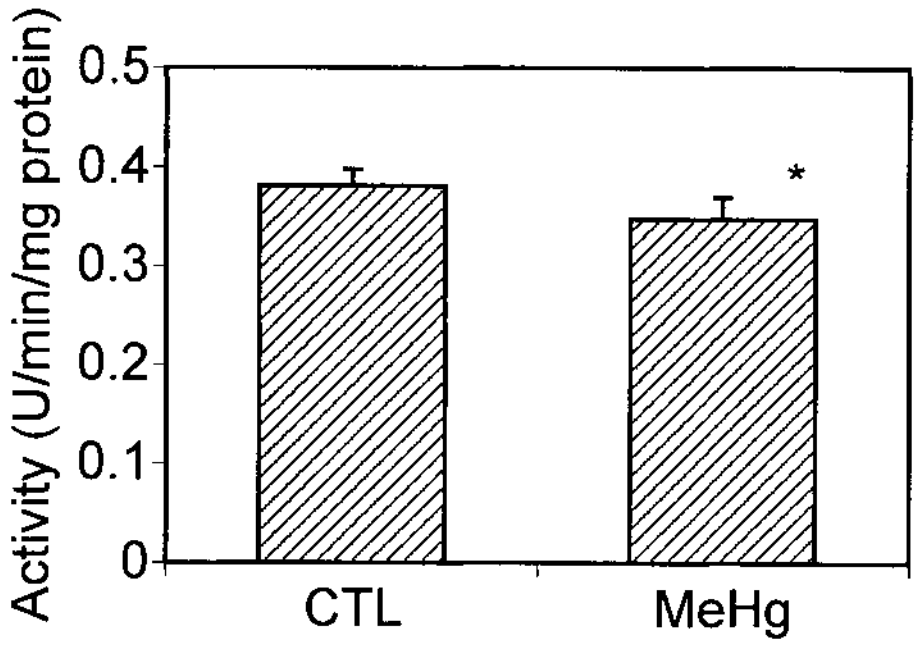


Fig.3. Effects of MeHg on renal γ-GTP activity in mice. Mice were orally administered MeHg (160 μmol/kg) or saline. Twenty-four hours after administration, renal γ-GTP activities were examined according to Orlowski and Meister (1963). Each value represents mean ± SD obtained from 5 mice.
* Significantly different from control mice ($p < 0.05$).

DISCUSSION

GSH is the major functional thiol that undergoes dynamic intra- and interorgan metabolism. The liver is well known for its high GSH secretion to bile and circulation (Sies et al. 1978; Akerboom et al. 1982). On the other hand, the kidney is the major organ responsible for GSH degradation because it has the highest r-GTP activity among various organs (Inoue et al. 1977; Spater et al. 1982). Moreover, MeHg has a high affinity for the SH group of GSH (Simpson 1961), so its *in vivo* fate is closely related to GSH metabolism (Hirayama et al. 1987; 1990; 1991; Naganuma et al. 1988). Accordingly, a considerable portion of the circulating MeHg secreted from the liver as GSH conjugate would be taken up by the kidney following the degradation of the peptide moiety by the membranous enzymes. The MeHg thus accumulated in the kidney of female C57BL mice caused an acute renal dysfunction, such as inhibition of phenol red elimination, if the dose level exceeded 160 μmol/kg (Yasutake et al. 1990). The present results further demonstrated that MeHg could induce various alterations in the mouse GSH metabolism not only in the kidney but also in the liver.

Because of a high affinity for SH group, the binding of MeHg to critical SH groups in functional proteins is supposed to trigger its adverse effects on

bioorganisms. Since both γ-GCS (Meister 1974) and GSHS (Oppenheimer et al. 1979) are known to be inhibited by thiol reagents, MeHg may possibly inhibit these enzyme activities. At 24 hr after MeHg administration with a dose level of 160 μmol/kg, liver and kidney accumulated 55 and 86 μg Hg/g tissue, respectively (Yasutake et al. 1990). In the preliminary experiment, however, even if the homogenates of liver and kidney obtained from intact mice were incubated with MeHg dose levels of up to 100 μg Hg/g tissue, no alteration was observed in the activities of either enzyme (data not shown). This suggested that MeHg accumulated in liver and kidney in these conditions would be too low to cause any alteration in the enzyme activities. It should be noted, however, that MeHg treatment caused a significant activation of γ-GCS in the kidney, though the hepatic enzyme activity remained unchanged. The selective activation of the renal enzyme might be due to organ specificity of the enzyme or difference of tissue Hg accumulation levels. Recently, Woods et al. (1992) found that renal mRNA of γ-GCS was induced in rats which were continually given MeHg-contaminated water. They suggested that the induction would be one of the defense mechanisms against the oxidative challenge by the heavy metal. Interestingly, another heavy metal, Cd, was also reported to show quite a similar effect on cultured rat mesangial cells (Chin and Templeton 1993). In this context, stimulation of the renal γ-GCS might be a common response to some toxic heavy metals in several animal species.

