

PROCEEDINGS OF NIMD FORUM '99

October 12-13, 1999 Venue:National Institute for Minamata Disease Minamata City, Japan

Organized by National Institute for Minamata Disease

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Preface

During the past three decades, advanced science and technology have been two of the most decisive driving forces behind economic development. However, technological developments can be polluting and wasteful, and can create serious potential risks to environmental health.

The case of Minamata disease shows how activities that give top priority to economic goals but lack proper attention to the environment, can cause irreparable damage. It has left us with an invaluable understanding of the importance of taking thoroughgoing measures to prevent health damage from mercury pollution. Based on the lessons learned the hard way from Minamata disease, we must reflect seriously on how crucial it is to take the environment into consideration, and we must contribute cooperatively to international work in the field of environmental health. Attention is placed on recent enormous advancements in analytical chemistry and the epidemiological approach, which have contributed much to hazard identification and exposure assessment as well ad to environmental monitoring.

The main objective of the international workshop on "Minamata Forum '99" was to exchange knowledge, experience and information on the recent improvement of environmental protection measures and on promotion of investigation and research among scientists concerned with all aspects of the mercury compounds as global pollutants. The meeting was held at the National Institute for Minamata Disease, Japan on October 12-13, 1999. Participants included the Steering Committee members of the 6th International Conference on Mercury as a Global Pollutant which will be held on October 2001 in Minamata. The active discussions were extremely gratifying. All participants contributed greatly to obtain a consensus about the direction of the forum, focusing on fruitful cooperation in the future.

I would like to express my sincere gratitude to the invited speakers who prepared manuscripts for inclusion in these Proceedings which will be made available to a wide audience.

> Yukio Takizawa, M.D. Chairman NIMD Forum '99 Organizing Committee Director-General National Institute for Minamata Disease

Welcome Address

Ryo Takagi, M.D. Section Chief, Environmental Health Department Environment Agency of Japan

It's our great pleasure that the second forum of National Institute for Minamata Disease, NIMD Forum '99 has been held here in Minamata. Especially, I'd like to express our sincere appreciation to the people from the foreign countries.

Once, we had experienced the outbreak of Minamata Disease that is one of the most serious pollution, originated in discharge of water containing harmful substances from chemical plants. Nowadays, we believe that no situation or conditions exist for a new outbreak of Minamata Disease after the wide-ranging environmental protection policies and measures that we have developed. In 1995, the problem of compensation to Minamata Disease patients was settled by three ruling coalition parties, Liberal Democratic Party, Socialist Party and New Party Sakigake. However, Minamata Disease remains a lot of medical issues. For example, the health effects caused by continuous methylmercury exposure of very low level, especially the effects on fetus. So, we wish this Forum could have a useful opportunity for advanced studies and improved communication.

We hope National Institute for Minamata Disease provides its accumulated knowledge and enterprise related to the mercury poisoning incidences in Japan with other countries, and makes significant contribution in terms of international cooperation in the field of environmental health. We also hope that everyone of the foreign countries recognizes the importance of protection of environment, taking into account of this experience in Japan, and that environmental pollution will be successfully prevented in all countries.

We hear that the 6th International Conference on Mercury as a Global Pollutant will be held here on October in the first year of next century. We hope that this Forum in 1999 may become a first step to the 6th International Conference on Mercury as a Global Pollutant.

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Total and Methylmercury Levels in Hair of a Coastal Population from the Brazilian Northeast

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Abstract

A simple descriptive exposure survey was conduced at Santa Cruz Channel, Pernambuco, Brazil, to assess the possible risk of mercury contamination to which a coastal population could be exposed. The area was formerly know to be contaminated due to a chlor-alkali plant installed along one of the tributaries of the channel. The values for total and methylmercury were measured in human hair from inhabitants of Itapissuma and fish (*Mugil* sp.) catch in the area. Both human hair and fish muscle presented relatively low values, and were found to be well within the average when compared to other Brazilian coastal environments. The levels in oysters were also determined and showed slightly higher values of both mercury species measured than the fish sample. Sediments were found to be contaminated in the vicinity of the point source only.

Keywords: Mugil sp., total mercury, human hair, methylmercury, Santa Cruz Channel

Introduction

Santa Cruz Channel is a sheltered and shallow area, separated from the Atlantic Ocean by Itamaraca Island, 40 km north of Recife, in Northeast Brazil. The channel has about 22 km of extension and the average depth is in the order of a few meters. The whole area is bordered by highly productive mangrove swamps and fisheries (fish and shellfish) is abundant, both by catch and in man-made ponds. The channel has a number of tributaries, among the largest of them is Botafogo river (Figure 1).

During the last decade the communities along Santa Cruz channel have suffered sudden and severe changes in their ways to relate to nature, caused by unplanned development, irrational land occupation and resulting lost of environmental quality. Traditionally, the area was occupied by sugar cane and coconut plantations, associated to other subsistence cultures and artizanal fisheries. Presently the occupation of the land has been done mainly through seasonal tourism, in response to the natural talent of the region. Heavy industrialisation of the areas upstream the channel's tributaries has also contributed significantly for the decaying scenario. On top of that, unusually elevated population growing rates started to be registered in the areas along the channel. The rates ranged from 3.2 up to 4.2 % p.a., being the highest of Recife's metropolitan area in the 90's (Lima & Quinamo, 1998).

A chlor-alkali plant operates a few km up Botafogo river using the traditional electrodes method since 1963. From 1986 operations are combined with a precipitation basin, which only operated at its designated efficiency in 1991. The total mercury input to the channel up to 1987 may have been of the order of 22 to 35 tons. The present input is estimated at 0.03 ton/p.a.. This plant, together with its subsidiaries, is also responsible for the dumping of large amounts of chlorine in the river.

The presence of this plant has been the reason for the spreading of unfounded rumours about the contamination of Santa Cruz Channel with mercury. Two surveys made by the São Paulo State Environmental Agency (CETESB, 1981 and 1984) contracted by Pernambuco State's Government and one assessment made by the Pernambuco State Environmental Agency (CPRH, 1982) were inconclusive. Any of those works were made readily available to the public. Little or no attention

was paid to the combination of other discharges, and the resulting uncommon physico-chemical conditions in which the inorganic mercury was lost by the plant (Meyer, 1996).

An extensive work by Meyer was done in 1994/95 on the contamination status of Santa Cruz channel concerning the particulate matter, the oysters and the sediment. At the time she undoubtedly characterised Botafogo river and its vicinities as the areas most significantly polluted with mercury. On a tentative mass balance for determining the fate of mercury in Santa Cruz channel, she hypothesises that most of the mercury released by the plant has not settled in the mangrove system, but has been exported to the sea. The present amount in the sediments of the channel is estimated at 1.2 to 2.5 tons of mercury.

The aim of the present work is then to assess if some of the mercury remaining in the sediments and particulate matter which passes by the channel, could be assimilated by the biota and consequently put to risk the human populations which depend on the local fisheries for their survival.

Material and Methods

1. Sampling was conducted during two months: August and September 1999.

1.1 - Hair samples from 8 men and 84 women was sampled at the Health Centre in Itapissuma. This Health Centre has been traditionally dedicated to the fisherman and their families. Also a questionnaire about the subject's eating habits in respect to sea food and social issues, as well as their former and present occupations, was filled in.

1.2 - Fish (*Mugil* sp.) from Santa Cruz channel was collected with the help of local fishermen. This fish is widely consumed in the region due to its low price, US\$ 2.00 or less per kilogram. The better quality and higher priced catch is usually reserved for sale to local restaurants and in Recife. This gender has detritivorous feeding habit, eating from the bottom mud, and accumulating larger amounts of fat in the muscle. Measurement of the total length were made and the left lateral muscle was taken for analysis accordingly to the recommendations of FAO (FAO, 1983) and the NS&T Program (NOAA, 1987), and the sub-samples were individually frozen.

1.3 - Oysters (*Crassostrea rhizophorae*) were hand picked at stations 1, 2 and 3 (Figure 1), where Botafogo river reaches the channel, at low tide and refrigerated. In the lab they were processed as recommended by the NS&T Program (NOAA, 1987), taking as a measure of their age only the external length of the shell. The soft tissue for the composite samples (n=35, n=46 and n=37 individuals respectively) was frozen.

1.4 - Sediments were taken at points 1 to 7 (Figure 1) at low tide, refrigerated until arrival in the laboratory and then frozen until analysis. Sediments had their moisture and organic matter content determined as described in Loring & Rantala (1992).

Transportation of all the samples (except human hair) to Japan was made in dry ice.

2. The total and MHg analysis were performed at National Institute for Minamata Disease, Minamata, Japan, following the procedures developed at this institute which are fully described in Akagi (1997).

2.1 - Human hair and fish samples were finely chopped before analysis.

2.2 - The composite oysters samples and each individual sediment sample were homogenised with a blender after thawing immediately before analysis.

3. The analysis were made alongside determinations of TotHg and MHg in CRMs. Were used for this work IAEA 356 (total Hg; estuarine sediments) and IAEA 142 (total and methylmercury; mussel homogenate). CRMs for human hair were not available for this work, but the NIMD laboratory has taken part in a number of international calibration exercises in which its method and performance for total and methylmercury analysis in human hair has been tested and approved.

Results

The results of human hair analysis appear in Figure 2 a and b. The maximum, minimum and average values found for this analysis are listed on Table 1. In spite of the maximum value of the range for TotHg to be 12.5 ng Hg/mg, only two of the values (12.5 and 7.3 ng Hg/mg), corresponding to approximately 2% of the sampled population, were found to be above the 6.0 ng Hg/mg limit. Even then, these two individuals presented low MHg concentration in their hair (1.6 and 6.0 ng Hg/mg respectively).

	ng TotHg/mg	ng MHg/mg	% MHg
interval	0.1 - 12.5	0.1 - 6.0	13.0 - 100
average	1.9	1.2	70.2
stdev	1.6	1.0	29.9

10000 1. Summary of the results for the human main analysis $(10 - 75)$

The results for the fish sample analysis are shown in Figure 3 a and b. The maximum, minimum and average values obtained during this analysis are shown on Table 2. All the analysed individuals had both TotHg and MHg levels well bellow the maximum concentration permitted by the Brazilian regulation regarding human consumption (500 ng Hg/g). Only two individuals presented %MHg below 50%.

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	Length, cm	ng TotHg/mg	ng MHg/mg	% MHg
interval	27.2 - 36.6	4.6 - 167.0	1.8 - 91.6	5.7 - 100
average	30.6	26.9	19.6	80.0
stdev	1.9	26.1	16.0	23.8

Table 2: Summary of the results for fish (Mugil sp.) muscle analysis (N = 60).

Analysis of oysters composite samples showed values ranging from 247.6 - 412.6 ng TotHg/g and 94.5 - 130.5 ng MHg/g. These values are closer to the concentration limit stabilised by the Brazilian legislation for human consumption than the fish muscle values. Although the value for total mercury in oysters is within legal limits for consumption, the %MHg was found to be higher than expected for these animals, with an average value of 36.3%.

Sediment samples showed a marked contamination pattern with higher values at the mouth of Botafogo river and lower values at other parts of the channel, which agrees with the work of Meyer (1996). The levels present in surface sediments for the samples close to the mouth of Botafogo river (stations 1, 2 and 3) varied from 1190 up to 3490 ng TotHg/g, and showed no significant seasonal variation between June (end of dry season) and September (end of rainy season) in 1999. Sediments collected at stations 4, 5, 6 and 7 had their TotHg content in the range of 31 to 119 ng TotHg/g.

Discussion

The work from Meyer (1996), was a survey of the level of contamination of Santa Cruz Channel by inorganic mercury based on sediments and oysters total mercury contents. She has also produced a mass balance of the mercury in the channel, considering the total amount lost by the chlor-alkali plant in the last 35 years of its operation and the amount found in her survey. She concluded that less than 10% of the total mercury amount remains in the channel sediments today.

The rest of the mercury would have been exported in association with suspended solids to the sea. Part of the mercury could also have been transferred to other compartments of the environment. Among them the biota, including man, would be the one of biggest concern. The present work has then assessed the levels of mercury in two important compartments which are closely related to the sediments: a detritivorous fish and men, its direct consumer. Mercury analysis in edible parts of fish an human hair samples is widely recognised in the literature as important tools in the diagnosis of environmental mercury contamination.

The values found in the fish from the channel (4.6-167.0 ng TotHg/g w.w.) and human hair from residents of Itapissuma (0.1-12.5 ng TotHg/mg) were both within the national average for Brazilian and other tropical/sub-tropical coastal regions (Herut et al., 1996; Prudente et al., 1997; Kehrig et al., 1998; Vasconcellos et al., in this issue).

The MHg analysis results for human hair samples (0.1-6.0 ng MHg/mg) were also in this case (Vasconcellos et al., in this issue). The average %MHg for this population ($70.2 \pm 29.9\%$) suggests that this population is exposed to mercury mainly via fish/shellfish consumption. However, MHg reports on values for fish and shellfish from tropical/sub-tropical coastal areas are rare in the literature. The %MHg contents of this sample ($80.0 \pm 23.8\%$) seems to behave similarly to fish from tropical freshwater environments (Kehrig & Malm, 1999). The %MHg is typically higher than 50% in this sample.

The importance of these findings lays not only on the information as such, but also in the subside it is providing in the clarification of a long standing social issue. For many years the channel was believed to be contaminated with mercury in such a level that the consumption of its fish and shellfish would put at risk residents and other consumer markets. It might have been true in the past, but there was no survey of such conditions at the time, in spite of the public concern. This work is the first attempt to assess this risk. Although quite preliminary, this assessment made already possible to believe that there is no immediate risk to the population through the consumption of fish from Santa Cruz channel. The diminished present risk can be attributed to a number of causes, and not necessarily to the measures taken by the plant to clean its discharges. Among them, the rapid decline of the local artizanal fisheries in the last ten years or so due to impoverishment of environmental conditions and the male occupational shift towards tourism and industry; the improvement of fishermen and their families eating habits, with the introduction of other sources of animal protein; and also the efficient transport of the mercury to the sea by particulate matter. A deeper survey of the social and economic issues may confirm the two first hypothesis (Lima & Quinamo, 1998).

Under the lights of this results and some more which is being done, the issue of mercury contamination will be discussed with the community by the Federal University of Pernambuco/ Oceanography Department researchers to infor and clarify their doubts, as well as to thank for their collaboration in submitting to the hair sampling. Their complaints about physical disconfort will be further investigated, but presently they are being attributed to occupational stress, due to the rough nature of the work in the mangroves, especially for women and children, and to other sorts of aquatic pollution.

The results from the oysters analysis show that these organisms are still safe for human consumption in respect to mercury. The higher %Mhg could be the result from these animals to be feeding on particulate matter which originates from ressuspended sediments. In intertidal mangrove sediments it seems to happen conditions for mercury methylation, transforming the inorganic mercury lost by the plant before it reaches the channel. Unfortunatelly, other environmental stresses have degraded this resource in the vicinity of Botafogo River. The chlor-alkali plant also makes chlorine discharges into the river. In each of these ocasions the oyster population becomes heavily compromised. This fact has driven oyster pickers away from the oyster banks of the most heavily mercury contaminated area.

The mercury content measured in the sediments were found to be in good agreement with the results of Meyer (1996) for the same area, and confirm that Botafogo river is a point source for mercury to Santa Cruz channel.

Conclusions

- ✓ The present work was the first attempt to assess the level of contamination of the populations living around Santa Cruz channel and to speciate the mercury contents of the local fish and shellfish consumed by the local population and sold in the markets.
- ✓ It has also pointed the direction which future work could follow in analising more carefully the dynamic of methylation in sediments and its effects on oysters.
- ✓ The human subjects have shown normal levels of total and organic mercury content in their hair when compared to an average group of Brazilians.
- ✓ The fish muscle mercury contents for both mercury species analysed was found to be within normal limits.
- ✓ In spite of not reaching the 500 ngTotHg/g limit established by the Brazilian legislation, the oysters showed an unexpected high %MHg level of 32 to 39%.
- ✓ The sediments at Botafogo river and its vicinities are significantly contaminated with mercury, but the other locations of the channel show normal mercury levels in the sediments for a tropical estuarine area.
- ✓ This work will be shortly followed by a feedback work with the local community, especially those fishermen and their families from whom the hair has been sampled.

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Figure 1: Botafogo River, 1 to 7 sampling stations (oysters and sediments samples) location, Itapessuma City (human hair samples) and Canal de Santa Cruz (fish samples) in Pernambuco, Northeast Brazil.



(a)

Figure 2 a and b: Total and Mhg concentrations and % Mhg in human hair from Itapissuma, Pernambuco, Brazil, in August 1999.



(a)



Figure 3 a and b: Total and Mhg concentrations and % Mhg in fish (Mugil sp.) from Santa Cruz Channel, Pernambuco, Brazil, in August 1999.

STUDIES ON MERCURY EXPOSURE OF SOME BRAZILIAN POPULATIONAL GROUPS LIVING IN THE AMAZONIC REGION BY MEANS OF HAIR ANALYSIS

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It is well known that intense gold exploration activities are occuring mainly since the 1980's in the Amazonic region and a considerable environmental impact is being detected in the biota, soils, sediments and atmosphere due to the use and disposal of mercury employed for gold amalgamation.

In the present work, mercury exposure of Brazilian populational groups living in two main locations in the Amazonic region was studied, by means of hair analysis: the Xingu Indian Park and the State of Amapá.

Total mercury was determined in the hair of thirteen Indian groups living in the Xingu Park and also in residents of the State of Amapá, by instrumental neutron activation analysis, at the Radiochemistry Division of IPEN/CNEN-SP (São Paulo, Brazil).

Methylmercury was determined in the hair of about half of the Indian groups by CVAAS, after separation of mercury species by ion exchange, at the Department of Environmental Sciences of the Jozef Stefan Institute (Ljubljana, Slovenia).

The results obtained were compared to those of a control group, consisting of individuals not exposed to mercury occupationally or environmentally and with low fish consumption.

In all the thirteen Indian tribes of the Xingu Indian Park very high amounts of total mercury and methylmercury were found, as compared to controls, and a similar trend was observed for many individuals residing in three localities of the State of Amapá.

The results obtained for the mercury analysis in hair are discussed in terms of possible sources of contamination and related to fish consumption of these populations.

Introduction

Environmental contamination by mercury due to gold exploration activies in the Amazon has become a world wide concern and has been receiving very special attention from many international research groups in the last 15 years. Considering the annual inputs, high concentrations of mercury have been reported in nearly all natural compartments of the regions ecosystems⁽¹⁾.

Biomonitoring of human populations has been carried out in many studies mainly by hair analysis, which is considered as a reliable indicator of mercury body burden, mainly for methylmercury⁽²⁾.

Malm et al⁽³⁾ have analyzed mercury in fish and hair, in the Tapajós, Madeira and Negro River basins, in the Brazilian Amazon. The Tapajós and Madeira river basins

have suffered impacts of gold mining, while in the Negro river gold mining is rarely documented.

High mercury values were found in predatory fish in all the three basins, with maximum values of 3.8, 3.2 and 4.2 mg/Kg, respectively. Also high values for mercury were found in hair samples, in the Tapajós and Madeira riverine populations, due to their high fish consumption. An average of 18.6 mg/Kg and maximum of 176 mg/Kg was found at Tapajós and corresponding values of 8.9 mg/Kg and 71 mg/Kg at Madeira river basin. The authors call atention to the complexity of these kinds of studies in the Amazon, due to the tremendous diversity in fish species and also to seasonal variations.

Forsberg⁽⁴⁾ et al studied also mercury in fish and human hair in the Negro river basin (Brazilian Amazon) and found high concentrations. For predatory fish, more than half of the values were higher than 0.5 ppm, the maximum values set by WHO for human consumption⁽⁵⁾.

As for hair, the concentrations were exceptionally high and more than half of the values exceeded 50 mg/Kg, the concentration at which sensitive individuals begin to show signs of mercury in toxication⁽⁵⁾.

The authors point out that these elevated levels of mercury might reflect a high natural background of mercury in the Negro River, due to its very special organogeochemical characteristics, such as high concentration of DOC, low pH and conductivity and high density of hydromorfic podsols. Also these conditions could amplify the effects of anthropogenic influence from other regions, including the atmospheric transport of mercury vapour.

Besides analysis of total mercury in hair, it is also very important to assess the methylmercury content, specially of riverine populations which consume fish very frequently. Akagi et al⁽⁶⁾ have determined concentrations of total mercury and methylmercury in human hair and fish samples from fishing villages of the Tapajós river basin. Very high amounts of mercury in hair were found, the predominant form being methylmercury in the riverine populations, while in goldminers and goldshop workers mercury was mostly in the inorganic form. The fish analyzed presented mercury levels up to 3.82 mg/Kg mainly in the form of methylmercury and most of the fish from downstream exceeded the limit value of 0.5 ppm in Brazil.

Akagi et al⁽⁷⁾ studied also more completely the human exposure to mercury due to goldmining in the Tapajós River Basin by doing speciation of mercury in human hair, blood and urine.

High levels of mercury were observed in hair and blood from the fishing villages investigated and more than 90% was in the form of methylmercury, in both kinds of samples. In the gold mining areas, on the contrary, the values were much lower and the percentages of methylmercury varied widely. In the urine of goldshop workers, mercury was found mostly in the inorganic form. A good correlation was found between mercury in hair and blood in the fishing villages.

A very important question that arises from these findings is to which point hair mercury levels that are not so high as those found in Minamata and Iraq can be related to neurotoxic and other adverse effects.

Lebel et al⁽⁸⁾ have studied the neurotoxic effects of low-level methylmercury contamination in the village of Brasília Legal, in the Tapajós River, a tributary of the Amazon. The subjects studied were 91 inhabitants of the village, with hair mercury levels below 50 mg/Kg. Performance on a neurofunctional test battery and clinical manifestations of nervous system disfunction were examined in relation to hair mercury concentrations.

It was verified in this work⁽⁸⁾ that the near visual contrast sensitivity and manual dexterity decreased significantly with hair mercury levels. Also it was concluded that hair mercury levels were significantly higher for persons who presented disorganized movements on a alternating movement task and for persons with restricted visual fields. According to the authors, there results suggest dose-dependent nervous system alterations at hair mercury levels below 50 mg/Kg, previously considered as a threshold for clinical effects.