Despite the unchanged GSH synthetic activity in the liver, the hepatic GSH level was significantly lowered by MeHg treatment. The reduction of the tripeptide might be caused by the altered elimination rate from the tissue. Since the half-life of the hepatic GSH shortened after MeHg treatment, the efflux rate of the tripeptide would possibly be enhanced. GSH, particularly in its reduced form, is secreted mostly in the circulation (Bartoli and Sies 1978). The fact that both the total and reduced forms of plasma GSH levels were significantly increased after MeHg administration, not to mention the more marked increase of the reduced form, might support the MeHg-enhanced GSH secretion in the circulation. Unlike the plasma level, the blood level was decreased to a significant extent. Since GSH S-adducts of xenobiotics in the erythrocytes were suggested to readily be secreted to the extracellular space (Board 1981), a great accumulation of MeHg in the cells would accelerate GSH efflux as its S-MeHg conjugate. This might also contribute to the increased GSH distribution in the plasma.

Most GSH in the circulation is hydrolyzed at the kidney by the membranous enzymes (γ-GTP and aminopeptidase M) followed by renal absorption. Since MeHg

treatment caused no change in GFR determined by urinary elimination rates of ^{14}C-inulin, the amount of blood passing through the kidney would remain the same after the challenge by the heavy metal. Accordingly, the amount of GSH supplied to the kidney would be increased after MeHg administration because of its elevated plasma level. The renal γ-GTP, which functioned as the key step enzyme in hydrolysis of the circulating GSH, was slightly (9%) but significantly inactivated by MeHg treatment. However, the extent of this inactivation would scarcely affect the rate of GSH degradation, because the activity of this enzyme in the kidney is much more than enough to hydrolyze the circulating GSH (Hahn et al. 1978). Thus, the rate of GSH degradation in the kidney would scarcely be affected by MeHg treatment. These results suggested that the increase in the plasma GSH level induced by MeHg was mostly responsible for the stimulated secretion from liver and/or erythrocytes as described above.

In this context, the supply rates of the constituent amino acids for GSH synthesis to the kidney would depend on the plasma levels of GSH and constituent amino acids, among which CySH was a rate-limiting one (Tateishi et al. 1974). MeHg-induced elevation of the plasma thiols suggested that the amount of CySH supplied to the kidney would be more abundant in the MeHg-treated mice than in control. The MeHg-induced activation of the renal γ-GCS would also be responsible for the elevation of the GSH levels in the kidney. Furthermore, an elongation of GSH half-life observed in the kidney of MeHg-treated mice might also lead to the increase in the GSH level. Thus, three factors, enhanced supply of CySH, stimulated γ-GCS activity and suppressed efflux, would together serve to increase the renal GSH level.

The suppressed secretion of renal GSH observed here would probably be one of the toxic effects of highly accumulated MeHg. This seems compatible with the previous result that urinary phenol red elimination was slightly inhibited by MeHg treatment of the same dose level (Yasutake et al. 1990). GSH in the renal cells was suggested to be secreted in the luminal space as an anion (Inoue 1985), and the anionic phenol red would share the same transport system. This system in the renal tubule might be one of the most sensitive targets for MeHg acute toxicity. Previously, we found that inorganic Hg had no involvement in the renal dysfunction observed in the MeHg-treated mice (Yasutake et al. 1991). Accordingly, the MeHg-induced alterations in the GSH metabolism observed in the kidney would be also responsible for MeHg itself.

The alterations in the GSH metabolism observed in this study might reflect

one of the defense mechanisms of bioorganisms challenged by organic Hg. Especially in the liver, the enhanced GSH secretion might help to protect the organ against MeHg toxicity by stimulating its elimination as a GSH adduct. Bile was one of the major excretory routes of MeHg (Norseth and Clarkson 1971; Refsvik and Norseth 1975). And bile secretion of GSH was expected also to be enhanced under the present circumstances. However, further study will be necessary to discuss this point in detail.

Acknowledgement. The authors thank Miss Maki Sogawa for her valuable assistance.

REFERENCES

Akerboom, T., Bilzer, M. and Sies, H. (1982) The relationship of biliary glutathione disulfide efflux and intracellular glutathione disulfide content in perfused rat liver. *J. Biol. Chem.*, **257,** 4248-4252

Bartoli, G.M. and Sies, H. (1978) Reduced and oxidized glutathione efflux from liver. *FEBS Lett.*, **86,** 89-91

Bartoli, G.M., Haeberle, D. and Sies, H. (1978) Glutathione efflux from perfused rat liver and its relation to glutathione uptake by the kidney. *In* "Functions of glutathione in liver and kidney" (Sies, H. and Wendel, A., eds), pp89-91. Springer Verlag, Berlin/Germany.

Board, P.G. (1981) Transport of glutathione S-conjugate from human erythrocytes. *FEBS Lett.*, **124,** 163-165

Chin, T.A. and Templeton, D.M. (1993) Protective elevations of glutathione and metallothionein in cadmium-exposed mesangial cells. *Toxicology*, **77,** 145-156

Di Simplicio, P., Gorelli, M., Ciuffreda, P. and Leonzio, C. (1990) The relationship between gamma-glutamyl transpeptidase and Hg levels in Se/Hg antagonism in mouse liver and kidney. *Pharmacol. Res.*, **22,** 515-526

Hahn, R., Wendel, A. and Flohe, L. (1978) The fate of extracellular glutathione in the rat. *Biochim. Biophys. Acta.*, **539,** 324-337

Hirayama, K., Yasutake, A. and Inoue, M. (1987) Effect of sex hormones on the fate of methylmercury and glutathione metabolism in mice. *Biochem. Pharmacol.*, **36,** 1919-1924

Hirayama, K., Yasutake, A. and Inoue, M. (1990) Role of glutathione metabolism in heavy metal poisoning. *In* "Amino Acids: Chemistry, Biology and Medicine" (Lubec, G. and Rosenthal, G.A. eds), pp. 970-975. ESCOM Science Publishers, Amsterdam/Netherlands.

Hirayama, K., Yasutake, A. and Adachi, T. (1991) Mechanism of renal handling of methylmercury. *In* "Advances in Mercury Toxicology" (Suzuki, T., Imura, N. and Clarkson, T.W. eds), pp. 121-134. Plenum Press, New York/USA.

Inoue, M. (1985) Interorgan metabolism and membrane transport of glutathione and related compounds. *In* "Renal Biochemistry" (Kinne, R.K.H. ed), pp. 225-269. Elsevier Sci, Amsterdam/Netherlamds.

Inoue, M., Horiuchi, S. and Morino, Y. (1977) Affinity labeling of rat kidney r-glutamyltranspeptidase. *Eur. J. Biochem.*, **73,** 335-342

Jacobs, M.B. Yamaguchi, S., Goldwater, L.J. and Gilbert, H. (1960) Determination of mercury in blood. *Am. Ind. Hyg. Assoc. J.*, **21,** 475-480

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193,** 265-275

Meister, A. (1974) Glutathione synthesis. *In* "The Enzymes, Vol. 10" (Boyer, P.D. ed), pp. 671-697. Academic Press, New York/USA.

Naganuma, A., Oda-Urano, N., Tanaka, T. and Imura, N. (1988) Possible role of hepatic glutathione in transport of methylmercury into mouse kidney. *Biochem. Pharmacol.*, **37,** 291-296

Nardi, G., Cipollaro, M. and Loguercio, C. (1990) Assay of γ-glutamyl-cysteine synthetase and glutathione synthetase in erythrocytes by high-performance liquid

chromatography with fluorimetric detection. *J. Chromatogr. Biomed. Appl.*, **530,** 122-128

Norseth, T. and Clarkson, T.W. (1971) Intestinal transport of ^{203}Hg-labeled methyl mercury chloride: role of biotransformation in rats. *Arch. Environ. Health,* **22,** 568-577

Oppenheimer, L., Wellner, V.P., Griffith, O.W. and Meister, A. (1979) Glutathione synthetase: Purification from rat kidney and mapping of the substrate binding sites. *J. Biol. Chem.*, **254,** 5184-5190

Orlowski, M. and Meister, A. (1963) γ-Glutamyl-p-nitroanilide: A new convenient substrate for determination and study of L- and D-γ-glutamyltranspeptidase activities. *Biochim. Biophys. Acta.*, **73,** 679-681

Refsvik, T. and Norseth, T. (1975) Methyl mercuric compounds in rat bile. *Acta Pharmacol. Toxicol.*, **36,** 67-78

Sarafian, T. and Verity, M.A. (1991) Oxidative mechanisms underlying methyl mercury neurotoxicology. *Int. J. Dev. Neurosci.*, **9,** 147-153

Sies, H., Bartoli, G.M., Burk, R.F. and Waydhas, C. (1978) Glutathione efflux from perfused rat liver after phenobarbital treatment, during drug oxidation, and in selenium deficiency. *Eur. J. Biochem.*, **89,** 113-118

Simpson, R.B. (1961) Association constants of methylmercury with sulfhydryl and other bases. *J. Am. Chem. Soc.*, **83,** 4711-4717

Spater, H.W., Poruchynsky, M.S., Quintana, N., Inoue, M. and Novikoff, A.B. (1982) Immunocytochemical localization of r-glutamyl transferase in rat kidney with protein A-horseradish peroxidase. *Proc. Natl. Acad. Sci. (USA),* **79,** 3547-3550

Tateishi, N., Higashi, T., Shinya, S., Naruse, A. and Sakamoto, Y. (1974) Studies on the regulation of glutathione level in rat liver. *J. Biochem.*, **75,** 93-103

Tietze, F. (1969) Enzymic method for quantitative determination of nanogram

amounts of total and oxidized glutathione: Applications to mammalian blood and other tissues. *Anal. Biochem.*, **27**, 502-522

Woods, J.S., Davis, H.A. and Baer, R.P. (1992) Enhancement of gamma-glutamylcysteine synthetase mRNA in rat kidney by methylmercury. *Arch. Biochem. Biophys.*, **296**, 350-353

Yasutake, A., Hirayama, K. and Inouye, M. (1990) Sex difference in acute renal dysfunction induced by methylmercury in mice. *Renal Failure*, **12**, 233-240

Yasutake, A., Adachi, T., Hirayama, K. and Inouye, M. (1991) Integrity of the blood-brain barrier system against methylmercury acute toxicity. *Jpn. J. Toxicol. Environ. Health.*, **37**, 355-362

Roles of Glutathione and γ-Glutamyltranspeptidase in Renal Uptake of Mercury Compounds in Mice

Akira Naganuma*, Toshiko Tanaka-Kagawa¶ and Nobumasa Imura¶

**Department of Molecular and Biochemical Toxicology, Faculty of Pharmaceutical Sciences, Tohoku University, Sendai 980-77, and ¶Department of Public Health and Molecular Toxicology, School of Pharmaceutical Sciences, Kitasato University, Tokyo 108, Japan*