In the present paper, biomonitoring of mercury exposure was performed in two main regions of the Brazilian Amazon: The Xingu Indian Park, located in the State of Mato Grosso (Central Brazil) and the State of Amapá, located in the Eastern Part of the Amazon. In the State of Amapá, three main localities were object of the study: Serra do Navio, Vila Nova and Tartarugalzinho.

The Xingu Indian Park is situated in na area of 2700 square Kilometers, in the North of the State of Mato Grosso, close to the frontier with the State of Pará and there are 17 Indian Groups living there, totalizing about 6000 inhabitants.

The locality of Serra do Navio, in the State of Amapá, the second area studied in the present work, is not affected by the gold extraction activities, but is partially degraded by the mining of manganese, while the locality of Tartarugalzinho, an area also studied, is clearly affected by the "garimpos" activities.

Experimental

Collection and washing of hair samples

The hair samples were collected and washed according to the protocol recommended by the IAEA⁽⁹⁾. The samples were cut using stainless steel scissors, from the occipital area of the head and as close as possible to the scalp in an amount corresponding to about 2g.

The hair was then cut with the scissors into segments as short as possible and transferred to a glass vial to be submitted to the recommended procedure of sequencial washing with acetone and water, followed by drying at room temperature.

Determination of total mercury in hair and reference materials by instrumental neutron activation analysis (INAA).

About 100-200 mg of the prepared hair samples and of the reference materials (RMs) were weighed in clean polyethylene envelopes and submitted to a thermal neutron flux of about 10^{12} n.cm⁻² .s⁻¹, in the IEA-R1 nuclear research reactor, together with mercury standards.

After about 70h of decay time, necessary for the decay of interfering activities, the radioactivy of 197 Hg ($t_{1/2} = 64.1$ h), was measured in a gamma-ray spectrometer, constituted of a hyperpure Ge detector coupled to associated electronics.

The more detailed procedure has already been described elsewhere (10,11).

Determination of total mercury and methyl-mercury in hair by cold vapour atomic absorption spectroscopy

A part of the hair samples collected from the Indians of the Xingu Park was sent to the Nuclear Chemistry Department of the Jozef Stefan Institute (Ljubljana, Slovenia), for analysis of total mercury and methyl-mercury. The method used for hair analysis is basically the technique described by May et al, which uses an anion exchange separation of extracted inorganic from organic mercury species, followed by destruction of organic species by UV irradiation, with the usual CV-AAS finish.

Results and discussion

In Table I, a Summary is presented of the results obtained for analysis of total mercury in hair of the control population and of thirteen Indian groups of the Xingu Park.

The control population comprised adult individuals living in São Paulo, with low fish consumption and with no history of mercury exposure, either environmentally or occupationally. The arithmetic mean, the median and the geometric mean were very close to 1 mg/Kg. These results are similar to the ones found by other authors for Brazilians⁽¹⁰⁾.

The concentrations found for the thirteen Indian groups analyzed were statistically significantly different from the controls, as proved by applying the ANOVA test at 95% confidence level and the geometric means varyied from 3.2 to 21 mg/Kg.

Table	I.	Summary	of th	ете	esult	s obtain	ed for t	total m	hercury cos	nten	ts in the	hair of th	he
		controls	and	of	the	Xingu	Indian	Park	(mg/Kg),	by	neutron	activatio)n
		analysis ⁽	12)										

· · · · · · · · · · · · · · · · · · ·		<u> </u>			
Populational group		<u>s</u>	Median	xg	Range
CONTROLS	1.1	0.6	1.0	0.9	0.3-2.9
INDIAN GROUP 1	18.5	5.9	18.0	17.1	6.9-34.3
INDIAN GROUP 2	12.0	4.0	10.7	11.4	6.5-21.6
INDIAN GROUP 3	8.7	3.0	8.2	8.2	4.5-18.5
INDIAN GROUP 4	13.2	3.8	13.0	12.7	4.8-25.3
INDIAN GROUP 5	10.6	3.9	11.5	9.4	1.7-15.1
INDIAN GROUP 6	20.6	10.0	18.8	19.0	8.1-57.3
INDIAN GROUP 7	16.5	5.5	15.8	15.5	2.5-30.2
INDIAN GROUP 8	17.2	6.0	16.2	16.3	2.1-31.7
INDIAN GROUP 9	21.8	6.1	20.8	21.0	12.4-34.2
INDIAN GROUP 10	8.1	9.0	2.8	4.7	1.5-33.1
INDIAN GROUP 11	18.2	7.8	16.2	16.7	5.5-41.8
INDIAN GROUP 12	12.2	3.1	12.5	11.8	6.6-18.8
INDIAN GROUP 13	3.6	2.4	2.6	3.1	1.2-11.1

In Table II are presented the results for methylmercury contents in hair of six of the Indian groups studied. It can be concluded that most part of the mercury found in hair of these populations is present as methylmercury and this fact can be attributed to the very frequent fish consumption of these populations.

			ł		
Populational group	x	S	Median	xg	Range
INDIAN GROUP 1	15.6	4.5	15.0	14.9	4.8 25.7
INDIAN GROUP 2	10.2	1.8	10.5	10.1	7.6 - 12.9
INDIAN GROUP 9	15.9	3.9	15.1	15.5	10.0 - 23.7
INDIAN GROUP 10	12.4	8.3	10.0	10.6	5.5 – 24.2
INDIAN GROUP 11	16.9	7.0	14.2	15.5	4.4 - 32.8
INDIAN GROUP 12	10.6	2.8	11.2	10.1	4.3 - 15.3

Table II. Summary of the results obtained for methylmercury contents in the hair of the Xingu Indian Park residents (mg/Kg)⁽¹²⁾

Table III presents a summary of the results obtained for total mercury in the three localities of the State of Amapá (Serra do Navio, Vila Nova and Tartarugalzinho).

The results for Hg in hair were also significantly higher than for the controls, specially in Tartarugalzinho, where the impact of gold extraction activities was more pronounced.

Table III. Summary of the results obtained for mercury contents in the hair of the Serra do Navio, Vila Nova and Tartarugalzinho (mg/Kg)

Region	- x	S	Median	- xg	Range
SERRA DO NAVIO	3.73	3.63	2.11	2.44	0.21-20.58
VILA NOVA	5.42	2.27	5.32	5.02	2.61-8.62
TARTARUGALZINHO	11.34	9.80	6.60	7.34	1.19-28.62

The Figures 1,2 and 3 show the mercury concentrations versus fish consumption, in the three localities studied.

It can be observed that there is a trend of increase of mercury concentration in hair with fish consumption, for the three localities in the State of Amapá.



Fig. 1 - Total mercury concentrations in hair vs fish consumption -





region of Vila Nova (State of Amapá)



Fig. 3 - Total mercury concentrations in hair vs fish consumptionregion of Tartarugalzinho (State of Amapá)

Conclusions

The concentrations of total mercury in the thirteen tribes analyzed, living in the Xingu Indian Park, were significantly higher than the control population, and the geometric mean varyied from 3.2 to 21 mg/Kg Hg.

In six of the Indian tribes analyzed for methylmercury, it comprised almost the totality of mercury found in hair.

In the three localities analyzed in the State of Amapá, the total Hg concentrations were also much higher than for the controls.

There was a trend of increase of total mercury concentrations in hair with frequency of fish consumption.

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Mercury Environmental and Human Contamination in Brazilian Amazon; An Overview.

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A gold rush happen in South American Amazon during the last 20 years. Artisanal gold mining was first happening mainly in Brazil and nowadays is more spread over other countries as Venezuela, Colombia, Bolivia, French Guyana, Guyana, Ecuador and Peru since the 1980's. Mercury was extensively used in Spanish South America during colonial time along nearly 300 years, as total amount by a factor of 70 times more than in the recent mining activities. But now environmental and human circumstances are quite different. Piscivorous fish has been frequently found downstream from gold mining areas with Hg concentrations usually higher than 0.5 μ g.g⁻¹ and with MeHg percentages usually over 80%. This was found also in fish from other main river basins in Amazon even some with no gold mining story. Daily fish intake in most of riverine communities easily exceeds 100g and then estimated ingestion doses also recommended monitoring programs. Hair-Hg levels also showed to be in many places higher than the established level that already offer risk for the foetus being formed and percentages of MeHg were also over 80%. Main studies from our group as well as from other more representative ones, on fish, human hair, air and human urine samples on major Amazon river basins are discussed.

Mercury in the Amazon: A Comprehensive Review with Special Emphasis on Bioaccumulation and Bioindicators

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ABSTRACT

About 13 million people in 55 countries worldwide are directly engaged in artisanal mining activities. Gold represents the main mineral extracted by rudimentary techniques. Poor mining practices generate enormous social and environmental problems in most developing countries. In Latin America as many as 1 million artisanal gold miners are emitting about 200 tonnes of mercury annually to the environment. The majority of these illegal or informal operations are in the Amazon region. Misuse of mercury to amalgamate fine gold is an insidious occupational hazard for miners and for the environment; only recently have other sources of mercury been recognized. Forest fires, erosion of the river banks, flooding, vegetation and soil degassing are also responsible for releasing or mobilizing mercury into the Amazon environment. Assuming that the average atmospheric deposition rate from all sources of mercury emission the Amazon is between 10 and 16 $\mu g/m^2/a$, in an area of 5 million km², the Brazilian Amazon alone has been receiving 50 to 80 tonnes of Hg/a from different sources. As a consequence, a number of monitoring programs have reported high levels of methylmercury in fish in areas not even disturbed by mining activities.

A better knowledge of the reactions of metallic mercury with organic acids from sediments and darkwater systems is a key step to understand the factors which catalyze the methylation process. The mechanism in which organic complexes are directly bioaccumulated or transformed into methylmercury is still unknown. An understanding of mercury sources and methylation reactions are important to support the scientific background of the problem and establish solutions.

This paper raises some key points that must be addressed to understand mercury bioaccumulation in the Amazon. Specific issues evaluated include: different sources of mercury emission; Hg cycle components which are still poorly understood; the influence of organic matter on complexation of metallic mercury and its subsequent bioaccumulation. Earthworms are suggested as simple and effective bioindicators for fast, inexpensive assessments of the bioavailable species of mercury in contaminated soils. The methodology is discussed and results of preliminary tests with Hgcontaminated soils and synthetic Hg-tannate complexes are presented.

Keywords: mercury bioaccumulation, bioindicators, earthworm, artisanal mining, gold mining

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INTRODUCTION

Artisanal Gold Miners

Artisanal mining is the main environmental and social problem related to mining activities in developing countries. The economic structure of artisanal miners is not different from any other capitalist activity. The driving force for artisanal mining is maximum profit with minimum investment. However, the environmental damage caused by this attitude is now being recognized. Most people in developing countries become miners to escape complete social marginalization as a result of poor rural policies established by Governments (Veiga, 1997). Artisanal mining activities are usually an island of prosperity in a sea of poverty. The existence of informal mining is largely due to poverty, lack of alternative employment and a "get rich quick" mentality (Suttill, 1995).

In 1993, it was estimated that about 6 million of the world's 30 million mineworkers were engaged in artisanal mining in more than 40 countries extracting over 30 different types of mineral substances (Noetstaller, 1995). The International Labour Organization estimates that currently the number of artisanal miners is around 13 million (Table 1) in 55 countries and rising, which suggests that 80 to 100 million people worldwide depend on this activity for their livelihood (ILO, 1999). Due to its unique characteristic of being easily sold and not subjected to monetary instability of local Governments, gold is by far the main mineral being extracted.

Continent	Number of Miners
	(million)
Asia/Pacific	6.7 - 7.2
Africa	3.0 - 3.7
Latin America	1.4 - 1.6
Developed countries	0.4 - 0.7
Total	11.5 - 13.2

Table 1 - Employment in artisanal mining (source ILO, 1999)

A wide range of mining and mineral processing activities are classified as <u>artisanal mining</u>. This ranges from individual panning to large dredging operations. Quite often the terms artisanal and peasant miners are applied to make reference to low-tech manual panners. Even in large-scale operations, most of those miners do not follow conventional technical approach adopted by organized mining companies. The manner in which the work is carried out is the most significant identifying factor. Artisanal miners work based on instinct, need for feeding his family and paying bills. There is no previous "classical" geological exploration, no drilling, no proven reserves, no ore tonnage establishment and engineering studies (Table 2). The concept of survival is the constant, driving force for those miners.

Conventional Mining	Artisanal Mining
geology, drilling	feeling, testing
reserves	subsistence
engineering	curiosity, feeling
control	results
feasibility study	pay bills
sophisticated equipment	homemade devices

Table 2 - Main characteristics that differentiate Conventional Mining from Artisanal Mining

The term <u>artisanal</u> miners generally encompasses all small, medium, large, informal, legal and illegal miners who use <u>rudimentary</u> processes to extract gold from secondary and primary ore bodies. In many texts and legislations, the term "small-mining" is used to describe this type of activity but in fact, there are many mills and dredges in the Amazon with the capacity to process 3 to 4 million tonnes of ore. History has shown that without technical support and investment, primary ores are the worst nightmare for artisanal miners. So, artisanal activity is "naturally" controlled by the type of ore deposit.

The establishment of constant gold production by artisanal mining is a difficult task. Production numbers fluctuate considerably due to the illegal nature of the operations, existence of foreign currency black market and money laundering. In Latin America, virtually all countries have artisanal gold mining activities. It is estimated that as many as 1 million artisanal miners are currently mining for gold in Latin America and their production can be as high as 200 tonnes (6.4 Moz) of gold annually (Veiga, 1997). According to Gold Fields Mineral Service Ltd. (1999), the world gold production in 1998 totaled 2555 tonnes The contribution of artisanal miners is not reported but this might range from 500 to 800 tonnes/a.

Use of Mercury in Artisanal Gold Mining Operations

Although the use of mercury is illegal in most countries, mercury amalgamation is the preferred method employed by artisanal gold miners. When used correctly, mercury is an effective, simple and very inexpensive reagent to extract gold (1kg of Hg costs 1g of Au). Despite this, some miners are using cyanidation (*e.g.* Ecuador, Bolivia). A variety of mining and amalgamation methods are used in artisanal mining operations. The extent of mercury losses from a specific site is defined by Au-Hg separation procedures; mercury often is discharged with contaminated tailings and/or volatilized into the atmosphere.

A common practice in many countries is to amalgamate the whole ore, either spreading mercury on the riffled concentration boxes or by using the old plate amalgamation method. In a few places where hydraulic monitors are used, some miners spread large amounts of mercury on the ground with the belief that the "quicksilver" will move on the dirt to catch all gold available. Amalgamation actually occurs after, when the riffled sluices retain mercury droplets and gold specks pumped with the ore. This gives the impression that gold is amalgamated on the ground. When this crude method is applied, the losses can be higher than 3 parts of mercury lost to 1 part of gold produced and the chance to trap gold is remote. Nowadays, several miners are amalgamating only gravity concentrates. This is an important evolution in artisanal mining methods, enabling significant decreases in Hg consumption and emissions.

When gravity concentrates are amalgamated, the mineral portion is separated from the amalgam by panning either in water boxes or in pools excavated in the ground or at creek margins. The heavy mineral-rich amalgamation tailings frequently contain 200 to 500 ppm of residual mercury, which creates "hot spots" when dumped into adjacent water bodies. In dredging operations, amalgamation is done on board using a blender and amalgamation tailings are steadily dumped into the rivers.

The most common method applied to remove excess mercury from amalgams is by manually squeezing it through a piece of fabric. The remaining amalgam usually consists of about 60% gold. When the amalgam is centrifuged the mercury content in the amalgam drops to 20-30%. Only a few miners in Venezuela are using this centrifuging method.

Once the amalgam is obtained, it is retorted or simply burnt in pans. Retorts can be used to capture volatilized mercury and condense it, allowing the mercury to be recycled. This leads to Hg recovery above 95% and significant reductions in air pollution and occupational exposure. There are many types of retorts. Some are made with stainless steel while others use inexpensive cast iron. Mercury losses during retorting depend on the type of connections or clamps used. Unfortunately, the usual practice to separate Hg from gold is to burn the amalgam in a pan or shovel with a blowtorch. When this happens, Hg is accumulated in the miner's lungs, as evidenced by high Hg content in urine.

In some countries, miners recover mercury from amalgams through dissolution in nitric acid. Mercury can then be precipitated from solution using an aluminum or zinc wire. The major problem with this technique is the fact that, after precipitation, the solution still has some mercury and must be treated before disposal. Unfortunately, this never happens. In addition mercuric nitrate fumes are highly toxic. Human beings have a tolerance of only 0.05 mg per cubic meter of air for the prevailing compound in the process, mercury pernitrate - $Hg(NO_2)_2.H_2O$. A very serious risk is also present when mercury pernitrate contacts alcohol as fulminate (Hg (CNO)₂) can be produced. This compound explodes readily when dry and is used in blasting caps and detonators. Currently, gold miners in some parts of the world, such as Colombia are not precipitating mercury from nitrate solution. They simply discharge all mercuric solution into the water streams. Mercury in this form is readily available to be biotically or abiotically methylated.

When the amalgam is retorted, a gold doré is obtained. This is sold in villages to gold shops that melt the gold to get rid of some impurities before paying the miners. In fact, the doré still contains about 20 g of mercury per kg of gold, which is later released when gold is melted. This operation is usually carried out by the gold buyers under the miner's supervision. Mercury levels in the interior of these shops are extremely elevated. Fume hoods used for this are usually very rudimentary, consisting only of a fan which blows the mercury vapours out into the urban atmosphere. The exposure to mercury vapour creates an extremely serious hazard to innocent people living in cities near those gold dealers. In the video documentary "The Price of Gold", the BBC (1993) profiled a case of severe mercurialism in the Amazon caused by vapours emitted from a gold shop. A 60 year-old citizen who lived in an apartment on top of a gold shop received toxic vapours for 10 years. As a result, his neurological functions were dramatically reduced and he suffered from extreme muscle tremors.

When gravity concentrates are amalgamated properly (*i.e.* when retorts are used and gold is melted in adequate furnaces), mercury emissions are almost insignificant (Table 3). An example of effective and creative amalgam processing is currently being applied in Venezuela, where Amalgamation Centers have been constructed to increase gold recovery and reduce mercury emissions. Miners bring their gravity concentrates to these private or state-owned centers to be properly amalgamated, retorted and melted by specialized operators (Veiga and Beinhoff, 1997).

Amalgamation Method	Hg lost : Au produced
Whole ore	3
Concentrates, no retort	1
Concentrates, with retort	0.001

Table 3 - Mercury losses depend on the amalgamation method

In summary, mercury emitted by miners includes both the fraction lost to the atmosphere when the amalgam is inappropriately burned *and* the portion discharged with amalgamation tailings into aquatic environments. As much as 80% of the mercury initially introduced during amalgamation is

lost to the atmosphere when no retort is used (CETEM, 1989). As well, the amount of mercury dumped with tailings is more significant when the whole ore is amalgamated.

Mercury Emissions in the Amazon

Gold mining activities are not the only source of mercury emissions. Other sources of mercury are usually underestimated in tropical environments. As mercury is extremely volatile compared to other metals, the atmosphere is a key compartment to be investigated. Some other natural and manmade sources of Hg emission and/or mobilization in the Amazon are listed below:

- geologic weathering and erosion
- evaporation from waters and soils
- run-off waters
- ancient gold and silver mining
- plant transpiration and decomposition
- waste incineration
- forest fires
- diffuse emissions

Soils are important sinks for atmospheric mercury deposition from all sources. Roulet and Lucotte (1999) studied the importance of soil erosion as a form to carry Hg from lithogenic and anthropogenic sources associated to particulate matter into the aquatic systems in Amazon. Organic matter can play a significant role in solubilizing mercury in these environments (Melamed, 1997)

Forest fires are believed to mobilize Hg contained in biomass and redistribute it into the atmosphere, either as vapour or attached to particulate. Today, with the high rate of deforestation by fire in South America, Hg emissions derived from wood combustion must be significant. The amount of Hg annually emitted by deforestation in the Amazon has been estimated between 8 and 80 tonnes of Hg (Veiga *et al.*, 1994; Lacerda 1995). To a large extent, the estimate depends on the biomass distribution, the area burned and Hg levels in plants and organic matter (ranges from 0.02 and 0.3 mg/kg). Regardless of differences in emission estimates, the significance of the forest fire as a vector for Hg emissions in Amazon is indisputable. Concentrations as high as 1,000 mg/kg Hg were measured in smoke particles smaller than 2.5 μ m in a forest fire in Amazon (Kaufman *et al.*, 1992).

In a comprehensive review, Porcella (1995) describes the mercury emission and deposition rates in the North and South Hemispheres. He believes that much of the background emissions are in the form of elemental Hg (Hg°) that evades from water surfaces, soils and vegetation. Conversely, forest fires and other high temperature emissions are likely to emit at least partially oxidized Hg in particulate and gas-phase forms of Hg. Upon evaluation of data compiled from different sources, the author estimates that the global emission of mercury from all sources is between 5000 and 6000 tonnes/a. Porcella also estimated that the mercury deposition rate in the northern hemisphere ranges form 11 to 14 μ g/m²/a and in the southern hemisphere, where industrial activities are less intense, from 5 to 7 μ g/m²/a. In wet conditions, such as in forested areas, mercury deposition rates can double. In the central part of Brazil, von Tumpling et al. (1996) estimated a deposition rate of 67 to 151 μ g/m²/a associated with mining activities and grassland fires. Lacerda and Marins (1997) considered the mercury deposition rate in the Amazon around 16 μ g/m²/a, particularly near mining activities. In recent work using lake sediment cores, Lacerda et al. (1999) estimate the current Hg deposition in the Amazon basin ranges from 10 to 12 µg/m²/a. Fosberg et al. (1999) analyzed Hg in rain water and estimated the annual deposition of Hg in the Negro River basin, a region with very little mining influence, as 14.7 μ g/m²/a. Assuming the average deposition rate from all sources of mercury emission the Amazon is between 10 and 16 $\mu g/m^2/a$, in an area of 5 million km², the Brazilian Amazon alone has been receiving 50 to 80 tonnes of Hg/a from different sources.