Abstract Complex of glutathione (GSH) with methylmercury (CH_3Hg^+) has been found in several tissues such as brain, liver, erythrocytes and bile. Although the binding of MeHg to GSH is reversible, the complex formation may play an important role in transport of CH_3Hg. Specific depletion of hepatic GSH by pretreatment of mice with 1,2-dichrolo-4-nitrobenzene (DCNB) reduced renal accumulation of MeHg or inorganic mercury (Hg^{2+}). The renal uptake of mercury in mice receiving GSH complex with CH_3Hg^+ or Hg^{2+} intravenously was significantly higher than that in mice receiving CH_3HgCl or $HgCl_2$. Although complex of GSH with CH_3Hg^+ or Hg^{2+} has hardly been detected in plasma after administration of mercury compounds, our results described above suggest the possibility that the GSH released from the liver into plasma plays a important role in the renal accumulation of mercury compounds. Inhibition of γ-glutamyltranspeptidase (γ-GTP) by acivicin pretreatment also reduced renal mercury uptake and increased urinary excretion of mercury and GSH. These facts indicate that mercury compounds are transported to the kidney as GSH complex and the mercury is incorporated into the kidney by a γ-GTP dependent system.

Key words: methylmercury; inorganic mercury; renal uptake; glutathione; γ-glutamyl-transpeptidase

INTRODUCTION

The highest concentration of mercury after administration of inorganic mercury or methylmercury to animals *via* any route is generally found in the kidneys. Several studies have been carried out to clarify the mechanism of the renal uptake of mercury, but very little is actually known. Mercury compounds have high affinity for SH groups (Simpson, 1961; Bach and Weibel, 1976; Hughes, 1951). Glutathione (GSH) is the most abundant naturally occurring thiol compound in mammalian tissues. Methylmercury-GSH complex has been found in the liver (Omata *et al.*, 1978), kidneys (Yasutake *et al.*, 1989), brain (Thomas and Smith, 1979), bile (Refsvik and Norseth, 1975; Naganuma and Imura, 1984; Urano *et al.*, 1988) and red blood cells (Naganuma *et al.*, 1980; Naganuma and Imura, 1979). However, the role of GSH in tissue distribution of mercury has not been elucidated. Therefore, we examined roles of GSH in renal uptake of mercury compounds in mice.

MATERIALS AND METHODS

Effect of pretreatment with GSH depletor on renal mercury uptake. Male ICR mice (n=4) were treated intravenously (i.v.) with ^{203}Hg-labeled CH_3HgCl or $HgCl_2$ (0.37 MBq/5 μmol/kg). Mice were kept in metabolism cages (one mouse/cage) to collect urine samples. DEM (3.1 mmol/kg) or DCNB (2.5 mmol/kg) in olive oil was administered intraperitoneally (i.p.) 30 min prior to the injection of CH_3HgCl or $HgCl_2$. Mice were sacrificed at 30 min after injection of mercurials and tissues (kidney, liver, brain, heart, lung, spleen, plasma and red blood cells (RBC)) were removed. Effect of pretreatment with DCNB (0.5, 1.5, 3.0 mmol/kg, i.p.) on renal mercury uptake at 10 min after injection of CH_3HgCl or $HgCl_2$ (0.37 MBq/1 μmol/kg, i.v.) was also investigated. Mercury contents in tissues

and urine were determined by measuring the radioactivity of ^{203}Hg using an Aloka Auto Well gamma system (Aloka Japan).

Renal mercury uptake of mice injected with mercury-GSH complex. Mice were injected i.v. with ^{203}Hg -labeled CH_3HgCl or $HgCl_2$ (0.37 MBq/5 μmol/kg) premixed with GSH (10 μmol/kg). Mercury-GSH complex formation was confirmed by gel chromatography using Sephadex G-15, and ion exchange chromatography using DEAE-Sephacel. At 5 min after injection of methylmercury-GSH or inorganic mercury-GSH, the mice were sacrificed and tissues were removed. The content of mercury in the tissues was determined.

Determination of tissue concentrations of GSH. GSH concentrations in the liver, kidney and plasma of mice treated with DCNB were measured by high performance liquid chromatography (HPLC) using ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F) as a fluorogenic reagent (Toyo'oka and Imai, 1983).

Effect of acivicin pretreatment on renal mercury accumulation. Male ICR mice (5 weeks old) from Charles River Japan Inc. (Atsugi, Japan) were kept in metabolism cages (One mouse/cage). Mice (n=4) were treated with acivicin (0.25, 1.0, 2.5 mmol/kg, i.p.). At 30 min after acivicin injection, mice were injected with ^{203}Hg–labeled CH_3HgCl or $HgCl_2$ (0.37 MBq/18 μmol/kg, i.v.) and sacrificed at 2 hr after injection of mercurials and tissues were removed. The content of mercury in the tissues and urinary excretion of mercury were determined by measuring the radioactivity of ^{203}Hg using an Aloka AutoWell gamma system (Aloka Japan).

Determination of renal γ–GTP. Renal γ–GTP activity in mice at 30 min after acivicin treatment was determined. Kidneys were weighed and homogenized in 20 volumes of 50 mM Tris-HCl (pH 8.0). The homogenate was centrifuged at 800 x g for 10 min. The supernatant was used for the assay of γ–GTP activity according to the method of Tate and Meister (1974) using γ–glutamyl-*p*-nitroanilide as a substrate. Protein was determined colorimetrically using Coomassie blue binding (Bio-Rad Protein Assay, Bio-Rad Laboratories, Richmond, CA, U.S.A.) with γ–globulin as a standard. An enzyme unit (U) was defined as 1 μmol of *p*-nitroaniline produced per min.

RESULTS

Pretreatment of mice with DEM 30 min prior to injection of CH_3HgCl or $HgCl_2$ significantly decreased the renal accumulation of methylmercury and inorganic mercury, as reported earlier in the case of rats (Richardson and Murphy, 1975; Baggett and Berndt, 1986). DEM has the ability to decrease the GSH concentration in both the liver and kidneys. After several series of experiments in a search for a reagent that specifically depresses hepatic GSH, DCNB was selected among the various GSH-binding substances tested. As shown in Fig. 1, DCNB administration significantly decreased the GSH concentration in the liver without affecting the renal GSH levels at least during the 1 hr after the treatment. The renal accumulation of methylmercury or inorganic mercury decreased with the DCNB-induced depression of hepatic GSH, similarly to the case of DEM treatment. Further, dose-related decreases in hepatic and plasma GSH were observed by DCNB treatment (Fig. 2A). Although renal GSH was not significantly depressed, renal mercury accumulation at 10 min after administration of CH_3HgCl or $HgCl_2$ was significantly reduced by DCNB treatment (Fig. 2B). The renal mercury content of DCNB-treated mice was significantly correlated with the GSH concentration of the liver and plasma (Fig. 3). No significant correlation between renal mercury content and renal GSH content was found (Fig. 3). Thus, hepatic and plasma GSH appear to be more important determinants of renal

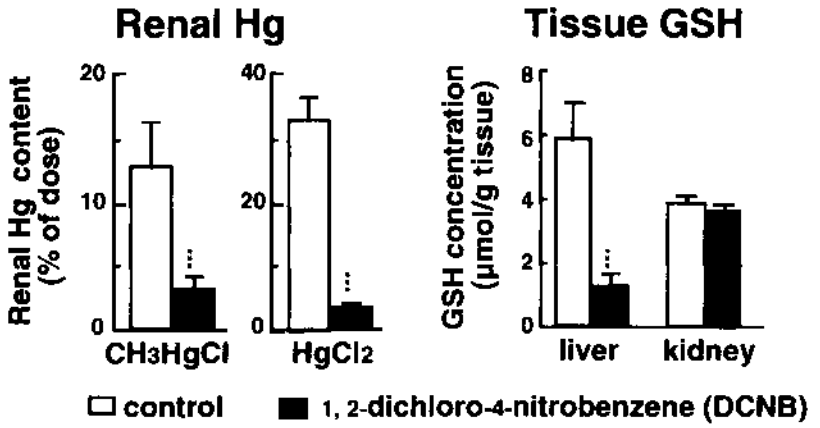


Fig. 1 Effect of pretreatment with DCNB on renal Hg accumulation and tissue GSH concentration. DCNB (2.5 mmol/kg, i.p.) was injected to mice 30 min before administration of mercurials (5μmol/kg, i.v.). Renal Hg content was determined 30 min after mercury injection. Tissue GSH concentration was determined 30 min after DCNB treatment.

uptake of these mercurials than renal GSH.

It is well known that hepatic GSH is released into the plasma as a major source of plasma GSH, and preferentially extracted by the kidneys. Figure 4 shows the renal uptake of mercury after i.v. injection of methylmercury-GSH or inorganic mercury-GSH complex. In comparison with the case of mice administered CH_3HgCl or $HgCl_2$, significantly higher renal accumulation of mercury was observed in mice receiving the GSH complexes.

To examine the role of γ–GTP in renal accumulation of mercurials, acivicin, an inhibitor of γ–GTP, was administered to mice 30 min prior to injection of CH_3HgCl or $HgCl_2$. The extent of inhibition of renal γ–GTP assayed 30 min after administration of acivicin is shown in Table 1. This acivicin treatment decareased γ–GTP activity by 92-98% during the subsequent 4 hr (data not shown). Acivicin pretreatment caused a decrease in the renal mercury accumulation in a dose-dependent manner within a dose of 1 mmol/kg at 2 hr after injection of CH_3HgCl or $HgCl_2$ (Fig. 5) without changing the level of mercury in the other tissues (brain, heart, lung, liver, spleen, plasma and RBC). This result indicates that acivicin may increase the urinary excretion of mercury. It has been reported that acivicin