It is not clear if all these calculations have taken into consideration the high levels of mercury emitted by artisanal miners in the Brazilian Amazon, but experts believe that about 3000 to 4000 tonnes of mercury were emitted by miners to the environment over the last 2 decades. In the same period it is estimated that 5,000 tonnes of Hg were emitted in all of Latin America by artisanal miners (Veiga, 1997). Most calculations of mercury emission from gold miners are extremely rudimentary but are usually based on the regional ratio of Hg_{emitted} : Au_{produced} = 1. Based on a similar ratio, Lacerda and Marins (1997) estimated that the annual mercury emission from mining activities in Brazil should be around 78 tonnes/a. Due to the scarcity of easily exploitable ores, the low price of the metal and high operating costs, artisanal gold activities have declined since the beginning of the 90's. Consequently, it is very difficult to establish the current gold production from artisanal miners and thus the amount of Hg being emitted.

Whether or not all mercury emitted to the atmosphere by miners travels long distances or is deposited near the emission source is a controversial point. According to Marins *et al.* (1991), the majority of Hg emitted from 32 gold smelting shops is deposited near the emission source (*i.e.* within 1 km). In Alta Floresta, a town in the South of the Amazon Basin, neither air analyses nor soil samples up to 500 m from gold shops show significant Hg concentrations in samples analyzed (CETEM, 1991). A simulation model of mercury emissions from gold shops in the same town concluded that Hg concentrations in air decrease quickly with distance (< 2km) from the source (Artarxo *et al.*, 1999). Even when deposition is near the source, mercury from miners and gold shops can be re-emitted when a fire is ignited (Fig.1). The use of fire to clear forests or to control pest on pastures is actually a common practice in the Amazon. No detailed speciation has been conducted to characterize the forms of Hg being emitted from miners and gold shops.



Fig. 1 - Forest fires redistributes Hg emitted by amalgam-burning

The recent discovery of water-soluble species of mercury in the atmosphere, named reactive gaseous mercury (RGM), has heightened concerns of toxicologists. Source measurements have indicated that RGM is formed in combustion processes (Lindberg, 1999). The nature of RGM is believed to consist of one or more simple Hg (II) compounds, such as HgCl₂. In Tennessee, the RGM form of mercury represents 3 to 5 % of the total gaseous mercury in the atmosphere (Lindberg and Stratton, 1998). In Florida, this species of mercury represents the dominant form of total mercury in the atmosphere associated with dry deposition (S. Lindberg - personal communication). Lindberg and Stratton (1998) indicated seasonal trends might exist in RGM concentrations; this

variability was primarily associated with temperature, solar radiation, O_3 , SO_2 , and TGM. This research also suggested that vegetated areas may act as important sinks of RGM and, due to the high water solubility of the compounds, rainfall events are significant to RGM's removal from the atmosphere.

It seems reasonable to believe that most mercury emitted by miners is in Hg^{\circ} form and the majority of this is deposited near the source. It is difficult to predict if a small portion of gaseous Hg^{\circ} travels long distances, as the rainfall in the mining areas of the Amazon region is seasonal. From November to March the monthly precipitation is usually 100 to 300 mm and from June to August <10 to 30 mm. Reactive gaseous mercury might have a significant role in mercury deposition from other emission sources such as forest fires or other diffuse forms. However, little is known about how Hg^{\circ} emitted by miners can be transformed into RGM and what is its relation to fish contamination in areas with no influence of gold mining.

Mercury Bioaccumulation in Darkwater Systems

The first evidence of mercury bioaccumulation in Amazonian fish was reported in 1984 by the Jacques Cousteau Society as a result of an expedition of the scientist to Serra Pelada in 1982 (Hacon, 1990). In 1991-92, an international team comprised of Brazilian and British scientists analyzed blood and urine from residents of Jacareacanga, an area not directly influenced by mining activities (Fig. 2). Fish is the main diet of this community, which is located 250 km upstream of the Tapajós River where "garimpo" activities in the Itaituba region are abundant. Considering normal Hg blood levels range from 6 to $12 \mu g.L^{-1}$ (Krenkel, 1971), the gravity of the situation is evident.



Fig. 2 - Hg in blood and urine of fish-eating people from Jacareacanga. (from GEDEBAM, 1992)

A number of monitoring expeditions took place in the following years. Many studies have established the extent of Hg contamination in fish in the Amazon region. Levels higher than 0.5 ppm Hg are mainly encountered in carnivorous species. Albert Rogerio B. Silva (personal communication), ex-director of the Secretariat of Industry, Commerce and Mining from State of Pará, gathered results of 8333 samples of sediments, water, and biological subjects from at least 30 research institutes from 10 different countries. Many of these studies determined that methylmercury is the main form of mercury found in the aquatic biota, constituting more than 70% of total Hg in muscles.

It has been recognized that methylmercury is mainly produced in sediments and subsequently released into the water column where it is rapidly accumulated by biota (Jensen and Jenerlov, 1969; D'Itri, 1972). The methylmercury production rate seems to primarily depend on (a) mercury complexing characteristics, (b) microbial metabolic activity of the sediment and (c) total inorganic mercury concentration in the sediment (Bisogni and Lawrence, 1975). The availability of Hg (II) in an environment is generally regarded as a limiting factor to the formation of methylmercury by biotic processes. A radiometric method, originally developed by Canadian scientists in the 80's, was adapted to tropical conditions by Guimarães *et al.* (1995) to determine the rate in which 203 HgCl₂, as a source of Hg (II), is methylated in a sediment or in other substrates, such as aquatic plant roots (Guimarães *et al.*, 1998). In the Amazon, higher methylation rates (10^{-2} %.g⁻¹.h⁻¹) were found in organic rich sediments in dark water forest streams than in rivers with cloudy or clear waters. High methylation rates have commonly been associated with low pH characteristic of organic sediments and dark waters (Lacerda *et al.*, 1995).

Some authors believe that the pH of sediments has less influence on methylmercury production, than the distribution of methylmercury between sediments and the water column (Miller and Akagi, 1979). A decrease in pH of one or two units has been found to double the amounts of Me-Hg released from sediment into the overlying water, but not actually stimulate methylation. As well, field observations have shown more Hg accumulation in fish living in acidic waters in Canada, Sweden and Finland (Verta, 1986; Lindqvist *et al.*, 1991; Andersson *et al.*, 1995; Hintelmann *et al*, 1995). In many areas in the Amazon basin, far away from mining activities, fish samples have shown high levels of mercury (CETEM, 1991; Fosberg *et al.*, 1999; Fadini and Jardim, 1999; Brabo *et al.*, 1999; Kehrig and Malm, 1999; Meili *et al.*, 1999). High levels of Hg has been encountered in fish from acidic dark waters (Nakazono *et al.*, 1999) confirming previous predictions (Veiga, 1994; Tromans *et al.*, 1996; Meech *et al.*, 1998).

It is well known that dissolved organic matter (say fulvic acid) forms more stable and predominant complexes than any of the inorganic species (Duinker, 1980; Xu and Allard, 1991). The presence of fulvic acids (FA) is an important parameter that enhances solubility of organic matter and associated mercury. Schnitzer and Kerndorff (1981) have shown that over a large range of pH (4 to 9) when more than 20 ppm of FA is added to solution, Hg becomes very soluble. The authors pointed out that Hg interacts with fulvic acid in partly hydrolyzed forms. Melamed *et al.* (1999) experimentally demonstrated that humic acid solutions increase the solubility of metallic mercury but the presence of calcium ions inhibits Hg solubilization.

When organic acids contact metallic mercury, in interstitial waters for example, soluble complexes are formed at lower Eh levels than those observed in the Eh-pH diagram for inorganic soluble mercury species (Tromans *et al.*, 1996). As oxygen is likely the main electron donor in the complex formation reaction, Hg oxidation is controlled by oxygen diffusion in water. So, when Hgcontaminated sediments ("hot spots") exist in shallow creeks with considerable dissolved oxygen available or atmospheric Hg is deposited on top of organic soils the possibility of formation Hg-rich soluble complexes is high. The run-off waters can easily transport these contaminants to water streams. For deep sediments, available oxygen is likely to be extremely low and non-replenished (Meech *et al.*, 1998). The formation of Hg-organic complexes when reactive gaseous mercury (RGM) is deposited in darkwater systems may be a significant mechanism worthy of detailed investigation. However, no information is currently available on this matter. It seems reasonable to assume that the RGM-organic matter reactions occur preferentially in the water column and less intensely in the sediments, although the residence time of these complexes in the water is not known. Currently, most studies addressing interactions of Hg with organic matter focus on understanding the chemistry and bioaccumulation of these Hg-organic complexes.

How these Hg-organic complexes transform into methylmercury is unclear. Since fulvic acids are known to be methyl-group donors, methylation of these complexes seems to be feasible through either biotic or abiotic processes (Mannio *et al.*, 1986; Verta *et al.*, 1986). The soluble Hg complexes can also be adsorbed by colloidal organic matter, which serve as substrate for methylating bacteria.

An intriguing aspect that deserves special attention is the potential for direct bioaccumulation of these Hg-organic complexes. Since most Hg found in fish flesh is already methylated, if these complexes are directly bioaccumulated into invertebrates (Fig. 3) or in fish, then methylation may be occurring in the intestines of the organisms. Rowland *et al* (1977) showed that Hg (II) ingested as a chloride can be methylated in less than 20 hours by intestinal bacteria. They estimated that the total methylmercury synthesized from ingested inorganic mercury in man is approximately 0.4 mg/day. No information is available on intestinal methylation of Hg-organic complexes.



Fig. 3 - Simplified diagram indicating the possibilities of transformation of metallic Hg into Hg-organic complex and its bioaccumulation

Mercury Bioindicators

All studies in the Amazon have shown that carnivorous (piscivorous) fish accumulate more Hg than other species; however, it is difficult to compare Hg levels in fish from different sites due to different migration habits of species. Another problem is that many carnivorous fish have omnivorous habits and consume other sources of food, such as seeds. Black piranhas (*Serrasalmus rhombeus*) seem to be an ideal bioindicator as 80% of their diet is fish-based, they do not make long migrations, and they mainly live in quiet waters (Goulding, 1980). Unfortunately, black piranha is not found in all areas of the Amazon.
Roulet *et al.* (1999) have found that some carnivorous fish (tucunaré-*Cichla ocelaris*, traíra-*Hoplias malabaricus* and piranha caju-*Serrasalmus nattereri*) from the Tapajós River show very good correlations between Hg content, weight and standard length. Consequently, these researchers believe it is possible to use some of these species as bioindicators of Hg contamination from different Amazonian sites. Rondon and Perez (1999) adopted the 250g *Hoplias malabaricus* as a bioindicator while studying 15 dams in the interior of Venezuela. Incidentally, these researchers found high concentrations of mercury in 7 lakes, 5 of which had no evidence of mining influence.

CETEM (1991) used aquatic snails as bioindicators in Poconé, Brazil. These freshwater mollusks (*Mariza sp.*), with a diameter from 5 to 15 cm, are herbivorous with low mobility and an enormous water filtering capacity. The test procedure consisted of analyzing a group of 15 of snails (30 grams as total wet weight) after they lived in cages for 15, 30, 45 and 60 days in contact with highly Hgpolluted ferruginous sediment. The organisms showed low incorporation of mercury over the days, probably because the Fe-rich sediments had adsorbed any reactive mercury.

Intuitively, the best bioindicator for methylmercury (Me-Hg) bioaccumulation is human beings; however, there are ethical issues associated with collecting biological samples from individuals as, in many cases, the donors do not have any knowledge of the results. Although hair analysis is affected by external factors, such as use of dyes and Hg° vapour exposure, the simplicity of sampling and analysis make it an amenable indicator for toxicological assessment of Me-Hg exposure. Assessments of Hg concentrations in blood and fish suggest that a direct relationship exists between the two. Clarkson (1973) compiled results from other authors which showed that, for a 70 kg individual, Hg in blood (ppb) = $0.95 \times Hg$ (mg) daily intake from fish. Swedish individuals, who are considered to have reached the equilibrium between dietary intake and body burden of mercury, exhibit a direct relationship between Hg in blood and hair. Hair values are about 300 times higher than blood, although this depends on which part of the hair is sampled (Nelson *et al.*, 1971). In this case, a correlation between Hg in the hair in ppm (H), mass of fish consumed daily in grams (W_t) and Hg concentration in fish in ppm (F) is approximately obtained: H = $0.285 \times W_f \times F$.

So, a 70-kg person consuming 200 g daily of "non-contaminated" fish containing 0.3 μ g/g Hg, as commonly observed in riverine populations of the Amazon (Barbosa *et al.*, 1995; Castilhos and Bidone, 1999), would be expected to consume 0.86 μ g of Hg per kilogram of body weight and may show around 11 ppm of Hg in hair samples. This is clearly an approximation since many site-specific variables must be taken into account. The time following fish consumption also plays an important role in Hg blood levels. The most recent guideline from Health Canada recommends as the allowable daily ingestion level something below 0.2 μ g of Hg per kilogram of body weight for women of child-bearing age and children and 0.47 μ g for men

The normal Hg level in hair is less than 6 ppm and signs of Me-Hg intoxication can be observed at 50 ppm. Methylmercury readily crosses placental barriers and is considered to be a developmental toxicant (Grandjean, 1999), thus hazardous effects to the fetus are possible when only 20 ppm is analyzed in the hair of pregnant women (Krenkel, 1971; Malm, 1991). Levels of 10 ppm must be considered as the upper limit for pregnant women (Skerfving, 1973). Many studies have shown levels of mercury above 50 ppm in riverine populations and Aboriginal people in the Amazon with fish dependent diets (Barbosa *et al.* 1997; Malm *et al.*, 1997; Kehrig *et al.* 1997). Methylmercury is usually above 70% of the total mercury analyzed in hair (Vanconcellos *et al.* 1999).

Bioindicators play an important role in identifying the factors controlling Hg toxicity and bioavailability and can ultimately be used to evaluate hazards where Hg pollution is present. Recent

studies have demonstrated that the bioavailability of metals in terrestrial and aquatic systems is dependent upon a number of geochemical and biological factors. The presence of organic matter (Gagnon and Fisher, 1997, Standley, 1997), colloidal particles and certain minerals, such as sulphides (Melamed, 1997) or Fe, Mn oxides (Gagnon and Fisher, 1997), influence speciation and/or sorption mechanisms and therefore bioavailability of various metal compounds (Benoit *et al.*, 1999; Wen-Xiong *et al.*, 1998). Organism physiology, internal solubilization capabilities (Gagnon and Fisher, 1997), food quality (*i.e.* nutrients) and feeding behavior also affect the assimilation efficiency of a metal (Lawrence *et al.*, 1999, Wen-Xiong *et al.*, 1998). Thus, an appropriate bioindicator organism must be reasonably well understood in terms of biological qualities and responses to be broadly applicable to various external (*e.g.* geochemical) conditions. Earthworms may be a viable alternative to traditionally applied organisms (*e.g.* fish, people) as they are simple, well-studied creatures that can provide indications of bioavailability in a short time frame at relatively low costs.

Earthworms as Bioindicators

Substantial evidence indicates that earthworms accumulate heavy metals from polluted soils and other media (Edwards and Bohlen, 1996; Goats and Edwards, 1988; Rhett *et al.*, 1988; Neuhauser *et al.*, 1985; Ireland, 1983). Earthworms are particularly suitable for the assessment of contaminant bioavailability for a number of reasons. They ingest large quantities of soil and are in full contact with the substrate they consume. They constitute up to 92% of the invertebrate biomass of soils and participate in many food chains, acting as a food source for a wide variety of organisms including birds, fish, insects, various mammals, and reptiles (Ireland, 1983; ASTM E1676-95). In addition, they are easily bred, have been extensively studied, and are approved for use in toxicity testing by the US EPA, the European Economic Community and the Organization for Economic Cooperation and Development (ASTM E1676-95). Despite these factors, little information exists concerning the uptake of Hg and MeHg in these organisms. Very few studies (Braunschweiler, 1995; Rhett *et al.*, 1988; Marquenie and Simmers, 1988; Martin and Coughtrey, 1982) have documented Hg concentrations in earthworm tissues and even fewer (Lawrence *et al.*, 1999; Yongcan *et al.*, 1998; Beyer *et al.*, 1985) have addressed the biological and physiological elements that influence Hg bioavailability in these organisms.

In this paper, we present a methodology using the earthworm Eisenia foetida, commonly known as the barnyard or tiger worm, to evaluate the bioavailability of Hg in both tailings and aqueous solutions. This methodology can also be applied to soils and sediments, as well as a wide range of inorganic and organic contaminants. Results indicate that E. foetida are capable of accumulating Hg and a positive correlation exists between Hg concentrations in worm tissues, the substrate they consume and the length of exposure (a dose-response relationship). Depuration (i.e. post-exposure starvation) time was also compared to ensure analytical results were indicative of Hg in tissues and not material retained in the gut. In addition, this research program investigated the effect of natural organic acids as mediators of Hg bioavailability. Two series of tests were conducted wherein metallic Hg was dissolved in tannic acid and "fed" to the worms in a substrate of paper and silica sand. Total Hg and MeHg were analyzed to assess whether methylation of Hg was occurring in the substrate, directly within the worms (e.g. in the intestines), or in the tannic acid-Hg solution. The results of analysis revealed that the ratio of MeHg:Total Hg was up to 160 times higher in worm tissues than both the tannic acid-Hg solution and the substrate at the conclusion of the test periods. This result is particularly important in darkwater systems, such as the Amazon, where naturally occurring organic acids may be facilitating methylation internally within organisms. An additional series of jar tests was completed wherein worms were put in direct contact with heavy metal laden tailings. Mercury and other metals (e.g. Cd, Pb, Zn) in this media were in a sulphide form and were consequently not bioavailable.

METHODS AND MATERIALS

Organism Culturing and Selection. E.Foetida were initially acquired from a local composting cooperative and cultured in a dark plastic, ventilated bin on a diet of either alfalfa pellets or a mixture of vegetable food waste. Worms were hand-selected for testing on the basis of sexually maturity, as evidenced by the presence of a clitellum, size (0.25 to 0.3 g wet weight), and liveliness. Prior to use, the chosen worms were stored for 24 hours on damp filter paper to void their gut contents.

Tannic Acid-Hg Jar Tests. Two separate series (TA1, TA2) of these tests were conducted. Metallic mercury (TA1: 3.03 g and TA2: 6.18 g) was added to 0.005M tannic acid (0.3 and 1.0 L volumes) and vigorously stirred for one (TA1) and three (TA2) days to promote dissolution. Total Hg concentrations in the tannic acid solutions were 696 ppb (TA1) and 1150 ppb (TA2). Prior to full scale testing, 3 to 5 worms were exposed to the pure tannic acid solution (pH 4.1) in Petri dishes to provide some indication of acute responses. Most specimens died within two hours. Subsequent adjustment of the pH (to 5.85 and 6.02, respectively) enabled long term habitability for the worms.

For the full-scale tests, 25g of shredded, kaolin-based paper and 175g (TA1) or 100g (TA2) of fine silica sand were added to nine 500mL acid washed, glass jars. The pH adjusted tannic acid-Hg solution (80 mL) was then added and jars were manually shaken to homogenize. Groups of 25 to 30 worms were weighed and added to each jar. The populations were left relatively undisturbed for the duration of the tests (14 or 28 days). Two additional jars were completed without silica, but the moisture content was inhabitable for the earthworms – all specimens in these jars died within 5 days. Silica sand not only retains some moisture, but is also used by worms for grinding during the digestion process.

At the conclusion of the exposure period, worms were removed from each jar, carefully washed and dried, counted and weighed. Observations such as motility, light sensitivity and physical qualities (*e.g.* discolouration) were documented to provide some indication of toxic responses. Cleaned worms were then placed in Petri dishes with damp filter paper for either 24 or 72-hour depuration periods, then re-washed and re-weighed. Worms from two jars (OCT99-7, OCT99-8) were kept in mixtures of 15g of clean paper towel and 50g of silica saturated with 50 mL of distilled water for a period of 5 days. Prior to analysis these worms were also depurated for 24 hours.

Nine jar tests were completed with the following experimental specifications:

- Two Jars: 14 day test, 24 hour depuration;
- Two Jars: 14 day test, 72 hour depuration;
- Three Jars: 28 day test, 24 hour depuration;
- Two Jars: 28 day test, 5 day clean paper feeding, 24 hour depuration.

Sample Preparation and Analysis. Following post-depuration washing and drying, worms were placed in 250ml, acid washed Erlenmeyer flasks and digested in 20 mL of 0.7M nitric acid. Distilled water was then added until a volume of 120 mL was reached. Samples were split into 60 mL volumes, poured into acid-washed polyethylene containers and promptly frozen. One of the two samples was kept as a duplicate and the other submitted for total Hg (wet weight) analysis by CVAA. Samples submitted for MeHg analysis were not digested but frozen immediately following post-depuration washing and drying. Methylmercury was analyzed by Cebam Analytical Inc, Seattle, Washington, US.

Total Hg and MeHg in the tannic acid-Hg solution was analyzed directly by the aforementioned methods. At the conclusion of the 28 day test period, substrate material (*i.e.* tannic acid saturated paper and silica sand) was leached with distilled water. Leaching involved the addition of 300 mL of distilled water to 178g of substrate. The combined material was shaken vigorously for several minutes. Leachate (198 mL) was subsequently extracted using a vacuum filter and submitted for MeHg and total Hg analysis.

Hg-rich Mine Tailings Jar Tests. Earthworms were put in direct contact with heavy metal laden mine tailings from British Columbia to assess uptake of various metals including Hg. Tailings were combined with different media in three 900 mL jars as follows:

- 175g tailings, 25g shredded brown paper, 1g bread yeast;
- 175g tailings, 25g shredded brown paper;
- 175g tailings, 25g peat, 10.3g CaCO₃.

Peat was used as a source of organic acid to investigate its influence on metal availability. Deionized water (125 mL) was added to each jar. Like the tannic acid-Hg jar tests, worms were prepurged and cleaned, then placed in the substrate for periods of 16 and 29 days. Digestion and total Hg analysis of worms were conducted as described above. Full metals scan was also conducted using ICP-MS.