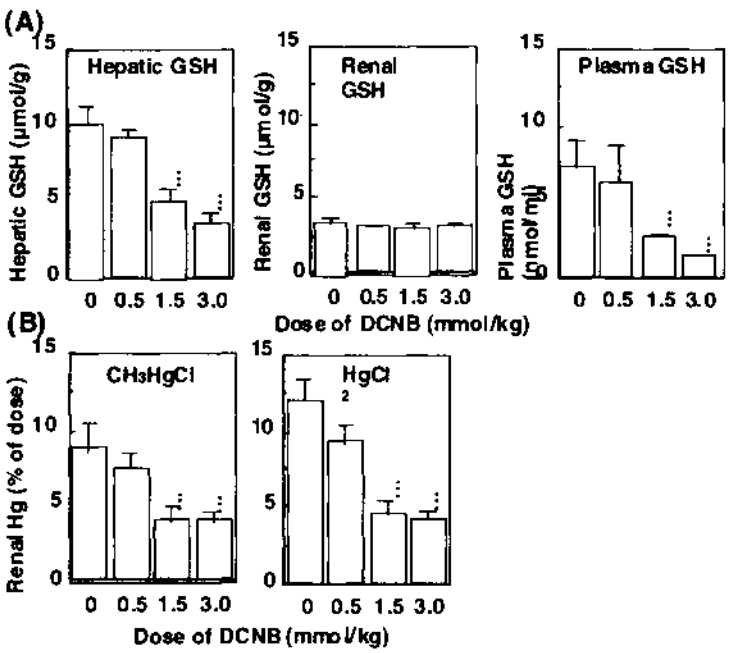


Fig. 2 (A) GSH concentration in mouse tissues 40 min after i.p. administration of DCNB. (B) Effect of pretreatment with DCNB on renal Hg accumulation 10 after injection of merucurials (1 μmol/kg, i.v.).

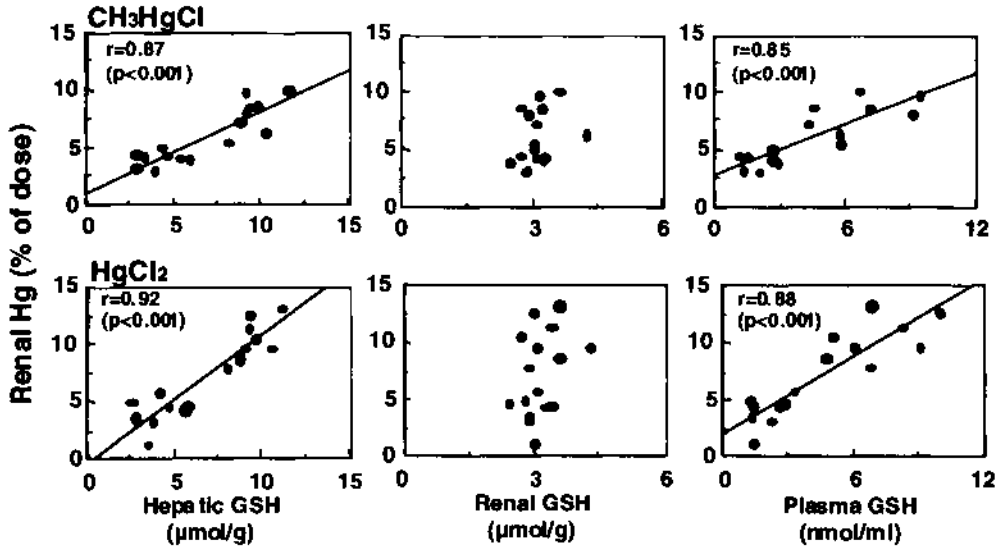


Fig. 3 Relationshiop between tissue GSH concentration and renal Hg content of mice 10 min after injection of mercurials (1 μmol/kg, i.v.).

treatment increases urinary excretion of GSH by inhibiting γ–GTP of the brush border membrane. In this study, the pretreatment of mice with acivicin significantly increased the amounts not only of GSH (320-1700% of control) but also of mercury (methylmercury 1800-9700%, inorganic mercury 600-1200% of control) excreted in the urine collected for 2 hr after the administration of CH_3HgCl or $HgCl_2$ as shown in Fig. 5. The increased amount of urinary mercury was almost the same as that of renal mercury decreased by acivicin pretreatment (Fig. 5).

DISCUSSION

Methylmercury and inorganic mercury can easily bind to GSH and other thiol compounds without the involvement of GSH S-transferase (Simpson, 1961; Bach and Weibel, 1976; Thomas and Smith, 1979; Naganuma and Imura, 1979; Refsvik, 1978). The binding of methylmercury or inorganic mercury with GSH is reversible and proteins can deprive the mercury-GSH complexes of mercury. Although methylmercury-GSH complex has been found in several GSH-rich tissues, most of the plasma methylmercury was bound to proteins and methylmercury-GSH has hardly been detected in plasma, which contains only a trace amount of free GSH. Inorganic mercury-GSH complex has not been detected in animal tissue other than rat bile (Ballatori and Clarkson, 1984). However, the present study using DCNB, a specific depletor of GSH in the liver, demonstrate that hepatic GSH plays an important role in the renal accumulation of methylmercury and inorganic mercury in mice. It is well

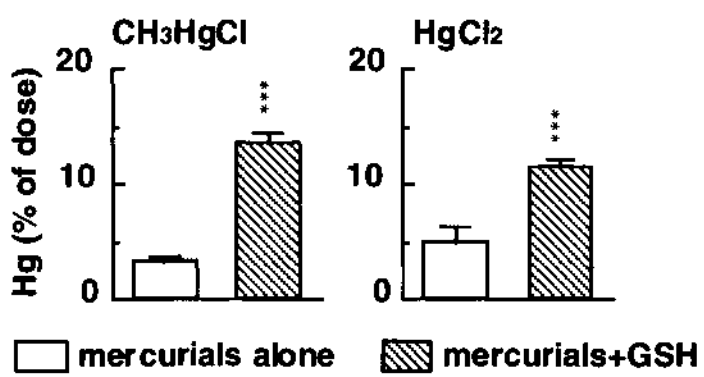


Fig. 4 Effect of co-administration of GSH with mercurials on renal Hg content 5 min after i.v. injection.

Table 1. Inhibition of renal γ-GTP by acivicin

Acivicin (mmol/kg)	γ-GTP activity (U/mg protein)	Inhibition (%)
0	0.376 ± 0.050	0
0.25	0.017 ± 0.001	95.6
1.0	0.006 ± 0.003	98.4
2.5	0.002 ± 0.001	99.4

Activity was determined 30 min after i.p. injection of acvicin.
Results were expressed as mean ± S.D. of 4 mice.

known that hepatic GSH, a major source of plasma GSH, and its S-conjugates with electrophilic xenobiotics are released from the liver into the plasma and preferentially extracted by the kidney (Inoue, 1985). In the present study, the correlation of hepatic and plasma GSH with the renal mercury uptake was observed (Fig. 3), suggesting that methylmercury and inorganic mercury are transported to the kidneys as a GSH complexes. Actually, methylmercury-GSH and inorganic mercury-GSH were easily taken up by the kidney (Fig. 4). The mercury-GSH complexes may be filtered through glomeruli as in the case of GSH itself, and the rate of filtration of this complexes may be influenced by plasma GSH levels. Takahashi *et al.* (1988) has revealed that a trace amount of methylmercury-GSH was actually detectable in rabbit plasma using an *invivo* blood-dialysis technique. As an explanation for the fact that mercury-GSH complexes cannot be detected in plasma, it is reasonable to consider that mercury bound to GSH is highly exchangeable and able to bind to plasma proteins, and that very small portion of mercury in the plasma can exist as mercury-GSH complexes which is filtered through the glomeruli.

In the kidney, extracellular GSH and its S-conjugates are hydrolyzed through the actions of γ-GTP and dipeptidase(s) into their constituent amino acids, which are rapidly taken up by kidney cells (Inoue, 1985; Inoue and Morino, 1985). GSH itself is not effectively transported into the cells (Hahn *et al.*, 1978). Pretreatment of mice with acivicin, a potent inhibitor of γ–GTP, significantly depressed the renal accumulation of methylmercury and inorganic mercury and increased the urinary excretion of the mercurials and GSH as shown in Fig. 6. This indicates that the complexation of mercurials with GSH and the hydrolysis of the GSH moiety by γ–GTP are important steps in the renal accumulation of methylmercury and inorganic mercury in mice, supporting the assumption that methylmercury and

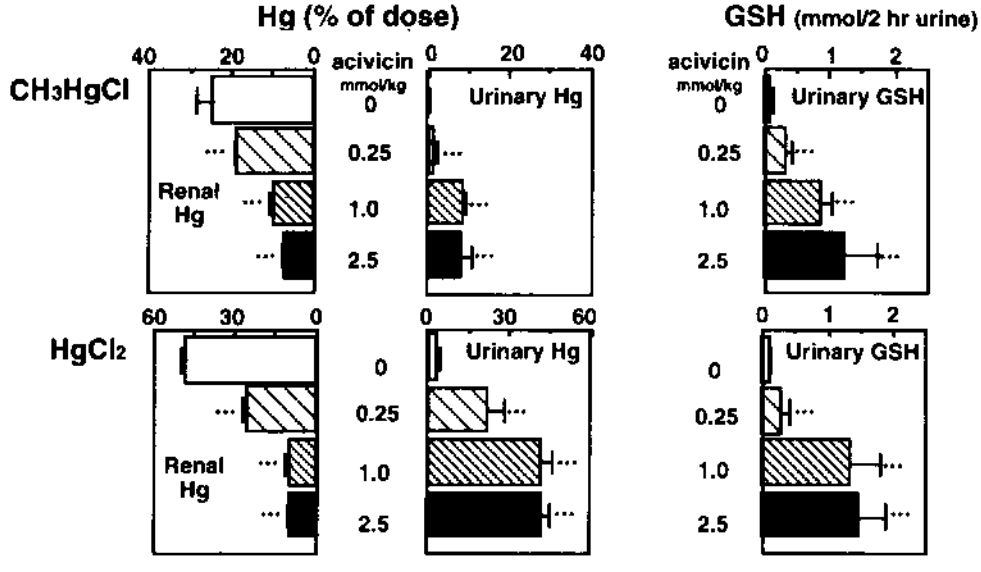


Fig. 5 Effect of pretreatment with acivicin on renal Hg accumulation and on excretion of Hg and GSH in the urine.

inorganic mercury are transported to the kidney as complexes with GSH. Moreover, the substantial increase in urinary excretion of methylmercury and inorganic mercury due to the inhibition of renal γ-GTP (Fig. 5) also suggests that the form of mercurials in the lumen of proximal tubules is mercury-GSH complexes.

Although renal γ-GTP activity was reduced to about 5% of control by acivicin (0.25 mmol/kg), more than 50% of renal mercury still remained as indicated in Fig. 5. Increases in the dose of acivicin resulted in further reduction of renal mercury uptake. These observations may be explained by the fact that renal γ-GTP activity is very high in the brush border membranes of renal proximal tubules (Inoue, 1985; Goldberg *et al.*, 1960; Meister, 1973), and even a small portion of the activity may be sufficient for the hydrolysis and subsequent cellular uptake of GSH in the lumen.

In conclusion, the present study demonstrated that both methylmercury and inorganic mercury are transported to the kidneys as a complex with GSH, and then incorporated into renal cells as a complex of Cys with CH_3Hg^+ or Hg^{2+} after degradation of the GSH moiety by γ-GTP and dipeptidase.