RESULTS AND DISCUSSION

The worms not exposed to any solution but cultured in the same medium as the test worms as well as the control worms which underwent the same test protocols, but fed a solution of tannic acid alone (<0.5 ppb Hg), had tissue concentrations below 40 ppb (Table 4). In the tannic acid-Hg jar tests, Hg concentrations in worm tissues ranged from 0.7 to 7.3 ppm. Bioconcentration factors, or the ratio of Hg concentrations in worm tissues to the substrate, averaged 3.7. The most bioconcentration occurred in populations which exhibited toxic responses to their environment (*e.g.* discolouration, impaired mobility). Conversely, lowest Hg concentrations can be primarily attributed to insufficient homogenization of the worms in the jars or the type of paper used. Successive tests intend to enhance mobilization of worms within jars and use a more absorbent paper capable of distributing solution more evenly. Table 4 summarizes results of the jar tests.

Sample No.	Test Description	Hg in Tissues (ppb)	Hg in Substrate (ppb)	Bioconcentration Factor
OCT99-1	14d, 24hr dep	2837	828	3.4
OCT99-2	14d, 24hr dep	2449	828	3.0
OCT99-5	14d, 72hr dep	3695	828	4.5
OCT99-6	14d, 72hr dep	1614	828	1.9
CAM99-1	28d, 24h dep	861	278	3.1
OCT99-3	28d, 24hr dep	2499	828	3.0
ОСТ99-4	28d, 24hr dep	749	828	0.9
ОСТ99-7	28d, 5d feed plus 24h dep	7305	828	8.8
OCT99-8	28d, 5d feed plus 24h dep	4055	828	4.9
14d Average	-	2649	828	3.2
28d Average	-	3094	718	4.1
Overall Average	_	2896	767	3.7
Control worms	28 d, 24 hr dep	35	<0.5	• ·

Table 4 - Results of Tannic Acid-Hg Jar Tests

Although 35 to 40% more Hg was, on average, accumulated during the 28-day tests, the populations were generally healthier and more responsive than the 14-day jar tests. The average weight per worm actually increased for the 28-day populations. This overall improved condition could be due to the reduced stress associated with the extended acclimatization period. As healthier, less stressed organisms are supposed to be more indicative of natural uptake, the 28-day tests are recommended for implementation of this methodology.

Depuration times (24h, 72h, 5days plus 24h) were also compared during this study. The average worm weight loss was about 30% greater in the worms fed clean paper for 5 days following the test; despite this, Hg concentrations were up to 40% higher in these populations. It is possible that during the additional 5 days, Hg containing gut contents were more thoroughly assimilated than other tests. The overall weight decrease may be indicative of more complete purging. As shown in Figure 4, Hg concentrations in tissues generally increased with purging time. The physiological explanations for these responses are currently being studied in greater detail – results of subsequent test programs should provide more insight into this matter. A 5 day "clean" substrate feeding (plus 24 hour depuration) following a 28 day test provides the most reliable results.



Fig. 4 - Total Mercury Concentrations in Worm Tissues versus Purging Time

Total Hg and MeHg were analyzed to assess whether methylation of Hg was occurring in the substrate, directly within the worms (*e.g.* in the intestines), or in the tannic acid-Hg solution. The results of analysis revealed that the ratio of MeHg:Total Hg was up to 2400 times higher in worm tissues (32.2 ppb) than both the tannic acid-Hg solution (0.059 ppb) and the substrate (0.013 ppb). This result is particularly important in darkwater systems, such as the Amazon, where naturally occurring organic acids may be facilitating methylation internally within organisms. Despite this, MeHg (32.2 ppb) constituted only around 1% of the total Hg in worm tissues (from a 28-day test), which is considerably lower than measured values in higher organisms. It is possible that, as earthworms are consumed (*i.e.* as the Hg moves up the food chain), it is subject to further methylation internally within other organisms.

The mine tailings jar tests suggest that these organisms can also be used to characterize the bioavailability of a range of heavy metals. Among other metals, Hg, cadmium (Cd), lead (Pb) and zinc (Zn) were accumulated by earthworms, but not bioconcentrated over the course of 16 and 29-

day tests. For example, Hg in tailings was measured at concentrations up to 19.2 ppm, but only reached concentrations of 0.071 ppm in worm tissues. Bioavailability was likely inhibited as most metals, including Hg, were present in the form of sulphide minerals. The presence of peat in the jars did not significantly influence results. This methodology can potentially be applied to the assessment of bioaccumulation of metals from mining wastes (*e.g.* metals mobilization associated with ARD). This could be conducted in conjunction with kinetic tests for a more comprehensive evaluation.

CONCLUSIONS

Misuse of mercury to amalgamate fine gold is an insidious occupational hazard for miners and for the environment; other sources of mercury are currently being investigated. Forest fires, erosion of the river banks, seasonal flooding, vegetation and soil degassing, as well as other industrial activities are also responsible for releasing mercury into the Amazon environment. The recently discovered species of atmospheric Hg, reactive gaseous mercury, may play a significant role in transferring Hg to the aquatic environment. In fact, its has been recognized over many years that fish from darkwater systems accumulate more Hg than fish from other environments, in both the presence and absence of mining activities.

The reaction of metallic mercury with organic acids from sediments and darkwater systems is definitely an important pathway for mercury bioavailability. The mechanisms in which organic complexes are directly bioaccumulated or transformed into methylmercury require further study.

Bioindicators play an important role in identifying the factors controlling Hg toxicity and bioavailability and can ultimately be used to evaluate hazards where Hg pollution is present. The development of easily implemented, low-cost, less sophisticated methods can be beneficial for rapid diagnosis of potentially hazardous situations, particularly in regions such as the Amazon where resources are limited and pollution is widespread. Earthworms (*E. foetida*) are capable of accumulating Hg and other metals. A positive correlation exists between Hg concentrations in worm tissues, the substrate they consume and the length of exposure (a dose-response relationship).

The methodology presented can be easily employed within a short period of time at a relatively low cost. Thus, this procedure can be applied as a tool for the assessment of Hg and other metal bioavailability in polluted soil, sediments, tailings and liquid effluents. Future research intends to focus on three main areas. First, the influence of naturally occurring organic acids on the facilitation of methylation, particularly within organisms, will be investigated in greater detail. Next, the physiological mechanisms controlling Hg and other metal intake and the possible synergistic or antagonistic effects of other elements (*e.g.* selenium, calcium) will be addressed. Finally, correlations between *E. foetida* and the substrate they consume, as well as between worms and various well-studied fish species (*e.g.* tucunaré, traíra and piranha) will be quantified. As many correlations already exist between these fish and people, if a link can be established with fish and worms a "gap can be bridged" and the earthworms can be used to assess human health hazards in impacted areas.

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BIO-PHYSICAL-CHEMISTRY OF MERCURY IN THE TROPICS

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Abstract

The utilization of mercury for gold recovery in the tropics has been contributing with large inputs of the metal into the aquatic environment. Although, in this activity, mercury is utilized in its elemental state, which is relatively immobile and inert, the transformations to methyl mercury occurring in natural systems are leading to mercury contamination of aquatic organisms and the food chain. This paper reports: i) the effectiveness and mechanisms involved in the solubility of mercury in the presence of humic acid, which is an important component of dark river waters in the tropics; the significance of physico-chemical parameters affecting mercury methylation in Eichhornia crassipes roots of a freshwater lagoon, in Rio de Janeiro; the testing of physico-chemical amendments, in different environmental compartments, to verify their potential to mitigate mercury contamination. Results show that the solubility of elemental mercury is enhanced due to the presence of humic acid through a solubilization-complexation mechanism, which was attributed to the presence of acid sites at the humic acid molecule, mainly the carboxil group. Sulfide addition reduces chemical methylation of Hg(II), but enhances the solubility of Hg⁰. The use of oxides, phosphate, and organic matter may be effective in the immobilization of Hg(II), depending upon Hg speciation. Calcium was effective in counteracting the solubility enhancement of Hg⁰ promoted by the presence of Aldrich humic acid. The effects of temperature, pH and electric conductivity on net mercury methylation by the roots of Eichhornia crassipes are: methylation increased from 10 to 35 °C, and decreased thereafter. The process was completely inhibited at 90 °C. At pH values of 6 and 7 methylation was stimulated and a significant decrease was verified at pH 8. Increasing KClO₄ concentrations led to a significant decrease of the methylation rates, while for KCl and CaCl₂ solutions, only a slight decrease was observed.

Introduction

The use of elemental mercury (Hg^0) in gold recovery as well as in other industrial applications is of concern, because mercury (Hg) is highly toxic and accumulates in living organisms, as methyl mercury (CH₃Hg), which damages the central nervous system of humans. Some of the pathways of the mercury cycle, leading to Hg contamination are:

- Direct input of Hg⁰ into river waters
- Volatilization of Hg⁰ following burning of Au-Hg amalgam
- Binding of volatilized Hg⁰ to aerosol and transport
- Deposition of Hg⁰ in soils and waters
- Oxidation of Hg⁰ by ozone or radiation and deposition of Hg(II) in soils and waters

• Adsorption of Hg(II) to water suspended particulate matter and sedimentation

• Immobilization of Hg by anoxic layer sulfide

• Reduction of Hg(II) to Hg^0 mediated by radiation mediated by organic acids and volatilization at the water air interface

• Solubilization of Hg⁰ through organic acid induced dissolution-complexation

• Leaching and transport of Hg(II) through the soil matrix leading to groundwater contamination

• Methylation of Hg(II) to methyl mercury

• Dimethylation of methyl mercury and volatilization at the water air interface

Procedures to minimize Hg bioaccumulation include:

• Precipitation of Hg with *selenium*, owing to the low solubility of mercury selenide. Additions of selenide may also release methyl mercury from its linkage to proteins.

• *Liming* water systems which increases pH and conductivity, reducing Hg uptake through gills.

• Additions of *activated charcoal* to remove inorganic Hg and methyl mercury from solution.

Additions of hydrous ferric oxides which have good Hg fixation capacity.

• Additions of *organic materials* such as fibbers, rubber, etc.. which have different adsorption capacities for Hg

• Addition of *sulfides* to precipitate Hg as HgS.

Recent studies have demonstrated that methyl mercury formation may be much higher in the roots of floating aquatic macrophytes than in surface sediments. Because several regions in the Brazilian Amazon and in the Pantanal floodplain areas are widely colonized by macrophytes, higher methylation rates in this compartment have important ecological implications. Methyl mercury produced in floating macrophytes roots is bioavailable due to its rapid water diffusion and to the dense and diverse biota living nearby the macrophyte stands. Little is known about methylation in macrophytes roots, despite its relevance. Since macrophyte stands support a diverse biota, methyl mercury produced in their roots may be regarded as highly accessible to other organisms. Biomethylation in aquatic macrophyte roots is favored by the intense microbial activity. This is associated with a significant production and accumulation of organic matter by the roots, a large contact surface area together with optimal physico-chemical conditions for microorganism growth. Microbial communities quickly respond to environmental changes, resulting in different mercury methylation rates. Thus, physico-chemical parameters of the system are of major importance in determining methylation rates.

In this paper, we show data that demonstrate the influence of physico-chemical parameters on the mercury cycle and on the effectiveness of the methylation process by aquatic macrophyte roots.

Results

Sulfide Control on Hg(II) Methylation

Figure 1 indicates that the relative strong bond or new phase formation between Hg(II) and sulfide prevents mercury methylation as reflected by the decrease in the production of CH₃HgCl from Hg(II).



Figure 1. Effect of sulfide on the production of methyl mercury from HgCl₂ solutions containing methylcobalamin, as a methylating agent.

Although the results of this and other studies indicate the efficiency of sulphide in avoiding the transformation of Hg(II) to methyl mercury, the addition of S as a technological tool should be taken with caution, because it has implications on oxireduction reactions and can enhance the solubility of Hg⁰, with the production of polysulfides in solution (Figure 2).



Figure 2. Solubility of Hg⁰ in the presence of sulfide (HgT- total mercury; HgS- mercury polysulfide)

Use of oxides for the immobilization of Hg

Figure 3 shows the adsorption isotherms of Hg(II) in the two clays from Rio Preto, Rio de Janeiro, confirming the relative high affinity of these clays for complexation with Hg(II).



Figure 3: Adsorption isotherms of Hg(II) in the clays of Rio Preto, RJ.

Although the mineralogy of the two soils are similar, the relative higher sorption capacity of Hg(II) depicted by the Hydromorphic soil (Figure 3) was attributed to its much higher organic matter content (2.9% vs. 1.1%). It is pointed out that, at the system pH, the major Hg species present is $HgCl_2^{0}$, which has great affinity for organic matter and low affinity for mineral surfaces.

Effect of Speciation and Phosphate on Hg(II) Mobility

The effect of KCl and KClO₄ electrolyte systems and the interaction with phosphate on Hg(II) adsorption and transport on an Oxisol soil is demonstrated in Figures 4 and 5. In general, a much higher adsorption of Hg(II) resulted in the KClO₄ system as compared to the KCl system. These results are in agreement with other studies of Hg(II) interaction with pure SiO₂. The formation of mercury chloride complexes promotes a much lower interaction of Hg at the soil/solution interface.

The effect of the presence of P on the transport of Hg(II) through the Oxisol soil under KCl and KClO₄ electrolyte systems is also demonstrated in Figures 4 and 5. Phosphate has practically no effect on Hg(II) adsorption in the KCl system. However, P enhances the adsorption of Hg(II) in the KClO₄ system considerably, due to the favorable surface potential and negative charge created. The complexation of P to the oxide surface of the Oxisol promotes an increase in the negative charge of the soil reflected by a lowering of the PZC (point of zero charge) from a pH value of 4.7 to 3.



Figure 4. Effect of P on Hg(II) adsorption on Oxisol under different electrolytes.

As a result, as demonstrated in Figure 5, the retention of Hg(II) in the upper half of the soil column is much higher in the KClO₄ system than in the KCl system. Mercury moves deeper in the soil column when chloride is present, due to the formation of the mercury chloride complex. The mobility of Hg(II) in presence of P is decreased more effectively in the KClO₄ system.



Figure 5. Hg(II) transport through Oxisol soil column in KCl and KClO₄ systems.

Effect of Ca on the counteraction of humic acid-induced solubility of Hg⁰

Figure 6 shows that the increased solubility of Hg^0 promoted by the *Aldrich* humic acid was reverted in the presence of Ca. These preliminary results need further studies for development of such a technology in the presence of natural organic acids. Furthermore, the data in Figure 6 indicate that Ca prevents the dissolution of Hg^0 rather than a competitive complexation mechanism.



Figure 6. Effect of Ca-humic acid interaction on Hg⁰ solubility

 Hg^{T} = total mercury, determined by oxidizing all forms of Hg in the supernatant with bromine chloride (BrCl), before reduction with stannous chloride (SnCl₂).

 Hg^{A} = mercury comprising dissolved metallic and inorganic-Hg, determined by reduction of all Hg in the supernatant with (SnCl₂).

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

Effect of physico-chemical parameters on Hg methylation by the water-hyacinth *Eichhornia crassipes*

The <u>Eichhornia crassipes</u> species is widely distributed in equatorial and tropical regions, bioaccumulates contaminants from water, including mercury. It is extensively used in domestic and industrial effluent treatment. The large and compact root system of <u>E</u>. crassipes acts as a filter, trapping suspended materials from the water, such as fine particulate matter. This environment support a diverse biota with a large biomass, including abundant microbial populations.

The most important factors influencing biological mercury methylation are the inorganic mercury bioavailability and the nature of the microbial community present in an ecosystem. Both are influenced by physical and chemical parameters such as temperature, pH, salinity, organic carbon and redox potential.

Most methylation studies have been carried out in temperate regions, where there are significant temperature variations during the year, and this parameter seems to be an important factor controlling the process, since it directly affects microbial activity and chemical reactions. Summer temperatures were associated with higher methylation rates in temperate lakes. In our study, results indicate an increase in methyl mercury production up to 32 °C. Methylation decreased thereafter, and the process was completely inhibited at 90 °C, probably due to the suppression of bacterial activity (Figure 7). The Tukey test showed that methylmercury production obtained at the lowest (10°C) and the highest temperatures (50 and 90 °C) were significantly lower (p<0.05) than those obtained at the other temperatures. These data are in agreement with similar tests on the influence of temperature on methyl mercury production in sediments of the Amazon region, showing increased methylation in the 35 – 45 °C range. Higher mercury methylation observed in this temperature range is relevant especially for equatorial and tropical water systems, where temperatures up to 40 °C are frequently reached.



Figure 7: Methylmercury production, in samples of *Eichhornia crassipes* roots, as a function of the incubation temperature. Vertical bars represent the 95% confidence interval (triplicate samples).

Methyl mercury seems to be formed under moderately acid and neutral conditions (pH from 5 to 7) and dimethyl mercury is mostly formed under alkaline conditions. The data reported here shows that net mercury methylation was highest (p<0.05) in both treatments at pH 6 and 7. Less methylation was observed at more acidic and alkaline pH values (Figure 8). A decrease in mercury methylation at pH 8 was expected due to increasing demethylation in alkaline waters, reducing net methyl mercury concentrations.



Figure 8: Methylmercury production, in samples of *Eichhornia crassipes* roots, as a function of pH, adjusted using HNO₃.Vertical bars represent the 95% confidence interval (triplicate samples).

Studies regarding the influence of electrical conductivity on mercury methylation are scarce. The biota from black-water Amazonian rivers, which are characterized by low pH values and reduced electric conductivities, have higher methyl mercury concentrations than the organisms from white water rivers, characterized by higher pH and electric conductivities. Increasing KClO₄ concentrations led to a significant (p<0.05) decrease in mercury methylation (Figure 9), while for increasing concentrations of KCl and CaCl₂ solutions, only a slight decrease was observed (Figures 10 and 11). At the lowest concentrations (1 mM), methyl mercury formation in the KClO₄ system (23.5 %) was higher than in the other systems (18.6 % for KCl and 18.4 % for CaCl₂). Significant differences (p < 0,05) in Hg methylation were observed between KClO₄ and the other electrolytes used, but no differences were verified between KCl and CaCl₂. (p < 0,05).



Figure 9: Methylmercury formation in samples of *Eichhornia crassipes* roots incubated under different electric conductivities modified by KClO₄



Figure 10: Methylmercury percentages in samples of *Eichhornia crassipes* roots incubated under different electric conductivities modified by KCl



Figure 11: Methylmercury percentages in samples of *Eichhornia crassipes* roots incubated under different electric conductivities modified by CaCl₂.

Conclusions

This paper shows and discusses the technologies that use physico-chemical manipulations to counteract or mitigate mercury pollution.

Results indicate the paradoxical action on the use of sulfides, due to the presence of Hg^0 , and the importance of speciation and complex formation with respect to the use of oxides, phosphate, and organic matter on the immobilization of Hg(II). Preliminary tests also show that the presence of Ca may counteract the solubility enhancement, induced by humic acid.

The net mercury methylation promoted by the roots of *Eichhornia crassipes* increases from 10 to 35 °C, and decreases thereafter. The process was completely inhibited at 90 °C. At pH values of 6 and 7 methylation was stimulated and a significant decrease was verified at pH 8. Increasing KClO₄ concentrations decreases the methylation rates, while only a slight decrease was observed by increasing KCl and CaCl₂ concentrations.

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Neurologic Features of Chronic Minamata Disease (Organic Mercury Poisoning) Certified at Autopsy

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Abstract

To better understand the neurologic events related to chronic Minamata disease (organic mercury poisoning), we studied data from 77 patients with Minamata disease as certified at autopsies performed from 1976 to 1994 (mean age: 72.3 years). Major neurologic findings included: sensory impairment in 80.5% of the patients which was limited to the extremities in 42.9%. Impairment of lower extremity coordination was present in 35.8% of the patients, constriction of the visual fields in 28.8%, and retrocochlear hearing loss in 15.3%. There was no correlation between the degree of cerebellar incoordination and the methylmercury concentration in the cerebellum. Compared with the classic type of Minamata disease, the incidence of major neurologic findings was markedly decreased. In light of these findings, supplemental examinations including brain CT, MRI, SSEP, or tremogram may be necessory to clinically diagnose Minamata disease, especially in atypical or mild cases.

Key words: Minamata disease, autopsy, cerebellar incoordination, methylmercury concentration

Introduction

Minamata disease, the first mass methylmercury poisoning in history, was caused by the ingestion of seafood from methylmercury-contaminated areas of Minamata Bay and the neighboring seas in Japan (1, 2). Residents of that area first showed evidence of the disease in 1953 (3, 4). They experienced concentric constriction of their visual fields, hearing loss, tremors, cerebellar incoordination, and sensory impairment of their tongue and lips or extremities. These findings are consistent with the features of organic mercury poisoning first described by Hunter et al in 1940 (5) and Hunter and Russell in 1954 (6). One hundred and seventy-eight patients in Minamata fell victim to this disease from 1953 to 1962, 144 from 1963 to 1972 and 1,935 between 1973 and 1994. For over 40 years, our colleagues have performed studies on Minamata disease patients, with special attention directed towards new or shifting symptoms (7-9). To better comprehend the characteristic neurologic features of chronic Minamata disease of late-onset, we investigated the neurologic findings in patients who were examined by neurologists prior to death and at autopsy.

Subjects and Methods

Based on data obtained from the Kumamoto Certification Council for pollutionrelated injuries in patients, neurologic status was determined using data from 77 Japanese patients with Minamata disease, as certified by autopsies performed from 1976 to 1994. This cohort consisted of 55 men and 22 women aged 36 to 96 years (mean: 72.3 years). The cerebral pathology in these patients was assigned to one of six grades classified by Takeuchi and Eto (10-14). All of the cases were classified as Grade I except for 4 cases of Grade II. The decrease in the number of neurons ("thinning-out") in the cortex was <30% in Grade I. In the 4 cases of Grade II, a 30 to 50% decrease in the number of neurons was evident in the vulnerable areas of the cerebral cortices. Glial cells, particularly astrocytes and macrophages, were increased in "thinning-out" areas of neurons. Changes in the cerebellum were consistent with Grade I in the classification system of Takeuchi and Eto (13, 14), except for one patient in whom the loss of granule cells was relatively well-recognized and apical scar formation was also apparent. Irregularity of the Purkinje cell layer also was clearly recognized and there was a slight gliosis with Holzer staining in this irregular area. Histochemically, mercury was identified in nerve cells and macrophages in the brain In peripheral nerves, the posterior roots, particularly in the lumbar area, (15). demonstrated some degree of abnormality in all patients. Changes in the anterior roots were less than those in the posterior roots. Methylmercury concentrations in the cerebellum (vermis) were measured by gas chromatography in 45 patients, and the correlation between the cerebellar methymercury concentration and the degree of cerebellar incoordination was evaluated by determining the absence (=0), suspicion or presence (=1.0) of ten cerebellar signs including: dysarthria. (=0.5), dysdiadochokinesis, impairment in finger-to-nose test, heel-to-knee test, and shin tap test, standing disturbance, dysstasia affecting one leg, impairment in simple gait and tandem gait, and intention tremor. The incidences of major neurologic features between classic Minamata disease patients reported by Tokuomi et al (7-9) were compared with diseases certified by autopsy

Results

Table 1 summarizes the incidence of major neurologic features in these 77 patients. The cardinal symptoms of constriction and depression of the visual fields were present in 28.8% and 50.0% of the patients, respectively. Hearing disorders included definite or possible retrocochlear deafness with deterioration in speech-hearing and auditory fatigue in 9/59 patients (15.3%); probable labyrinthine deafness with recruitment was noted in 22/59 patients (37.3%). Impaired cerebellar coordination was infrequent, including adiadochokinesis and impairment on the finger-to-nose, heel-to-knee, and shin tap tests observed in 17.6%, 29.0%, and 35.8% of the patients, respectively. Findings suggestive of a sensory disorder were found in 62/77 patients (80.5%). These sensory disorders included: a "glove and stocking" type of

hypesthesia (extremities type) in 33 patients (42.9%), systemic tactile and pain hypesthesia or anesthesia (systemic sensory disorder type) in 7, a hemisensory disorder in 9, an alternative sensory disorder (segmental sensory disorder or irregular type) in 10 and no impairment of superficial sensation in 15 patients. Figure 1 illustrates the correlation between the methylmercury concentration in the cerebellum (ppm) and the degree of cerebellar incoordination in 45 patients. The coefficient of correlation (r) was 0.106 (not significant). Figure 2 shows a comparison of the incidences of major neurologic features between classic Minamata disease patients and those with disease certified by autopsy.

Discussion

The classic type of Minamata disease includes those with a relatively acute or subacute onset from 1957-1960, corresponding to the period when seafood in Minamata Bay was heavily contaminated with methylmercury. Most patients suffered from concentric constriction of their visual fields, hearing loss, cerebellar ataxia, and a "glove and stocking" type of sensory disturbance, and hence were clinically diagnosed with methylmercury poisoning (Minamata disease). Follow-up studies were performed by Tokuomi et al from 1957 to 1960 (34 cases, mean age: 40.7 years), in 1969 (22 cases), and in 1978 (13 cases, mean age: 48.4 years) (7-9). Classic patients demonstrated concentric constriction of their visual fields (82%), hearing loss (80%), cerebellar incoordination (70%), and "glove and stocking" types of sensory disturbance (100%) even 20 years after disease onset (9). On the other hand, the subjects who were later diagnosed by autopsy had only complained of symptoms including: the gradual development of a sensory disturbance, leg weakness, headache, dizziness, a decrease in visual acuity, and hearing loss from 1957-1975; only rarely were these findings considered to be evidence of Hunter-Russell syndrome.

From 1981~1985, we analyzed neurologic features and complications in 171 patients with clinically documented Minamata disease who resided in Kumamoto Prefecture (16, 17). We found an increased incidence of Minamata disease patients presenting with mild and infrequent cardinal neurologic findings, such as constriction of the visual fields, hearing loss, and cerebellar ataxia; an increased incidence of agerelated complications was also noted. However, we have reported that the incidences of these complications (except for retinitis pigmentosa) differ little from the corresponding prevalences of these symptoms in the general population (17). In the present study, we investigated the neurologic findings in 77 Minamata disease patients, as certified by autopsies performed between 1976~1994 and compared these findings with those of classic Minamata disease patients reported by Tokuomi et al from 1957-1960 (1, 2, 7). Classic patients previously have demonstrated concentric constriction of visual fields in 82%, hearing loss (80%), cerebellar incoordination (70%), and sensory disturbance (100%) even 20 years following disease onset. However, in the present study, cardinal neurologic features such as concentric constriction of the visual fields and cerebellar ataxia accounted for less than 50% of the findings, except for dysstasia affecting one leg and a tandem gait disturbance. Most symptoms were mild except for sensory impairment. Although the incidence of abnormal deep tendon reflexes was increased in autopsy cases, it may be due to the

increased incidence of age-related complications such as cerebrovascular disease and cervical spondylosis, or diabetes (18). Compared with the data reported in the 1981~1985 survey, the incidence of main neurologic features in patients with this disease has decreased. In cases of acute intoxication with organic mercury, there is a correlation between the volume of ingested contaminated food and the severity of the clinical symptoms and signs (19). However, we found no correlation between the methylmercury concentration in tissues and the degree of clinical symptoms and signs The biological half-life period of in chronic Minamata disease patients. methylmercury is about 70 days and it is known that the methylmercury contents decrease and reveal nearly normal levels in the brain of mild prolonged cases with Minamata disease (13). We also found that these 77 patients with Minamata disease showed no significant difference in the frequency of main neurologic features except for visual constriction and sensory disorder from 78 non-Minamata disease cases who resided in Minamata district but had no characteristic pathology of Minamata disease (unpublished data). The presence of Grade I pathologic lesions (30% of "thinningout" of neurons) may not be related to the typical neurologic findings of organic Thus, it is difficult to make a precise clinical diagnosis of mercury poisoning. Minamata disease, in atypical or mild cases and supplemental examinations including: brain CT, MRI, SEP, or tremogram are necessary to confirm the clinical diagnosis in such cases (20).

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chronic Minamata disease [found/examined(%)]				
Abnormality of visual fields				
constriction	17/59 (28.8)			
depression	18/36 (50.0)			
Abnormality of ocular movements				
smooth pursuit movements	20/54 (37.0)			
saccadic movements	2/55 (3.6)			
Hearing disorders				
labyrinthine deafness	22/59 (37.3)			
retrocochlear deafness	9/59 (15.3)			
Abnormality of optokinetic nystagmus pattern	11/54 (20,4)			
Dysarthria	10/71 (14.1)			
Incoordination				
dysdiadochokinesis	14/74 (18.9)			
impairment in finger-to-nose test	13/74 (17.6)			
impairment in heel-to-knee test	20/69 (29.0)			
impairment in shin tap test	24/67 (35.8)			
Postural and gait disturbance				
dysstasia affecting both legs (standing disturbance)	11/63 (17.5)			
dysstasia affecting one leg	32/57 (56.1)			
positive Romberg test	11/60 (18.3)			
impairment in tandem gait	30/53 (56.6)			
simple gait	24/64 (37.5)			
Tremor				
intention tremor	10/76 (13.2)			
postural tremor	14/68 (20.6)			
resting tremor	9/77 (11.7)			
Muscle weakness	19/73 (26.0)			
Muscle atrophy	16/77 (20.8)			
Deep tendon reflexes				
normal	29/77 (37.7)			
increased	12/77 (15.6)			
decreased/absent	36/77 (46.8)			
Sensory disorder				
superficial sensation	62/77 (80.5)			
extremities	33/77 (42.9)			
extremities+hemisensory disorder	1/77 (1.3)			
extremities+systemic impairment of pain sens	e 1/77 (1.3)			
extremities+segmental sensory disorder	1/77 (1.3)			
systemic sensory disorder	7/77 (9.1)			
hemisensory disorder	9/77 (11.7)			
others	10/77 (13.0)			
none	15/77 (19.5)			
deep sensation	<u> </u>			

Table 1. Incidences of major neurologic features in 77 patients with

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Legends

Fig. 1. Relationship between the methylmercury concentration in the cerebellum (ppm) and the grade of cerebellar incoordination. Each grade was determined as the total score (absence (=0), suspicion (=0.5), and presence (=1.0)) of ten cerebellar signs: dysarthria, dysdiadochokinesis, impairment in finger-to-nose test, heel-to-knee test, and shin tap test, standing disturbance, dysstasia affecting one leg, impairment in simple and tandem gait, and intention tremor.

Fig. 2. Comparison of incidences of major neurologic features between classic Minamata disease patients and those with disease certified by autopsy.

Methylmercury concentration in cerebellum (ppm)







Radio and methylmercury sensitivity of cultured mammalian cells

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Abstract

Treatment of mammalian cell cultures (HeLa, Chinese hamster V79 and Chinese hamster ovary cells) by methylmercury (MeHg) or x-ray gave similar dose-response curves with a shoulder at a low exposure level. Although the curves were quite similar among the cell lines after MeHg exposure, they varied to a significant extent after x-ray irradiation. Furthermore, response of the cells showed variations after x-ray irradiation, such as changing to single cells without division, forming tiny abortive colonies including abnormal cells, and growing into large colonies. On the other hand, MeHg exposed cells showed a uniform dose-depend response, giving little abortive colony or growing into large colonies. Survival periods after treatment with lethal dose were also different between radiation and MeHg. It took for a few days for all the cells to observe cell death after 50 Gy x-irradiation. In the case of MeHg (10 μ M) the survival periods varied among the cell lines from a few hours to several days. Chinese hamster V79 cells, most sensitive cells examined, showed 50% cell death after only a few hours of treatment.

Key words: radiation, methylmercury, cell survival

Introduction

Radiation and methylmercury (MeHg) cause various effects on cultured mammalian cells. Some are similar and some are different between two treatments. Survival curve for proliferative mammalian cells are usually presented in the form with dose plotted on a linear scale and surviving fraction on a logarithmic scale by colony surviving assay (Hall 1973). For sparsely ionizing radiation such as x-rays, the dose-survival curves has an initial large shoulder followed by a steeper and straighter portion. This threshold type of response implies that damage must be accumulated before a lethal effect can become evident.

It appears that there are a number of critical sites that must be damaged within a cell

before it loses its reproductive capacity. This multiplicity of target sites may is supposed to account for the initial shoulder in the survival curve. A cell may have received an ionizing event in some, but not all its critical target site. Its has been damaged but it has never been killed. Given time, the cell may be able to repair (Hall 1973). In this study, we investigated the colony survival curves of MeHg-treated mammalian cells comparing to that of x-irradiated cells.

Materials and methods

Cell cultures

Mammalian cell cultures, HeLa, Chinese hamster V79 (V79) and Chinese hamster ovary (CHO), were maintained in Eagle's Minimum essential Medium (HeLa andV79) or RPMI1640 (CHO) supplemented with 10% fetal bovine serum (FBS), the cells are incubated in a 25 cm² culture flask at 37°C in a humidified atmosphere with 5% CO₂ and 95% air.

X-irradiation

Cells were irradiated at room temperature with the dose rate of 1.3 Gy/min with an x-ray generator (PANTAC, Shimazdu, Japan) equipped with an external filter of 0.3 mm Cu at 200 kVp and 20 mA and.

Treatment with MeHg

Stock solution of MeHg-cysteine (10 mM) was prepared by dissolving equimolar amount of MeHg chloride (Wako Pure Chemical Co., Japan) and L-cysteine (Wako Pure Chemical Co., Japan) in phosphate buffered saline and was stored at -80°C. Cells were plated in 60 mm plastic dishes or 96 well plates (Falcon), and after 24 hours MeHg was loaded

Colony survival assay

About 300 cells had been seeded into the 60 mm plastic dish and exposed to radiation or MeHg. After 8 or 10 days, cells were fixed by MeOH, stained by crystal violet, and colonies of surviving fraction were calculated.

Colorimetric assay for cell survival

Tetrazorium salt has been used to develop a quantitative colorimetric assay for cell survival (Korzeniewski and Callewaert 1983; Decker et al. 1988; Iwaki et al. 1995).

Cells seeded into 96 well plate were treated with MeHg for 24 hr, and colorimetric assay tests were performed. Living cells and dead cells were assayed using WST-1 Cell Counting Kit (Dojindo Laboratory, Kumamoto, Japan) and LDH-Cytotoxic Test (Wako Pure Chemical Co., Japan), respectively.

Results and discussion

To investigate the effects of radiation on the cell cultures a dose-response curve is often employed, and a colony survival assay is commonly used to evaluate the response (Hall 1973). In the present study, we determined dose-response curves of MeHg exposure to the cell cultures using a colony survival assay, and compared them with that of radiation.

When mammalian cells were irradiated by x-ray the survival curves showed shoulders at lower dose levels as shown in Fig. 1. In the case of neutron radiation, however, the shoulders became much smaller (data not shown). The size of the shoulders depended on the cell lines (Aramaki and Yoshinaga 1979) and was supposed to represent ability of repair from the radiation injury (Elkind and Sutton 1960). Exposure of the cells to MeHg caused similar shoulders in the survival curves at lower dose levels (Fig. 2). However, difference in the curves among the cell lines was not so significant as x-ray irradiation.

Fig. 3A shows colonies of V79 cells that were exposed to x-ray. The size of the colonies became smaller when dose levels was increased. In general, when growing cultured mammalian cells are x-irradiated they respond in various manners (Kura et al 1978). Some cells remain single and never divide, some cells manage to complete one or two divisions to form tiny abortive colony, and some cells grow into large colonies that slightly differ from the unirradiated control cells. The last cells are said to have 'survived' (Hall 1973), though they contain abnormal cells (Kura et al. 1978).

In the case of MeHg exposure, however, the size of the colonies did not reduced even if the dose levels were increased, though a number of colonies was decreased. The survived cells have later grown into the large colonies that look normal and were indistinguishable from the untreated control cells (Fig. 3B).

To know a cell death, we often use "trypan blue exclusion test." When the cells die the cell membrane is destroyed, and the dye enters the cells. When mammalian cells were irradiated by lethal dose levels (50 Gy) of x-ray, blue-stained cells could be observed through a microscope. A number of the stained cells (died cells) increased gradually with time. It needed as long as several days until the fraction of died cell exceeded 50% in all the cell lines examined (Guo et al. 1998). A typical feature from
HeLa cells was shown in Fig. 4A. On other hand, in the case of MeHg exposure, sensitivity seemed quite different among cell lines. The period that needed for 50% cell death varied from a few hours to several days. The most sensitive cell line is Chinese hamster V79, half of these cells have died within 2 hr after MeHg exposure of lethal dose levels (10 μ M, Fig. 4B). However, HeLa cell lines are less sensitive. This cell line survived for a few days even after exposure to the lethal dose (data not shown). Interestingly, survival rate of HeLa cells depended on the length of exposure period to MeHg (Fig. 5A). Cell death observed after short-term exposure (1 or 2 days) were much less than after 10-days exposure. CHO cells showed a similar feature HeLa cells (data not shown). On the other hand, in the case of V79 cells, the survival curves were independent on the length of MeHg exposure period (Fig. 5B).

As mentioned previously, trypan blue exclusion test depends on a microscopic observation. Recently, several colorimetric methods using a microplate reader have been developed to evaluate cell death. Among them, we employed WST-1 and LDH assays. WST-1 assay depends on function of the mitochondria (Iwaki et al. 1995). In LDH assay, an activity of enzyme (lactate dehydrogenase) released from damaged cells are determined (Korzeniewski and Callewaert 1983; Decker et al. 1988). We compared the two colorimetric assays and the classical colony assay of CHO cells that were exposed to MeHg for 24 hr (Fig. 6). In WST-1 and LDH assays, Y-axis represents living and dead cells, respectively (Fig. 6A,B), and both results showed good agreement, showing drastic change around 8 μ M MeHg. When the cells were treated for long period (8 days) MeHg levels that caused cell death, evaluated by colony survival assay, was one order lower than 24-hr exposure (Fig. 2). Together with the results in HeLa and V79 cells, these results showed that HeLa and CHO cells would possibly be repaired from MeHg cytotoxicity at low levels, but V79 cells would not. These difference were not observed in the single colony survival curves.

In summary, the colony survival curves of the cultured mammalian cells showed similar large shoulders both after x-ray irradiation and MeHg exposure. In the case of x-irradiation, some cell lines, such as Chinese hamster cells, that have larger shoulder may be repaired to a complete recovery from the sub-lethal damage caused by low exposure. However, other cell lines with relatively small shoulders may be repaired to a small extent. In the case of MeHg exposure, the survival curves after long-term exposure were similar among three cell lines (HeLa, V79 and CHO). However, only the cell lines that showed different curves after short-term exposure, such as HeLa and CHO cells, would recover from toxic effects of MeHg. To explain the cell line-dependent variation of toxicity further study should be conducted.

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Figure legend

Fig. 1. Survival curves of different cell lines of cultured cells exposed to 200 kVp X-rays. (Redrawn from Aramaki and yoshinaga)

Fig. 2. Survival curves of different cell lines of cultured cells exposed to MeHg.

Fig. 3. Colonies obtained with CHV79 cells after X-ray irradiation (A) and MeHg treatment (B). After X-ray or MeHg treatment, the cells were allowed to grow for 10 days before staining.

Fig. 4. Rates of cell death after 30 Gy X-ray irradiation (A) and exposure to 10 MeHg (B) evaluated by trypan blue exclusion test.

Fig. 5. Colony cell survival curves obtained from MeHg-treated cells for various period. (A) HeLa cells, (B) CHV79cells.

Fig. 6. Colorimetric assay curves of CHO cells. Twenty-four hours after MeHg treatment, the cells were assayed by WST-1 (A) and LDH (B).



Figure 1



Figure 2





DOSE (RAD)













Figure 5



Figure 6

Mercury pollution of natural origin in Kagoshima bayside area.

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Introduction

Mercury pollution of natural origin has been reported throughout the world. In Kagoshima Prefecture, two polluted areas have been found. Suzuki has reported in 1971 that the content of mercury in the hair of the inhabitants of Tokara Islands, south of Kagoshima, was high in general. The cause of the high content of mercury in the hair was due to consumption of large sea fish containing mercury. The other area was the Kagoshima bayside area. In 1973, a high content of mercury in fish from Kagoshima Bay was detected. In 1974, a high content of mercury in the hair of the fishermen living in the Kagoshima bayside area was also detected. The highest value was 128.9 ppm. No mercury of artificial origin was detected in Kagoshima Prefecture. Volcanic activity at the bottom of Kagoshima Bay was thought of as the source of mercury. The inorganic mercury, emitted by the volcanic activity at the bottom of the sea, turned to organic mercury, and fish were contaminated. The content of mercury in the hair of the fishermen was high enough to induce health damage, therefore, we reexamined the content of mercury in the hair of the inhabitants living along Kagoshima bayside area and also conducted a neurological investigation in order to clarify the effect of mercury pollution of natural origin on health.

To elucidate the health effect on human being with fish feeding with natural mercury pollution from Kagoshima Bay was an important problem. For this purpose, we performed cats experiments fed with fish harvested from Kagoshima Bay. The purpose of the following experiments is to elucidate that "Does natural Mercury pollution affect human health?"

1. The content of mercury in the hair of the inhabitants in the Kagoshima bayside area

Among 80 people living in the Kagoshima bayside area and whose content of total mercury in the hair was more than 40 ppm in 1975, we chose 15 fishermen (aged 46 to 70, mean 54.7) and measured the content of mercury in their hair 5 times during the following 4 years. In some cases, long hair was picked and cut every 1 cm, and the content of total mercury in each piece was measured. The total mercury was measured by using atomic absorption spectrophotometer according to Magos's method.

Until 1975, few cases of high content of total mercury beyond 100 ppm were recognized, but the mean value of the content of total mercury has gradually been lowered (Table 1). This decrease in the content of mercury in the hair is thought to be the effect of a warning by the government (1975) to abstain from catching and eating fish containing a high amount of mercury. The monthly fluctuations in the total mercury in the long hair of a fisherman living in the Kagoshima bayside area (samples were picked in Mar. 1975) are observed. The maximum content was 143.0 ppm in December and the minimum was 32.3 ppm in March. December is the peak fishing period in this area.

Year	Max.	Min.	Mean
1974	116.9	40.0	68.3
1975	126.2	39.6	87.4
1975	128.9	3.6	38.1
1976	55.0	6.0	32.2
1978	42.2	8.2	28.5
1978	39.0	10.7	21.7

Table 1. Total mercury in hair of inhabitants. (Kagoshima Bayside area, ppm)

2. Neurological findings in the inhabitants of the Kagoshima bayside area

We conducted a neurological investigation on the 15 fishermen described above. The clinical investigation was conducted neurologically, ophthalmologically and otologically. The neurological investigation consisted of neurological status, motornerve conduction velocity (MCV) of the posterior tibial nerve, sensory conduction velocity (SCV) of the sural nerve and spine xray. The ophthalmological investigation consisted of an examination of the visual field by the Goldman perimeter and an examination of the ocular movement by electro oculography (EOG). The otological investigation consisted of an examination of the hearing by audiometer. The periods of the investigation and the number of cases were Feb. 1975: 10 cases, Sep. 1975: 14 cases, Dec. 1976: 15 cases and Feb. 1978: 11 cases. The mean age for the 15

cases in the clinical investigation of 1976 was 56.7 years (48 to 72).