REFERENCES

Bach, R. D., and Weibel, A. T. (1976). Nuclear magnetic resonance studies on anion-exchange reactions of alkylmercury mercaptides. *J. Am. Chem. Soc.* **98,** 6241-6249.

Baggett, J. M., and Berndt, W. O. (1986). The effect of depletion of nonprotein sulfhydryls by diethyl maleate plus buthionine sulfoximine on renal uptake of mercury in the rat. *Toxicol. Appl. Pharmacol.* **83,** 556-562.

Ballatori, N., and Clarkson, T. W. (1984). Inorganic mercury secretion into bile as a low molecular weight complex. *Biochem. Pharmacol.* **33,** 1087-1092.

Goldberg, J. A., Friedman, O. M., Pineda, E. P., Smith, E. E., Chatterji, R., Stein, E. H., and Rutenburg, A. M. (1960). The colorimetric determination of g-glutamyltranspeptidase with a synthetic substrate. *Arch. Biochem. Biophys.* **91,** 61-70.

Hahn, R., Wendel, A., and Flohe, L. (1978). The fate of extracellular glutathione in the rat. *Biochim. Biophys. Acta.* **539,** 324-337.

Hughes, W. L. (1957). A physicochemical rationale for the biological activity of mercury and its compounds. *Ann. N. Y. Acad. Sci.* **65,** 454-460.

Inoue, M., and Morino, Y. (1985). Direct evidence for the role of the membrane potential in glutathione transport by renal brush-border membranes. *J. Biol. Chem.* **260,** 326-331.

Inoue, M. (1985). Interorgan metabolism and membrane transport of glutathione and related compounds. *In* "Renal Biochemistry" (Kinne, R. K. H., ED), pp. 225-269. Elsevier Science Publishers B. V., New York.

Meister, A. (1973). On the enzymology of amino acid transport -Transport in kidney and probably other tissues is mediated by a cycle of enzymic reactions involving glutathione-. *Science.* **180,** 33-39.

Naganuma, A., and Imura, N. (1979). Methylmercury binds to a low molecular weight substance in rabbit and Human erythrocytes. *Toxicol. Appl. Pharmacol.* **47,** 613-616.

Naganuma, A., Koyama, Y., and Imura, N. (1980). Behavior of methylmercury in mammalian erythrocytes. *Toxicol. Appl. Pharmacol.* **54,** 405-410.

Naganuma, A., and Imura, N. (1984). Species difference in biliary excretion of methylmercury. *Biochem. Pharmacol.* **33,** 679-682.

Omata, S., Sakimura, K., Isii, T., and Sugano, H. (1978). Chemical nature of a methylmercury complex with a low molecular weight in the liver cytosol of rats exposed to methylmercury chloride. *Biochem. Pharmacol.* **27,** 1700-1701.

Refsvik, T., and Norseth, T. (1975). Methyl mercuric compounds in rat bile. *Acta Pharmacol. Toxicol.*

36, 67-78.

Refsvik, T. (1978). Excretion of methyl mercury in rat bile: The effect of diethylmaleate, cyclohexene oxide and acrylamide. *Acta Pharmacol. Toxicol.* **42,** 135-141.

Richardson, R. J., and Murphy, S. D. (1975). Effect of glutathione depletion on tissue deposition of methylmercury in rats. *Toxicol. Appl. Pharmacol.* **31,** 505-519.

Simpson, R. B. (1961). Association constants of methylmercury with sulfhydryl and other bases. *J. Am. Chem. Soc.* **83,** 4711-4717.

Takahashi, H., Wada, S., Suda, I., Totoki, S., and Ohota, J. (1988). Toxicological and metabolic aspects of organic mercury poisoning. *J. UOEH.* **10,** 117-126.

Tate, S. S., and Meister, A. (1974). Interaction of g-glutamyl transpeptidase with amino acids, dipeptides, and derivatives and analogs of glutathione. *J. Biol. Chem.* **249,** 7593-7602.

Thomas, D. J., and Smith, J. C. (1979). Partial characterization of a low-molecular weight methylmercury complex in rat cerebrum. *Toxicol. Appl. Pharmacol.* **47,** 547-556.

Toyo'oka, T., and Imai, K. (1983). High-performance liquid chromatography and fluorometric detection of biologically important thiols, derivatized with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate (SBD-F). *J. Chromatogr.* **282,** 495-500.

Urano, T., Naganuma, A., and Imura, N. (1988). Methylmercury-cysteinylglycine constitutes the main form of methylmercury in rat bile. *Res. Commun. Chem. Pathol. Pharmacol.* **60,** 197-210.

Yasutake, A., Hirayama, K., and Inoue, M. (1989). Mechanism of urinary excretion of methylmercury in mice. *Arch. Toxicol.* **63,** 479-483.

PROCEEDINGS OF

NIMD Forum '97

NIMD
003-1997

平成9年12月24日発行

発行　環境庁　国立水俣病総合研究センター
〒867-0008　熊本県水俣市浜4058番18号
TEL　0966-63-3111
FAX　0966-61-1145

Published by National Institute for Minamata Disease
4058-18 Hama, Minamata City, Kumamoto 867-0008, Japan
TEL +81-966-63-3111
FAX +81-966-61-1145

December 1997

問い合わせ先：国際・総合研究部
Inquiry: Department of International Affairs and Environmental Sciences

印刷　有限会社　水俣旭印刷所
熊本県水俣市栄町2-1-25