In the results of our clinical investigation of 15 cases in 1976 (neurological findings, MCV, SCV, findings of spine X-ray, ocular movement and hearing), 6 cases were neurologically normal, while the other 9 cases showed some abnormalities. The abnormalities were glove and stocking type sensory disturbance (4), finger tremor (4), hemiplegia (1), muscle atrophy of a lower extremity (1). No case showed any of the abnormalities typical of Minamata disease such as ataxia, oculomotor disturbance, and constriction of the visual field and hearing disturbance. Cases 1, 2, 3, 4 exhibited a slight glove and stocking type sensory disturbance. The content of total mercury in the hair of 1, 2, 3 was less than 50 ppm in 1974 (40.9, 43.2, 44.7ppm). Spine x-ray of these 3 cases showed spondylosis deformans. We diagnosed the cause of this sensory disturbance as myeloradiculopathy due to spondylosis deformans. Case 4 showed a slight glove and stocking type sensory disturbance and finger tremor in his first clinical investigation in 1976, but there were no signs of sensory disturbance in the following two clinical investigations. His spine X-ray showed a very slight spondylosis deformans. He was an excessive alcohol drinker. Though the content of total mercury in case 4 was 116.9 ppm in 1974, we diagnosed the cause of the sensory disturbance and finger tremor in case 4 as being due to alcoholism. Cases 6, 9, 13 were also excessive alcohol drinkers, and we diagnosed the cause of their finger tremor as being due to alcoholism. Case 15 revealed a high content of total mercury in the hair (104.2 ppm), but showed none of the signs and symptoms of Minamata disease. The muscle atrophy was due to polyomyelitis contracted when he was a child. No case was diagnosed as intoxication of organic mercury in this area in 1976 nor in any other of our three clinical investigations.

3. Clinical and pathological study intoxicated cats fed with fish harvested from Kagoshima Bay.

Most of fishes harvested in Kagoshima Bay contained high mercury levels, especially fish living in the inner parts of Kagoshima Bay. The cats were divided into three groups; group A consist of 7 cats fed with fish pellet; group B consist of 7 cats fed with normal food contaminated with the same dose of methylmercury; and group C consist of 4 cats as controls. The experimental period was 760 days. The total and methylmercury contents were measured in the visceral organs, cerebrum, cerebellum, peripheral nerve and fur. Pathological studies were performed in the sural nerve and brain. In the sural nerve, the fiber density of the myelinated fibers was measured with

toluidine-blue staining. In the brain, light microscopic studies were performed with hematoxiline-eosin staining and in some parts, quantitative analysis were performed using computer. Detailed methods were described elsewhere1).

The rate of weight gain during experiments showed no difference between group A and B. The total mercury intakes were 51.0 ± 16.3 mg/cat in group A and 43.1 ± 20.6 in group B. At the end of the experiments, no clinical symptom was revealed in any groups. The average mercury levels of organs and tissues in three groups were shown in table 2. Average mercury levels of organs and tissues were higher in group A than group B and C.

	Total Hg			Methyl Hg		
	Group A	Group B	Group C	Group A	Group B	Group C
Liver	77.5	55.4	2.45	5.66	1.76	0.29
Spleen	11.2	2.28	0.15	2.79	0.6	0.08
Pancreas	1.95	0.66	0.08	1.08	0.24	0.04
Kidney	6.88	4.26	0.3	2.2	0.68	0.04
Peripheral Nerve	2.13	1.77	0.24	2.51	0.91	0.09
Cerebellum	5.11	2.02	0.16	3.21	0.84	0.14
Occipital lobe	5.67	1.92	0.13	3.14	0.75	0.1
Spinal cord	1.17	0.62	0.06	1.22	0.33	0.04
Muscle	5.09	1.10	0.24	4.44	0.98	0.2
Fur	115.4	66.2	3.18	108.1	54	2.99
Blood	4.05	1.51	0.06	0.25	0.11	

Table 2. Average mercury level in cat organs and tissues.

(ppm)

In the pathological studies, fiber densities of myelinated fibers in sural nerves did not show any difference among these three groups. The structures of occipital lobe cortex in cats from group A were well preserved. In the cerebellum, there was no decrease in the number of granular cells and no increase in the number of glial cells. In the liver, except mild lymphocytic infiltrations around Glisson sheath, no abnormality was seen in all groups. In the kidney, no abnormalityw was found in the glomeruli and renal tubules. In the morphometric analysis of the cerebellum on 1 um-thick epon sections, the numbers of the nuclei of granular cells in the same area of cerebellar hemisphere and vermis were counted. In group A, cell counts in vermis granular layer were decreased in 3 out of 5 compared to controls. In group B, cell counts in vermis granular layer were decreased in 2 out of 5 compared to controls. However, no decrease of cell counts was seen in hemispheric granular layer.

4. Pathological study on cats living along the inner parts of Kagoshima Bay.

We performed pathological studies in 9 cats lived along the inner parts of Kagoshima Bay. Three control rats living in Kagoshima city were served as controls. The fiber density of myelinated fibers of sural nerve was measured. We also performed microscopic investigation in the occipital lobe and the cerebellum with hematoxiline-eosin staining. Furthermore, we analyzed the number and area of cells in the striated cortex 2nd-3rd layers of occipital lobe and nuclear density of cerebellar granular layer. Detail methods were described elsewhere2).

The mercury levels in ca'st tissues living along the inner parts of Kagoshima Bay were not different from controls (table 3).

Table 3. Mercury	level in	cat's tiss	ies living	along th	e inner	parts of	Kagoshima
Bay							

	Control	Inner parts	
Total Hg			
Occipital lobe	0.097 ± 0.106	0.156 ± 0.262	ns
Cerebellum	0.094 ± 0.098	0.165 ± 0.212	ns
Fur	4.064 ± 4.214	4.714 ± 10.014	ns
Methyl Hg			ns
Occipital lobe	0.089 ± 0.106	0.083 ± 0.156	ns
Cerebellum	0.087 ± 0.106	0.087 ± 0.164	ns
Fur	2.819 ± 2.88	3.821 ± 9.09	ns
			(ug/g)

Fiber densities of myelinated fibers in sural nerve of cats living along the inner parts of Kagoshima Bay did not show any significant decrease compared to controls. Microscopically, there were no abnormalities in the occipital lobe and cerebellum. Using the quantitative morphometrical analysis of the occipital lobe and cerebellum, the nucleus / background ratio (nuclear density) of cerebellar granular layer did not show any difference between two groups.

Conclusions

The content of mercury in the hair of the fishermen was possibly high enough to induce health damage, but no clinical cases were found in the course of our study.

These data suggest that mercury pollution of a natural origin is benign, compared with artificial pollution such as that which caused Minamata disease. The discrepancy in health hazards between mercury pollution of a natural origin and that of artificial origin may be explained by the effect of selenium in fish as Ganther suggested. On the other hand, the transient character of the high contamination is suggested by the fluctuations in the content of mercury in the hair of the fishermen living in the Kagoshima bayside area. Therefore, it was suggested that the transient nature of the extremely high contamination was one of the main reasons why no health damage was found in these areas.

We performed the cat experiments in order to study the effect on the health of animal by natural pollution of mercury. Results of our experiments are summarize as follows; (1) mercury level of organs and tissues were high in the cats feeding fish contaminated by mercury; (2) no clinical symptoms were revealed; (3) no pathological abnormality was found in peripheral nerve; (3) no apparent pathological abnormality was found in the cerebellum. In the quantitative morphometric analysis of the cerebellar granular layer in the first experiment, cell counts in vermis granular layer were decreased in 3 out of 5 in group A and 2 out of 5 in group B respectively. However, we could not conclude that the decrease of cell counts in vermis granular layer was significant or not because of the following reasons. First, we studied the small number of materials and second, no abnormality was revealed by routine microscopic study. Furthermore, in preliminary experiments in control group, an interindividual variation was noted.

Conclusion

In human subjects who ate contaminated fish and showed high mercury level were revealed in their hair did not show any clinical abnormalities. Furthermore, from the feeding experiments of fish harvested Kagoshima Bay, experimental animals did not show any clinical and pathological abnormalities. We conclude that natural mercury pollution may not affect the health of inhabitants.

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Subjective complations in a population living in the methylmercury polluted area.

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INTRODUCTION

Methylmercury (MeHg) pollution was first idetified near Minamata City in Kumamoto Prefecture in 1956. The pollution was widespread and many persons were affected. By 1997, over 2200 inhabitants had been certified by a Kumamoto and Kagoshima Prefecture government committee as having Minamata disease (MD) and thus qualified for compensation.

MD patients typically have neurological signs such as ataxia, speech impairment, constriction of visual fields, and sensory disturbance. But not every MD patient shows typical MeHg poisoning, and chronic MD patients in particular show subclinical features. In addition to the MD patients, more than 8000 people have been certified for compensation, not for MD but as victims also affected by MeHg pollution suffering from some degree of physical and psychological symptoms. A few studies have investigated subjective complaints and activities of daily living (ADL) in these MD patients. There report that MD patients have more subjective complaints and impaired ADL as compared to a control population. Moreover, neurological signs, subjective complaints, and ADL were exacubated by aging and complication accompanying aging. Moreover, several studies have noted a relationship between MeHg pollution and mortality or biological aging.

Some of the medical and social issues still being debated with regard to MD and MeHg pollution are the long-term influence of MeHg poisoning and its impact on the overall population in the MeHg- polluted area. Although it is likely to be very difficult to clarify these issues because about four decades have passed and the MeHg poisoning has possibly been modified by various factors, it is important to examine the health status not only of MD patients but also of other inhabitants of the polluted area. The aim of the present study was to document subjective complaints and to analyze their structure in a population in the MeHg-polluted area and to investigate relationships between subjective complaints and methylmercury exposure.

SUBJECTS AND METHODS

Study population

Town A is located in the southern part of Kumamoto Prefecture, next to Minamata City, and faces the Yatsushiro Sea. It had a population of about 5,800 in 1995, about 330 of whom were certified as MD patients. Although the number of MD patients in town A is the second largest next to Minamata city, the prevalence rate of MD is higher in town A. We analyzed the geographical distribution of the subjects because about 90% of the total number of MD patients in this locality have lived in fishing villages, where much fish and shellfish were consumed. Thus, it is practical to divide the locality into two areas—the fishing villages as the polluted area and others as an internal control area—although residents in this second area may also have been exposed via fish consumption.

In addition, an external control population in a nonpolluted area, town B was selected. This town had a total population of about 11,400 in 1995. Town B faces the Ariake Sea and is geographically separated from Yatsushiro Sea. MeHg pollution was restricted to the Yatsushiro sea, and no one in town B has been certified as an MD patient. Thus, town B is relatively free from MeHg pollution. Town A and town B are similar economically, with mostly fishing and agricultural industries, and therefore the lifestyles in the two towns are similar. In both town A and town B the population is stable, and most of the adults and elderly have been living there since MeHg pollution occurred. Figure 1 shows the geographic locations of town A and town B.

Interviews

Subjects in town A were those who voluntarily participated in annual mass multiple health examinations in the summer of 1995. The 1,317 subjects (537 males and 780 females) were all older than 40 years of age and were interviewed by experienced public health nurses at the time of the health examinations. Excluding identified MD patients among the participants, data from 1,304 subjects were used for the analysis in town A. In town B, annual health examinations were given for 9 days in 1995; on 3 days examination were conducted among the population living in fishing villages. In total, the 446 subjects (127 males and 319 females) older than 40 years of old were interviewed in the fishing villages of town B in the same way as the subjects in town A. The participatory rate in town A and the fishing villatge of town B was about 30% among people older than 40. The residential and sex distribution of subjects are shown in Table 1.

The questionnaires concerning subjective complaints were based on the health administration procedures established by the Japanese Environmental Agency and consisted of 65 subjective complaints. Table 2 and 3 show the items. The subjects responded "yes" or "no" to each subjective complaint query.

MeHg Exposure Indicators

Mercury concentration in red blood cells (RBC Hg) were measured in samples, collected in 1989 from about 1,200 adults who participated in health examinations in 1989. Fish consumption was computed by dietary consumption survey of about 1,700 adults who participated in health examinations in 1988 and 1989:

Data on the subjective complaints, RBC Hg, and fish consumption were linked by

residential identification numbers and names. As a consequently, linkages established between subjective complaints data, and RBC Hg data and between subjective complaints data and fish consumption data for 553 subjects (216 males and 337 females) and 916 subjects (350 males and 566 females), respectively.

Analysis

Sex-specific prevalence of each subjective complaint was calculated by residential area: fishing villages, others and town B. The differences in prevalence were compared among residential areas by sex using the Mantel-Haenszel test, stratifying by age.

The subjective complaints were classified into 16 categories, and points were assigned to each category according to the sum total of subjective complaints. The reliability coefficient (α index) of each category is shown in Table 2 and 3.

Factor analysis was carried out using the point totals of the 16 categories of town A data. The factors whose eigenvalues exceeded 1.0 were considered significant and Varimax factor rotation was performed. Subsequently, the mean of four factor scores of subjects was calculated and compared among residential areas, sexes, and agegroups using Student's t test or one-way ANOVA. For further analysis, the relationship between factor scores and MeHg exposure indicators, RBC Hg and fish consumption, were measured with Spearman's correlation coefficient.

By means of factor scores, the subjects were classified into five groups by K mean cluster analysis. The characteristics of each cluster, such as centers of factor scores, residential area, age, and sex, were investigated. Then multiple variance analysis was performed using the software package SPSS 7.5.

RESULTS

The prevalence of subjective complaints is shown in Table 2 and 3. Residens in fishing villages reported higher prevalence of many complaints than others residents in town A or those in town B. In males, for example, stiffness, dysesthesia, hand tremor, dizziness, loss of pain sensation, cramp, upper arm muscular atrophy, arthralgia, insomnia, and lumbago showed significant differences among residential areas. In females, more complaints differed significantly among residential areas than in males; these complaints included leg tremor, tinnitus, loss of touch sensation, leg muscular atrophy, and muscular weakness.

The results of factor analysis are shown in Table 4. Four factors were obtained and the total cumulative eigenvalue percentage was 47.0%. Factor 1, whose eigenvalue percentage was the highest (23.1%), consisted of staggering, vertigo and dizziness, heart complaint, stiffness, and dysesthesia. Factor 2 consisted of loss of touch sensation, pain sensation, and thermal sensation and tremor. Factor 3 was arthrederma, arthralgia, tinnitus and difficulty hearing, and urinary complaints. Factor 4 was muscular atrophy and weakness.

The results of factor scores by residential areas, sex, and age groups are shown in Table 5. Residents of fishing villages had higher scores than other residents on all factors. Females had higher scores than males on factors 1 and 4 but lower scores on factor 2. Except for factor 4, the factor scores tended to increase with age.

The levels of RBC Hg were 28.5 ± 11.5 ng/g in males and 20.7 ± 9.8 ng/g in females. The mean fish consumption (g/day) was 95.6 ± 72.3 in males and 62.5 ± 59.2 in females. There were significant differences by sex, but not by age. The results of the correlation with factor scores and RBC Hg or fish consumption by sex are shown in Table 6. A relationship between factor scores and fish consumption or RBC Hg for the first and third factor scores only in males is evident.

The characteristics of each cluster are shown in Table 7. Cluster 1 included 46 subjects and its factor 3 score was the highest. Cluster 2, whose factor 2 score was the highest, included only two subjects. Cluster 3, whose factor 1, 2, and 3 scores were relatively high, included 55 subjects. For cluster 4, all of the factor scores were lower, it contained 1,164 subjects, a majority of the total. Cluster 5 included 37 subjects, and its factor 4 score was high. Persons in cluster 4 were younger than those in other clusters. The inhabitants of fishing villages were predominantly in clusters 3 and 5. In clusters 1 and 5, the proportion of females was relatively higher, while males were dominant in cluster 3.

DISCUSSION

The first outbreak of MD occurred in the 1950s among fishermen and their families living around the Yatsushiro Sea. Dietary consumption of fish and shellfish contaminated with MeHg led to severe neurological disorders and even fatal poisoning. The signs and symptoms of the disease, such as ataxia, speech impairment, and constriction of visual fields, were often accompanied by hearing impairment and sensory disturbance. These findings corresponded to the clinical description of MeHg poisoning defined by Hunter and Russel (1954).

In addition to features typical of MeHg poisoning, previous studies have found that the symptoms and complaints of MD patients, other than neurological ones, also differs from those of a control population. Moreover, so-called chronic MD shows atypical and subclinical features unlike those of classical MD. Aging, complications accompanying aging, and sociopsychological factors may modify the clinical features of MeHg poisoning. The complexity of the facts surrounding MeHg poisoning in the Minamata areas led to difficulties in diagnosing MD clinically and in solving the social problems related to assisting the victims.

By excluding MD patients, this study has documented that the inhabitants in the MeHg-polluted area (fishing villages) have more subjective complaints than those in an internal control or an external control areas. Similar to the findings of previous studies, the results of this survey found that not only neurological complaints such as paresthesia, tremor, and loss of sensation, but also nonneurological complaints such as forgetfulness and insomnia show significant differences in reported prevalence by residential areas. Particularly, the items in the borderline area between neurological and nonneurological symptoms, such as stiffness and cramps, show large differences by residential area.

Females tended to report more complaints and had more items than males showing significant differences by residential areas. As there are more male MD patients than female ones, it was expected that males would be more likely than females to have subjective complaints due to MeHg exposure. The results of this study may be due in part to the fact that females report more complaints about physical discomforts than males. In addition, several diseases which higher morbidity in females than in males, such as osteoarthrosis, may increase reports of subjective complaints.

The results of factor analysis indicated that the subjective complaints consisted mainly of four factors. Factor 1 was related to staggering, vertigo and dizziness, heart complaints, cramps, stiffness, and dysesthesia, which we described as "non-specific". Factor 2 is interpreted as a "sensory" factor, factor 3 as "arthritic", and factor 4 as "muscular". Cumulative percentage variance of the four factors is about 50%, which shows that subjective complaints in these areas could be explained to some degree by these four factors.

Factor scores of all four factors were higher in residents of fishing villages than in those from the internal control area. These results suggest that MeHg pollution may increase not only sensory subjective complaints, which are strongly related to typical MeHg poisoning, but also muscular and arthritic complaints. For factor 1 and 3, females had significantly higher scores than males. For factors 1, 2 and 3, factor scores tended to increase with age. In addition to MeHg exposure, the frequency of subjective complaints was greatly modified by other factors such as sex, aging, lifestyle, and illness and occupational history.

Except for cluster 2 (which consisted of only 2 persons), the clusters with relatively many subjective complaints were divided into three types: clusters 1, 3 and 5. Because cluster 1 was highest in factor 3 (arthritic factor), we described cluster 1 as an "arthritic dominant" type. Cluster 3 was described as a "sensory dominant" type, and cluster 5 as a "muscular dominant" type. The sensory dominant and muscular dominant types were prominent in fishing villages, while the arthritic dominant type was uncommon in fishing villages. In the arthritic dominant type, females predominated over males (87%). In contrast, for the sensory dominant type reflected some of the features typical of MeHg poisoning, while the arthritic dominant type may have been influenced by factors other than MeHg exposure.

One of the most important limitations of this study is the difficulty in estimating individual MeHg exposure. While MeHg exposure in this area is due almost entirely to fish consumption, the fish consumption data used in this analysis were obtained in 1988 and 1989 and do not show cumulative or past MeHg exposure. Recent fish consumption data might be useful in estimating past exposure to methylmercury because dietary lifesryle has changed rebtively little. Hg concentration in blood, particularly RBC Hg, is an indicator of MeHg exposure because blood Hg concentration reflects Hg concentration in body stores. Sakamoto et al found that RBC Hg levels were related to fish consumption in this area. However the half-life of Hg in blood is about 70 days. Even those who had more than a 10-fold increase of Hg concentration in hair at the onset of poisoning showed normal limits after 10 years. So the recent RBC Hg concentrations and fish consumption data used in this study cannot serve as indicators of exposures during the 1950s and 1960s, though they may reflect the dietary consumption at the time of measurement.

A previous study using multivariate analysis investigated the influence of MeHg pollution on human health. This study analyzed neurological findings in MD patients and a control population, using multivariate analysis to establish a statistical diagnostic method of identifying MD. While it is important to clarify the overall health effects of MeHg pollution, this clarification may not benefit victims of MeHg as they age. Fortunately, the issues with regard to the recognition of MD patients have been politically resolved. The purpose of our analysis of subjective complaints and the structure of these complaints is to demonstrate objectively the health status of overall population in MeHg-polluted areas, to investigate the cause of these complaints, including both MeHg and others pathogens, and to contribute to improvement of overall health status.

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Fig 1. Map of study areas

	Males			Females		
Age	A town		B town	A town	B town	
	Fishing villages	Other		Fishing villages	Other	
40-49	33	79	20	46	97	53
50-59	28	57	28	66	107	87
60-69	65	107	48	107	171	124
70-	55	102	31	73	111	55
Total	181	345	127	292	486	319

Table 1 Age-and sex-distributions of subjects

	Males			Females		
Subjective complaint items	A tow	'n	B town	A tow	/n	B town
	Fishing villa	ges Others	Control	Fishing villa	ges Others	Control
Heart-complaint (0.42)						
Chest pain	4.2	2.3	3.9	9.2 o	4.0 f	5.0
Palpitation	11.3 o,c	6.2 f	3.1 ſ	13.0 o,c	8.7 f	4.1 f
Arrhythmia	5.8	2.7	5.5	4.8	3.9	2.8
Stiffiness (0.74)						
Shoulders	52.0 o,c	30.7 f	29.1 f	63.7 o,c	45.7 ſ	46.7 f
Neck	23.2 o,c	15.5 f	11.0 f	42.4 o,c	21.3 f	18.8 f
Back	21.2 o,c	9.0 f	6.3 f	23.1 o,c	9.2 f	9.4 f
Dysesthesia (0.62)						
Hands	37.3 o,c	12.7 f	7.1 f	49.5 o,c	16.8 f,c	8.5 f,o
Legs	33.0 o,c	7.5 f	7.1 ſ	32.0 o,c	10.8 f,c	4 .1 f,o
Staggering (0.40)		·				
At sitting	1.2	0.6	0.0	3.2 o	0.3 f	1.6
At standing	22.0 o,c	11.0 f,c	3.1 f,o	30.5 o,c	11.3 f	5.3 f
At walking	2.4	0.9	0.8	7.4 o,c	1.5 f	0.9 f
Tremor (0.50)						
Resting (hands)	12.2 o,c	2.3 f	0.8 f	7.7 o,c	2.4 f	0.9 f
Intention (hands)	6.6 o,c	2.0 f	1.6 f	8.5 o,c	2.6 f	0.9 f
Resting (legs)	0.5	0.2	0.0	2.9 o,c	0.0 f	0.6 f
Intention (legs)	1.7	0.2	0.0	1.8	0.4	0.3

Table.2 Prevalence (%) of subjective complaints by residential areas and sex (1)

f: p<0.05 compared with fishingvillages, c: p<0.05 compared with control, o: p<0.05 compared with others

(): Reliability Coefficients (a index)

	Males			Females		
Subjective complaint items	A town		B town	A town		B town
	Fishing villa	ges Others	Control	Fishing villa	ges Others	Control
Urinary-complaints (0.41)						
Difficulty urination	12.0 o,c	5.8 f	3.1 f	5.5	3.9	2.5
Urinary incontinence	0.0	1.0	0.8	4.2 o,c	0.0 f	0.9 f
Sense of residual urine	15.2 o	8.9 f	11.0	7.7	5.8	4.4
Nycturia (≥2 times)	18.9	14.8	18.1	17.8 o,c	11.7 f	13.8 f
Hematuria	0.0	0.0	1.6	0.5	0.6	4.4
Vertigo and dizzness (0.54)						
Vertigo	4.9	1.0	0.8	6.8 c	3.8	0.6 f
Dizziness	9.0 o,c	1.3 ſ	1.6 f	14.3 o,c	5.3 f	6.9 f
Dizziness (at standing up)	14.7 o,c	4.4 f	0.8 f	14.5 o,c	6.3 f	6.3 f
Dizziness (at face movemen	t) 1.1	0.2	0.0	2.1	2.4	1.6
Tinnitus and difficulty hearing (0.31	l)					
Tinnitus (high tone)	8.0	4.1	3.9	6.6	3.3	5.6
Tinnitus (low tone)	21.6 o	12.8 f	14.2	21.6 o,c	10.8 f	11.0 f
Difficulty of hearing	29.7	25.9	18.1	23.1 o,c	18.5 f	13.5 f
Loss of pain sensation (0.79)						
Hands	8.0 o,c	1.0 f	0.0 f	4.8 o,c	1.0 f	0.3 f
Legs	5.6 o,c	1.3 f	0.0 f	6.3 o,c	1.9 f	0.0 f
Loss of thermal sensation (1.00)						
Hands	0.0	0.0	0.0	0.5	0.0	0.0
Legs	0.0	0.0	0.0	0.5	0.0	0.0
Loss of touch sensation (0.87)						
Hands	0.0	0.0	0.8	8.6 o,c	0.9 f	0.6 f
Legs	0.0	0.0	0.0	7.4 o,c	1.8 f	0.3 f
N	181	345	127	292	486	319

Table.2 Prevalence (%) of subjective complaints by residential areas and sex (2)

f : p<0.05 compared with fishingvillages, c : p<0.05 compared with control, o : p<0.05 compared with others

(): Reliability Coefficients (a index)

	Males					
Subjective complaint items	A town		B town	A town		B town
	Fishing villag	ges Others	Control	Fishing villages Others		Control
Cramps (0.54)						
Hands	24.2 o,c	12.7 f	12.6 f	33.5 o,c	13.6 f,c	4.4 f,o
Limbs	40.1 o,c	16.9 f	15.0 f	55.9 o,c	34.5 f,c	21.0 f,o
Muscular atrophy (0.85)						
Hands	5.2 o	0.0 f	1.6	4.2 o,c	0.8 f	2.2 f
Upper arms	6.4 o,c	1.5 f	0.8 f	4.2	1.2	0.0
Lower limbs	2.9	0.2	0.8	2.1 o,c	0.3 f	0.0 f
Upper limbs	4.6	0.9	1.6	4.0 o,c	0.6 f	0.9 f
Arthralgia (0.52)						
Hands or elbows	11.1 o,c	2.7 f	2.4 f	8.5 c	4.9	2.8 f
Fingers	5.2 c	3.7	0.8 f	11.1 o,c	5.1 f	2.8 f
Foot or knee	24.2 o,c	11.7 f	11.0 f	37.2 o,c	16.5 f	19.7 f
Toes	1.7	1.5	0.0	5.7	2.1	1.3
Others	5.2	2.2	0.8	3.9	4.1	0.6
Muscular weakness (0.74)						
Lower limbs	4.1	0.8	0.8	6.8 o,c	2.6 f	1.3 f
Upper limbs	1.7	1.2	0.0	5.4 o,c	1.9 f	0.0 f
Fingers	4.7	1.0	3.1	8.2 o,c	3 .0 f	1.6 f
Upper arms	3.5	0.5	2.4	5.7 o,c	1.6 f	1.3 f

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Table.3 Prevalence (%) of subjective complaints by residential areas and sex (1)

f: p<0.05 compared with fishing villages, c: p<0.05 compared with control, o: p<0.05 compared with others

(): Reliability Coefficients (Éøindex)

	Males			Females	Females		
Subjective complaint items	A town Fishing villages Others		B town	A town		B town	
			Control	Fishing villag	es Others	Control	
Arthredema (0.34)		·					
Hands or elbows	0.0	0.0	0.0	0.5	0.0	0.0	
Fingers	0.0	0.0	0.0	0.0	0.0	0.0	
Foot or knee	1.8	0.3	2.4	6.3 o	3.0	2.8 f	
Tœs	0.6	0.0	0.0	0.4	0.0	0.0	
Others	0.6	0.0	0.0	0.4	0.2	0.0	
Others							
Clumsy movement of tongue	6.7	2.8	0.8	5.6 o,c	1.6 f	0.3 f	
Dysphagia	3.7	1.7	0.8	2.6	1.8	1.6	
Leg edema	11.0	4.5	3.1	20.5 o,c	16.2 f,c	8.5 f,o	
Abdominal pain	4.9	6.2	7.9	7.1	6.4	3.1	
Forgetfulness	34.9	20.2	26.8	55.0 o,c	34.0 f	30.7 f	
Insomnia	18.9	10.5	3.9	33.7 o,c	1 7.4 f	11.3 f	
Lumbago	45.9 o,c	30.9 f	37.0 f	54.2 o,c	42 .0 f	39.2 f	
Thirst	20.7 o,c	9.8 f	10.2 f	12.1	7.6	6.9	
Fatigability	20.7 o	12.3 f	12.6	32.6 o,c	16, 7 f	16.6 f	
Bloody excrement	3.0	2.2	0.8	1.2	1.4	0.3	
Loss of consciousness	3.1	0.0	0.8	2.6	0.7	0.0	
Visual dimness	36.8 o,c	21.8 f	1 7 .3 f	42.9 o,c	29.6 f	20.4 f	
N	181	345	127	292	486	319	

Table.3 Prevalence (%) of subjective complaints by residential areas and sex (2)

f : p<0.05 compared with fishing villages, c : p<0.05 compared with control, o : p<0.05 compared with others () : Reliability Coefficients (Éøindex)

Table.4	Results of factor analysis

	Factor 1	Factor 2	Factor 3	Factor 4
Staggering	<u>0.67</u>	0.12	0.09	0.11
Vertigo and dizzness	<u>0.62</u>	0.08	-0.08	0.09
Heart-complaints	<u>0.55</u>	0.14	-0.03	0.07
Cramps	<u>0.53</u>	0.18	0.27	0.04
Stiffness	<u>0.49</u>	-0.04	0.29	0.27
Dysesthesia	<u>0.42</u>	0.39	0.34	0.06
Loss of touch sensation	0.22	<u>0.76</u>	0.06	0.01
Loss of pain sensation	0.17	<u>0.74</u>	0.13	0.00
Tremor	0.21	<u>0.60</u>	0.15	0.13
Loss of thermal sensation	-0.09	<u>0.53</u>	-0.13	0.02
Arthredema	-0.20	0.04	<u>0.71</u>	0.13
Arthralgia	0,19	0.07	<u>0.68</u>	0.12
Tinnitus and difficulty hearing	0.41	0.06	0.42	-0.15
Urinary-complaints	0.31	0.01	<u>0.36</u>	-0.09
Muscular atrophy	0.17	0.01	-0.10	<u>0.82</u>
Muscular weakness	0.09	0.15	0.24	<u>0.76</u>
% of variance	23.1	8.9	7.7	7.2

	N	Factor 1	Factor 2	Factor 3	Factor 4
Area					
Fishing villages	473	0.32 ± 1.21	0.23 ± 1.50	0.20 ± 1.22	0.16 ± 1.41
Others	831	-0.19 ± 0.80	-0.14 ± 0.47	-0.12 ± 0.82	$\textbf{-0.09}\pm0.63$
Sex					
Males	526	-0.09 ± 0.90	0.06 ± 1.16	-0.12 ± 0.77	-0.09 ± 0.90
Females	778	0.06 ± 1.06	-0.04 ± 0.87	0.09 ± 1.12 NS	0.06 ± 1.06
Age				-	
40-49	255	-0.33 ± 0.72	-0.14 ± 0.31	-0.44 ± 0.42	$-0.03 \pm 0.57 \mathrm{NS}$
50-59	258	-0.05 ± 0.10	-0.13 ± 0.48 NS	-0.10 ± 1.04	$-0.04 \pm 0.69 \text{ NS}$
60-69	450	0.06 ± 1.01 NS	0.06 ± 1.07	0.08 ± 1.00	$0.04 \pm 1.09 \mathrm{NS}$
70-	341	0.20 ± 1.10	0.12 ± 1.42 NS	0.29 ± 1.16	$0.04 \pm 1.28 \text{ NS}$

Table.5 Factor scores by residential area, sex, and age

Significant difference between groups except NS

	Males		Females			
	Fish consumption	RBC Hg	Fish consumption	RBC Hg		
Factor 1	0.12 *	0.03	0.07	-0.02		
Factor 2	0.01	0.09	0.00	-0.04		
Factor 3	0.12 *	-0.03	0.01	-0.04		
Factor 4	-0.04	-0.03	0.04	0.10		

 Table 6 Correlation between factor scores and mercury exposure index

Spearman's correlation coefficience, *: p<0.001

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
N	46	2	55	1164	37
Centers of cluster					
Factor 1	-0.87	-2.26	1.21	-0.06	1.09
Factor 2	0.01	13.62	3.12	-0.17	-0.01
Factor 3	3.80	-3.33	0.48	-0.16	-0.32
Factor 4	0.80	0.59	-0.58	-0.15	4.74
Age	67.2±7.9	76.0 ± 5.7	68.4±7.5	59.8±13.2	67.2±9.5
Area					
Fishing villages	25	2	42	377	27
Other	21	0	13	787	10
Sex					
Males	6	1	29	477	13
Females	40	1	26	687	24

Table 7 Characteristics of each cluster

PAST AND CURRENT STATUS OF MERCURY RESEARCH CARRIED OUT BY THE JOŽEF STEFAN INSTITUTE, SLOVENIA

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Abstract

The paper provides a short review of mercury research activities carried out by the Department of Environmental Sciences of the Jožef Stefan Institute, mainly focusing on: (1) development and validation of analytical methods for mercury analysis and speciation, quality control, as well as methods for studying Hg transformation in the environment, (2) professional exposure to mercury vapour in exposed professional workers; for example, the Hg mine in Idrija and the chlor-alkali industry, (3) other human health-related studies involving mercury and MeHg where most attention is directed towards active transport of MeHg across the placental barrier to the foetus, (4) biomonitoring, and (5) the biogeochemical cycle and modelling of mercury in the environment, involving fresh water lakes, rivers and the coastal environment.

Key words: mercury, methylmercury, analytical methods, professional exposure, and biogeochemistry, transport of methylmercury

Introduction

Idrija in Slovenia is the site of the second largest Hg mine in the world which was in operation continually for 500 years until about 20 years ago. Over five million metric tonnes of Hg ore was mined, and much of the residues were spread around the town and its vicinity. It has been estimated that 73% of the Hg mined was recovered with the remainder dispersed into the environment. One unfortunate outcome of centuries of Hg mining activity has been the constant exposure of the denizens of the area to the toxic effects Hg, including high Hg levels in miners. The Idrija mine has severely enhanced the mobilisation of Hg by mining activities, and Hg-laden material remains in the region. Most importantly, the processing of Hg ore over the centuries and the venting of the mine shaft which releases naturally occurring native Hg (Hg⁰), caused extremely elevated levels of airborne Hg. Concentrations of Hg in air samples exceeded 2.5 µg/m³ during active mining periods; even today, airborne Hg levels near the abandoned smelter and around the mine shafts are very high at over 300 ng/m³. Hg levels in sediments and flood plain deposits in the area downstream are very elevated as well. Soils in the Idrija valley are also rich in Hg. Hence, although the ultimate source of Hg in the Idrija region is from base deposits, the majority of material that resides in surficial materials, including deep sediments and spent ore along the banks of the river, are derived primarily from Hg re-mobilised by mining activities, mostly smelting. Some recent studies have shown that the system even today continues to supply high quantities of Hg to the river system of the Idrijca and Soča, and reaches the Gulf of Trieste some hundred km downstream where the river system (Isonzo) empties into the NE Adriatic Sea. The tailings and contaminated soils are continuously eroded and serve as a continuous source for the river, the flood plains and the Gulf of Trieste. This is confirmed by the fact that even 10 years after the closure of the Hg mine, Hg concentrations in river sediments and water are still very high and there are no signs of the

expected decrease of Hg in the Gulf of Trieste. Recent estimates of the Hg balance in the Gulf of Trieste have shown that the annual input through the Soča river discharge is about one ton and a half.

Furthermore, it is well known that the Northern Adriatic is subject to serious pollution problems, accompanied with eutrofication, anoxic conditions at the bottom, and winter and summer temperature stratification. All these favour transformation of inorganic mercury to more toxic MeHg, which is responsible for the elevated Hg leveles in marine organisms which frequently exceed the value of 0.5 mg/kg, which is set as the maximum permissible level according to WHO and Slovenian legislation. Moreover, due to deteriorated water quality in the Gulf of Trieste, Hg tends to accumulate in some marine food from mariculture areas, which represent a social and economic problem for the local population.

These facts directed mercury research in Slovenia, which was initiated by the Jožef Stefan Institute in the early 60's. The paper will provide a short review of these activities, mainly focusing on the analytical methods, health-related studies, biomonitoring and biogeochemical cycling and modelling of mercury in various environmental systems.

1. Analytical methods and quality control

Different methods for total mercury determination were developed, as listed in Table 1. Evidently, a variety of methods were developed and optimised. Selection of a method depends on matrix type and the expected concentration of mercury and its compounds. Radiochemical neutron activation analysis (RNAA) is based on volatilisation separation of mercury as its radioactive isotope Hg-197 after neutron irradiation in nuclear reactor and also allows Se to be determined from the same sample aliquot. This method is therefore often used in health-related studies in order to investigate the interaction of mercury and selenium. An additional advantage of NAA is that it is contamination-free after the sample has been is sealed and irradiated. Instrumental NAA (INAA) is non-destructive method and is very useful for multielemental analysis. It can provide excellent results for total Hg in environmental and biological samples. However, due to gamma spectrometric counting interferences from other radionuclides and compton background, the limit of detection (LOD) is not sufficient for low level Hg measurements (less than 30 ng/g), and in samples where Hg is weakly bound (e.g. lichens, mosses) volatilisation losses can be a problem. In practice, RNAA is often used as a reference method for quality control purposes. CV AAS is widely used, and its LOD is good enough to determine Hg in almost all biological and environmental samples, including natural water. It should be noted, however, that the LODs are dependent on the overall analytical procedure prior to the final quantification step, including sample preparation. In the case of low level Hg and small amounts of sample it is preferable to use CV AFS, as it has a 10 times better LOD than CVAAS.

Different methods were developed for isolation of methylmercury (volatilisation, acid leaching, solvent extraction, distillation and ion exchange) in various biological and environmental samples over wide concentration levels, and for its determination by gas chromatography with ECD or via CV AAS and CV AFS. In practice, low level MeHg measurements (water, sediments, etc.) are done by CV AFS, while for other samples GC-ECD is mainly used. Routine measurements of MeHg in human hair, fish and other biological samples are done by ion-exchange CV AAS. This method separated and then quantifies total organic Hg, which was shown to correspond to MeHg in virtually all biological samples. In samples such as sediments, soil, and lichens systematically higher results are obtained due to the presence of other organic ligands binding mercury, as well as MeHg which pass through the anion exchanger without absorption. This method is therefore not recommended for these sample types.

Apart from monomethylmercury it has become increasingly important to develop techniques for the determination of other geochemically important mercury species (Hg²⁺, DMM, Hg^o). DMM and other volatile organic species can be simply purged by inert gas from the sample and trapped on an adsorber (e.g. Tenax, carbotrap, gold). Further method validation is needed in order to verify the by accuracy of such procedures, in particular to check whether the amounts measured represent natural occurring compounds or possible analytical artefacts.

Also, due to some evidence of possible artifact formation of MeHg during analytical procedures in difficult matrices such as soil, sediments and plants, further studies are in progress in order to improve the quality of data for MeHg (Quevauviller and Horvat, 1999, Quevauviller et al. 1999).

Table	1.	Analytical	methods	for	mercury	analysis	and	speciation	currently	in	use	at	the
Depart	ment	of Environ	mental Sci	ience	es								

Analyte	Method Pretreatment, separation		Matrix	References		
	RNAA	Irradiation, oxidative pyrolysis	Biological tissues and liquids; aerosols, soils, sediments, food	Kosta and Byrne, 1969; Byrne and Kosta, 1974, Dermelj et al. 1991, Dermelj and Byrne 1997		
Total Hg	INAA	irradiation	Tissues; soil; sediments; aerosols	Smodiš et al. 1993,1994; de Corte et al.1997,		
	CV AAS	Acid digestion, reduction, gold amalgamation	Biological tissues and liquids; water, aerosols; soils; sediments	Horvat et al. 1985, 1986 a, b, c; 1987, 1991; Horvat, 1996		
· ·	CV AFS	Acid digestion, reduction, gold amalgamation	Crude oil, gasoline, water; low level samples	Liang et al. 1996		
MeHg	CV AAS	Ion-exchange separation, UV digestion, reduction, gold amalgamation	Tissues and biological liquids	Horvat et al., 1988 b; c, 1990		
	CV AFS	Alkaline dissolution, or acid leaching, followed by solvent extraction; distillation; detection performed after derivatisation with NaBEt4, preconcentration, gas chromatography and pyrolysis	Water; low level biological samples; aerosols; flue gases; sediments; soil	Horvat et al., 1993, a, b; Liang et al., 1994 a, b, 1995, 1996, 2000; Horvat and Schroeder, 1995; Logar et al. 2000		
	GC-ECD	Volatilisation; solvent extraction (various modifications)	Tissues, biological liquids, plants; sediments and soils	Zelenko et al., 1973; Gvardjančič et al. 1978; Horvat et al. 1988 b; c, 1990, 1991		
Hg ²⁻	CV AFS, CV AAS	Derivatisation or reduction	Biological tissues, water	Horvat, 1989; Logar et al. 2000		
DMM	CV AFS	Purging, preconcentration on Tenax	water	Horvat et al. 1993 a, b		
Hg°	CV AFS, CV AAS	Preconcentration by amalgamation or on carbon traps, exhaled breath	Air, flue gases Zvonarić et al.19 Horvat, 1996			

As regards analytical quality assurance, the Department has participated in many intercomparison and certification exercises for total and methylmercury determination in various sample types (Kosta and Byrne, 1982; de Goeij, 1983; Byrne, 1992; 1993, 1994; Horvat and Byrne 1997) including those organised by the IAEA laboratories in Monaco (Horvat et al. 1994 a, b, 1997 Mee et al. 1992, 1994; Coquery et al. 1997, 1998, 1999; Horvat 1999) and in Seibersdorf, Austria (Kosta 1980; Byrne et al. 1984, Byrne et al. 1987; Horvat 1991; Smodiš et al. 1994: Stone et al., 1995). We have also been involved in certification and intercomparison exercises for methylmercury organised by BCR (- Community Bureau of Reference in Brussels), and in the certification programmes of NIST (NBS) Washington, as a co-operating laboratory (Lindstrom et al. 1990; Behlke et al. 1997) and National Institute for Environmental Studies (NIES) in Japan (Yoshinaga et al. 1997).

In our laboratory a reference method protocol for determination of total and methylmercury and selenium in human hair has been developed for the purpose of a project on evaluation of methylmercury in Mediterranean populations and related health hazards, within the framework of the long-term Programme for Pollution Monitoring and Research in the Mediterranean Sea, organised by WHO in collaboration with UNEP (*UNEP/WHO/IAEA Reference Method for marine Pollution studies No. 46: Determination of methylmercury, total mercury and selenium in human hair, 1987*). In addition, the laboratory significantly contributed for the production of a reference method for the determination of methylmercury in marine organisms (*UNEP/FAO/IAEA/IOC, Reference methods for Marine Pollution Studies No 13 rev.1, 1992*). For determination of low level mercury in natural water the laboratory was involved in the validation of the EPA Method No 1633 "*Determination of metry in natural water* and served as the second reference laboratory in an international intercomparison exercises (Bloom et al. 1994, Horvat, 1999).

Current and future research is directed towards improvements of LODs for total and MeHg in natural water samples based on dithizone extraction, where simultaneous determination of both inorganic Hg and MeHg will be optimised. This work will be conducted in collaboration with Dr. Akagi from the National Institute for Minamata Disease.

2. Biogeochemical cycle of mercury in the environment

a) Marine environment

Since the release of heavy metals into the aquatic environment has long been recognised as a major threat to aquatic fauna and human health, particularly in the vicinity of industrial and urban centres, in 1985 in the framework of UNEP MED POL Phase II we started a research project whose main objectives were the establishment of the distribution mercury between sediments, water and marine biota, the uptake of mercury by marine organisms, and bioaccumulation of mercury at different trophic levels in mercury polluted areas. Particular attention was paid to the heavy metals Hg, Cd, and Zn and their binding to proteins by the formation of inducible matallothionein-like proteins. On the basis of results obtained so far the transfer and bioaccumulation at different trophic levels in the area near a chlor-alkali plant can be demonstrated. In co-operation with Croatian marine research centres, namely the Institute of Oceanography and Fisheries (Split), the Marine Research Centre in Rovinj and the Ruder Bošković Institute (Zagreb) we have been engaged in studies of the pathways of mercury and MeHg in the contaminated coastal and open waters of the Adriatic Sea. Mercury and MeHg were determined in various compartments, such as sediments, waters and marine biota (Kosta et al 1978 a, b; Stegnar et al 1981 a, b; Horvat et al. 1989 a, b; Mikac et al. 1985, Vukadin et al., 1982, Zvonarić et al. 1986 a, b; 1987; 1989 b; Zvonarić and Stegnar, 1987). Vertical transport by settling particles in Kaštela Bay, Croatia was also investigated (Tudor et al.

1991). Special attention was directed towards studies of the uptake and distribution of Hg in marine organisms such as mussels, shrimps and various species of marine fish (Škreblin et al. 1985). Formation of inducible metal-binding proteins (e.g. metallothioneins) in selected organs and life stages of *Mytilus galloprovincialis* was thoroughly studied (Pavičić et al. 1985-1994, Tušek-Žnidarič et al. 1983-1986). Also, the use of this mussel as a biomonitor is currently being further investigated (Odžak et al., 2000). Surprisingly high mercury concentrations in some fish species (sea bream, striped mullet) in the southeastern part of the Adriatic (remote from anthropogenic sources) were found, where maximum permissible levels of mercury in seafood are exceeded (Horvat et al., 1989 a, b). This raises the question of possible health risks for populations with a high consumption of fish.

c) Fluxes and fate of Hg in the Idrijca – Soča - Gulf of Trieste system

Recent studies in the Idrijca – Soča - Gulf of Trieste region (Fig. 1) were mainly directed towards quantification of Hg fluxes and better understanding of the fate of mercury, its accumulation in the flood plain and final input to the marine environment. For this reason interdisciplinary collaborative research was initiated with the Marine Biological Station, Piran, Slovenia and the University of Trieste, Italy. An assessment of the extent of contamination of the Gulf of Trieste after the closure of the Hg mine was made. Mercury and methylmercury were measured in various environmental compartments (estuarine and marine waters, sediments, and organisms) during the period 1995-97. Data obtained show that even 10 years after closure of the Hg mine, Hg concentrations in river sediments and water are still very high and did not show the expected decrease of Hg in the Gulf of Trieste (Horvat et al., 1998, 1999). A provisional annual mercury mass balance (Fig. 2) was established for the Gulf of Trieste showing that the major source of inorganic mercury is still the River Soča, while the major source of methylmercury is the bottom sediment of the Gulf (Covelli et al. 1999, 2000; Širca et al. 1996-2000).



Figure 1. Map depicting locations of sampling sites in the rivers Idrijca and Soča and the Gulf of Trieste (Hines et al. 2000).

In addition, the US National Science Foundation is funding a project entitled: Mercury biogeochemistry in the Idrija River system, Slovenia: processes controlling Methylation and Demethylation, for a period of three years (1997 -2000). The USA partner is Prof. Dr. Mark E. Hines from the Department of Biological Sciences, University of Alaska, Anchorage, USA, The primary objective of this project is to investigate MeHg dynamics in the Idrija aquatic system, particularly the determination of factors that control net MeHg production and Hg dynamics. A multidisciplinary study was conducted in June 1998 on water samples collected throughout the Idrija and Soča River systems, and waters and sediments in the Gulf (Hines et al, 2000). As shown in Fig 3. total Hg in the Idrija River increased >20-fold downstream of the mine with methyl Hg (MeHg) accounting for ~0.5%. Concentrations increased again downstream and into the estuary with MeHg accounting for nearly 1.5% of the total. While bacteria upstream of the mine did not contain mercury detoxification genes (mer), such genes were detected in bacteria collected downstream. Benthic macroinvertebrate diversity decreased downstream of the mine. Hg methylation and MeHg demethylation were active in Gulf sediments with highest activities near the surface. MeHg was degraded by an oxidative pathway with >97% of the C released from MeHg as CO₂. Hg methylation depth profiles resembled profiles of dissolved MeHg. Hg-laden waters still strongly impact the riverine, estuarine, and marine systems. Macroinvertebrates and bacteria in the Idrija River responded to Hg stress, and high Hg levels persist into the Gulf. Increases in total Hg and MeHg in the estuary demonstrate the remobilization of Hg, presumably as HgS dissolution and recycling. Gulf sediments actively produce MeHg, which enters bottoms waters and presumably the marine food chain (Hines et al. 2000). The project has been extended for an additional 3 years, mainly focusing on the role of river bank soil Hg transformation mechanisms.
Recently a new Coordinated Research Programme of the IAEA was initiated entitled "Health impacts of mercury cycling in contaminated environments studied by nuclear techniques" in which our laboratory will further study the biogeochemistry of mercury in the contaminated environment of Idrija and the Gulf of Trieste, with a major focus on methylation and demethylation mechanisms using nuclear techniques.



Figure 2. Mercury mass balance in the Gulf of Trieste (Širca et al. 2000)



Figure 3. Concentrations of total mercury (Hg) and methylmercury (MeHg) in unfiltered samples of river and marine waters in the Idrija river - Gulf of Trieste ecosystem.

c) Mercury modelling

In collaboration with the Faculty of Geodetic and Civil Engineering of the University of Ljubljana extensive measurements in the Gulf of Trieste have been accompanied by the development of modelling of the transport and fate of mercury in the Gulf. Two- and three-dimensional models have been developed to include the influence of significant advective transport due to currents. It was shown that wind, thermohaline forcing, and the river Soča momentum are the most important forcing factors.

A two-dimensional model simulated the transport of non-methylated and methylated mercury in dissolved, particulate and plankton fractions. Mercury processes included the input of atmospheric mercury, sedimentation, reduction, methylation and demethylation. The model simulations gave basically what were the correct trends of the phenomena; quantitatively the measured and computed results are mainly within a factor of three. To simulate the non-uniform distribution of parameters over depth, an existing three-dimensional (3D) hydrodynamic and transport-dispersion (TD) model called PCFLOW3D was adapted and applied. As it was found that most mercury transport is related to suspended sediment particles, a new 3D-sediment transport module was also developed and included in the model. Comparison with measurements was only partly possible, but the computed and measured results were mainly within a factor of two and the simulations resulted in the proper trends of the phenomena. The combination of modelling and measurements has resulted in some interesting conclusions about the transport and fate of mercury in a coastal sea (Rajar et al. 1998, Širca et. 1998-2000, Žagar et al. 2000).

Similar work was conducted to study the fate and transport of Hg in Lake Velenje, Slovenia, that is impacted by the nearby Šoštanj coal burning power plant (ŠTPP). The mass balance of Hg and its compounds showed that over 350 kg of Hg is emitted from the ŠTPP annually (Kotnik et al., 1999). Measurements of Hg and its compounds in various environmental compartments (precipitation, air,

soil, lake water, sediments) showed that Hg emitted from the ŠTPP has very limited influence on Lake Velenje, but is rather transported over long distances.

c) Terrestrial environment

Mercury distribution and uptake by plants, including mushrooms, and various organisms of the contaminated Idrija region and control areas in Slovenia has been the subject of various studies (Kosta et al. 1972, 1974 a, b; 1978 b; Byrne and Kosta 1970, Byrne et al. 1971, 1975, 1976, 1995; Byrne and Tušek-Žnidarič, 1990, Božič et al. 1990, Stegnar et al. 1973). More recently (1992-1997), long-term monitoring of total and MeHg of the terrestrial soil-vegetation-herbivorecarnivore food web with regard to accumulation and transformation processes was studied (Gnamuš et al, 1995, Gnamuš and Horvat, 1999, Gnamuš et al. 2000). Assessment of the inhaled and ingested contribution of mercury from the environment in roe deer, the selected wild mammal species living in these areas, showed that while the ratio between these two routes of uptake is relatively constant, food intake of mercury in roe deer is much more important than inhaled mercury, which represents only up to 0.2% of ingested Hg. Although the plant species comprising roe deer foodstuffs were not active accumulators of mercury from soil or air, vegetation mediates significant transfer of Me-Hg to herbivores, and this becomes subject to further accumulation in the higher trophic levels of this food web. Beside roe deer other bioindicators such as chamois were selected to confirm the uptake of mercury from plants. Though the conclusions drawn from the carnivorous predators lynx and wolves are limited due to the limited number of subjects (8 and 2, respectively), the results and their comparison to other environmental data showed the transfer of Hg from soil (and air) to vegetation, herbivores and carnivores further up the food web. The results of the measurements, as well as concentration factors (CF) and bio-accumulation factors (BAF) show explicit accumulation of MeHg and less distinctive accumulation of total Hg at higher trophic levels of this terrestrial food web. Interestingly, higher accumulation of MeHg was observed in those environments polluted with high concentrations of inorganic mercury compared to less contaminated and control areas (Gnamuš et al, 2000).

Genotoxicity studies were also conducted in vitro in fish cells exposed to mercury, methyl mercury and selenium (Al-Sabti, 1994, 1995).

c) Biomonitoring of Hg in air

The use of epiphytic lichens as biomonitors of Hg concentrations in air was studied in two Hg contaminated areas; (1) the Hg mining area of Idrija, Slovenia, (Lupšina et al. 1992) and (2) near the natural gas treatment facilities at Molve, Croatia, where concentrations of Hg in natural gas are very high and therefore has to be removed from natural gas before further processing in order to prevent technological and environmental problems (Horvat et al., 1997, 2000). *In-situ* lichens *Parmelia sulcata, Xantoria parietina* and *Hypogymnia physodes* and transplanted lichen species *Hypogymnia physodes* were used. A good correlation between mercury concentrations in air and lichens was found. It was confirmed that lichens can successfully be used as bioindicators, provided careful experimental design is used, particularly the amount of lichens transplanted, the duration of exposure and the initial levels and homogeneity of the transplanted lichens. Further studies are currently in progress in order to explain the occurrence of MeHg in lichens, mainly to elucidate whether MeHg is deposited from precipitation and/or formed by the lichen species.

3. Exposure to mercury vapour

In Slovenia there exist several sources of mercury which influence the levels of this element in local populations or in exposed professional workers; for example, the Hg mine in Idrija, or several chlor-alkali plants where severe exposure to Hg vapour can occur in the workforce. Professional exposure of workers in dentistry was also studied (Gašperšič et al. 1972, Klemenc et al. 1992). In our laboratory we have developed methods for measurement of the degree of exposure, for improving protective measures and working practices, and of industrial hygiene (Kobal et al. 1996, 1999). Control includes measurement of mercury in blood, urine, expired and ambient air, as well as enzymatic studies of blood and urine. Ambient and biological monitoring was performed in two former Yugoslav chlor-alkali plants, and health protection measures were organised in order to improve the health and working capability of workers professionally exposed to mercury vapour. Possible methylation in vivo of inorganic Hg in professionally exposed workers was also studied in collaboration with Swedish scientists (Barregard et al. 1994).

A preliminary survey of lymphocyte chromosomal damage in Slovenian workers exposed to occupational clastogens, including exposure in the Hg mine, was also performed (Al-Sabti et al, 1992).

Studies of mercury uptake and its distribution in the organs of experimental rabbits (Stegnar et al. 1973) and rats were conducted in the Idrija mining region. The effect of mercury on the tissue and subcellular distribution of endogenous copper, zinc and selenium, and the presence of Hg, Cu, Zn - metallothionein in the kidney and brain of rats exposed to mercury vapour was followed (Kralj-Klobučar et al. 1991, Prester et al. 1993; Falnoga et al. 1993, Škreblin et al., 1989). Modelling and computer simulation of mercury toxicokinetics in Wistar rats exposed to elemental mercury was investigated during and after exposure (Falnoga et al.1994)

Recently, an international study conducted by IARC (International Agency of Cancer Research in Lyon, France) which also included 1589 workers of the Idrija Mercury Mine, found a relation between long-term exposure to Hg° vapour in mercury mining and increased mortality due to ischaemic heart disease (standardised mortality ratio 1.66, 95% confidence intervals 1.35 - 2.02). This result is specific only for workers in the Idrija Mercury Mine, and not for workers from the mercury mines and mills of Spain and Italy. We presume that the differences in mortality ratio of workers caused by ischaemic heart diseases between observed mines and mills could be connected with different national nutritional habits. Therefore the principal objective of our future research is to test the hypotheses that the accumulated level of Hg° in previous and present occupational and ambient exposure promotes lipid peroxidation and reduces the antioxidative capacity, which could increase the risk of cardiovascular disease (CVD). This work is being conducted in collaboration with Dr. Kobal from Idrija, Mercury Mine in Idrija, and the Faculty of Medicine in Ljubljana.

4. Other human health-related studies involving mercury

On the basis of our long term experience with analytical techniques, a Reference Method for the determination of Hg, Me Hg and Se in human hair was prepared for WHO, for use in connection with the joint project (MED POL Phase II) on biological monitoring of Me Hg in Mediterranean populations. As a reference laboratory for this project, we analysed I300 hair samples for Hg, Me Hg and Se from Italy, Greece and Slovenia (Buzina et al. 1995 a,b). Studies were also directed towards pregnant women (from the central Adriatic islands) and the foetus, identified as groups at special risk, as it is considered that prenatal life is much more sensitive to Me Hg than adults (Dermelj et al. 1987, Horvat et al. 1988a, 1989b, 1991). Results for total mercury, MeHg and Se in hair (scalp, pubic), blood (maternal, umbilical cord) and placenta at parturition were obtained. In

addition, analyses of some other elements which can also have an important role during pregnancy (As, Cd, Sb, Cu and Zn) in placental and blood samples were performed. Active transport of Me Hg across the placental barrier to the foetus was confirmed even at low concentration levels. The applicability of the Hg content of hair as an integrator of exposure over a period of time has been proved in many studies. The positive correlation between Hg concentration in hair and placenta, which was found in this work, suggests that more attention should be given to the significance of Hg and Me Hg concentrations in placenta as an estimate of exposure of the infant during pregnancy. Further discussion of this problem must await the results of additional studies, which are now in progress in our laboratory and elsewhere. In 1999 a new project was initiated by the University of Udine in order to assess the exposure of the critical population bordering the northern part of the Adriatic Sea (e.g. Marano and Grado Lagoons).



Figure 4. Hg/Se molar ratio as a function of the mercury concentrations in post mortem human organs collected in the last decade

Some twenty years ago we started to study the presence of mercury and some other elements in human organs obtained post-mortem from Idrija miners and Idrija residents (Kosta et al. 1975, 1985, Falnoga et al, 1997, 1998, 2000). We reported data showing that selenium was co-accumulated with mercury in an approximately molar ratio in human subjects highly exposed to inorganic mercury as mercury vapour in those organs which accumulated and retained the highest amounts of mercury, namely the pituitary, thyroid, kidney and some parts of the brain. Fig. 4 presents the Hg/Se molar ratio as a function of the mercury concentration in human organs collected in last decade. From Fig. 4 is evident that that the molar ratio was about 1 or higher in those tissue samples where the Hg concentrations were higher than $1 \mu g/g$ (fresh weight).

In recent studies we have also expanded our investigations on subcellular level after tissue homogenization and centrifugation. In one such investigation after tissue separation of samples obtained from retired miners the majority of mercury and selenium was found in the cell particulate phase (pellet) (Fig. 5), presumably in lysosomes or nuclei. Because of the absence of toxic symptoms it seems that the presence of co-accumulated endogenous selenium can protect from the harmful effects of accumulated and retained Hg. Regarding cellular defence mechanisms towards mercury ions beside selenium antagonism we have also studied the induction of protective

proteins - the metallothioneins (MT). In experiments with rats exposed to high concentrations of mercury vapour we identified the induction of Hg,Zn,Cu - thioneins in rat brain and in mercury exposed human found the presence of thyroid Cu,Zn,Hg – thioneins (Falnoga et al. 1993, 1997). Now we are examining the possibility of MT induction after exposure of experimental animals to high concentrations of MeHg.

In connection with selenium our most recent activities are focused on plasma selenoprotein P. In the recent literature it is hypothesised that plasma selenoprotein P (SelP) may play an important role in Hg sequestration in the blood stream and consequent accumulation. So at present determination of plasma selenoprotein P and its binding of Hg (or Cd) in populations exposed to elevated concentrations of Hg in their environment is under way.



* as the concentration in cytosol was under the detection limit (< 10 ng/g) the percentage distribution is given approximately; ND – nucleus dentatus

Figure 5. Hg and Se in human samples of retired miners: percentage distribution between cytosol and pellet

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Small- Scale Gold Mining and Its Environmental Impacts in Tanzania

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Abstract

Small-scale gold mining provides self-employment and means of subsistence to over 300,000 people in Tanzania. The mining has been largely semi-formal and sporadic and hence difficult to regulate. Annual gold production exceeded 4 tonnes during the gold-rush period between 1989-1995, but has declined in recent years due to the depletion of near-surface gold-rich deposits.

Despite of the positive contribution of the small-scale mining to the national economy, the mining operations have caused significant environmental impacts. Land degradation by extensive pitting and trenching, tree cutting for construction of shelters, firewood and charcoal burning and timbering of mine pits is among the negative impacts. Processing of gold-ore along rivers has caused siltation and diversion of seasonal rivers, apart from causing coloration and increasing suspended load in water.

The major chemical impact from the mining operations is the contamination of soil, rivers and air by mercury and the enhancement of heavy metal load in river sediments. The aqua regia leachable heavy metals Pb, Cu, Cr, Cd, and Zn already show enrichment factors of 1.5 to 16 in contaminated river sediments relative to uncontaminated sediments.

Mercury contamination factors relative to background mercury concentration vary from 1.1 to 138 in river sediments and from 93 to as high as 612 in highly contaminated soils around gold-mercury amalgam firing places.

Increased incidences of inorganic mercury exposure are reported in the gold miners, especially those who work in the amalgamation and burning of amalgams. In a recent study 36% of the occupational exposed group exceeded the WHO guideline concentration of 50 μ g Hg/g creatinine in urine.

Preliminary biological monitoring of mercury pollution in the Lake Victoria gold fields revealed suprisingly low total mercury concentrations in fish (2-20 Hg μ g/kg) and human hair (160-5400 μ g/kg) which reflect background levels. The fish-mercury concentration suggests extremely low background environmental methylmercury that is available for bioaccumulation in the Lake Victoria region.

The dynamics of mercury transformation, distribution and accumulation in various environmental compartments still need to be clarified in order to gain better understanding of the long-term impacts of mercury pollution due gold mining operations in Tanzania and other tropical regions of Eastern and Southern Africa.

Keywords: Gold mining; environmental impacts; methylmercury; Lake Victoria.

1. Introduction

It is estimated that more than 300,000 people were engaged directly or indirectly in small-scale gold mining activities in various parts of the country during the gold rush period between 1989 and 1995.

Official gold exports increased from 112 kg in 1989 to 3,779 kg in 1991 and up to 4,525 kg in 1992 (ESRF, 1997) due the liberalisation of gold trade and the involvement of the Bank of Tanzania (BoT) in the purchase of gold directly from the small-scale miners. The withdrawal of BoT from the gold buying business in the mining areas led to the decline of official gold exports from 3,364 kg in 1993 to 320 kg in 1995, as only few licensed buyers remained in the business.

Since 1995 joint-venture undertakings in mining between local miners and foreign companies and the acquisition of large tracts of land and gold prospects by big exploration companies have significantly reduced the operations of small-scale miners in the country.

Although haphazard small-scale mining has been publicly criticised for environmental destruction and pollution, very few studies have been done to evaluate and quantify the impacts of past and present mining activities on the physical, chemical, and biological environments and land use.

This paper reviews the existing information and data on the environmental impacts of small-scale gold mining in the country, charts out strategies for evaluating the impacts, and recommends measures for reducing or mitigating adverse impacts, with special attention to mercury pollution.

2. Gold Deposits and Mining

2.1. Gold Deposits

Gold deposits are concentrated in three major goldfields, namely the Lake Victoria goldfields (LVGF), Mpanda mineral field (MMF) and Lupa goldfield (LGF). In addition to these three main gold producing areas, gold mineralisation occurs in the central part of Tanzania in Dodoma region and in the southern part of the country in Mbinga district (Fig. 1).

The LVFG comprise of gold producing areas located to the east or south of the Lake Victoria, within the Archean Nyanzian-Kavirondian greenstone belts. Primary gold mineralisation occurs in quartz veins and replacement sulfide ores in shear zones. Primary gold is also found in disseminated form and veins in banded iron formation (BIF). Gold-rich deposits of secondary origin also constitute an important source of gold in the LVGF. Secondary deposits include eluvial, alluvial and lacustrine gold placers, and gold-bearing laterites (Barth, 1990).

The MMF is a polymetallic gold-base metal district within the Ubendian belt of western Tanzania. Primary gold mineralisation occurs in quartz or carbonate-quartz veins that are closely associated with Cu-Zn-Pb sulfides. Eluvial and alluvial gold placers are also being worked in the MMF.

The LGF is located within the Ubendian belt in the southwestern part of the country, south of Lake Rukwa. Gold is mined from primary vein deposits as well as from secondary deposits derived from the weathering of primary ores. Gold-rich alluvial placers are important sources of gold production in various parts of the LGF.



Figure 1. Map showing the distribution of artisanal gold mining centers in Tanzania.

2.2. Gold mining and ore-processing

Alluvial gold mining is carried out in old river channel deposits or in recent riverbed sediments. Opencast mining by pitting and trenching is widely used in the extraction of gold ore from the alluvial deposits. Gold recovery from the alluvial ore is accomplished by simple panning using gold-pans or by washing the ore on sluice boxes.

The mining of primary gold ore involves sinking a shaft (1 m x 1m) through a mineralised bedrock and extraction of gold ore through underground drifts and adits. The mining is labour intensive, carried out using rudimentary tools such as picks, shovels, chisels, steel rods, hand hoes and hammers.

The processing of primary gold ore involves manual crushing of the ore with hand hammers and grinding in wood mortars using old motor vehicle excels as pestles. In a few cases the grinding is done in locally fabricated ball mills connected to tractor or motor vehicle axles. After grinding, the ore is screened and the fine-grained fraction is washed on sluice boxes to obtain a heavy mineral concentrate with gold. The gold is finally recovered from the concentrate by mercury amalgamation.

After the amalgamation, the gold-mercury amalgam is fired in open air to evaporate the mercury, leaving behind gold bullion pellets or nuggets. At this stage the gold is ready for sale to brokers or dealers.

3. Environmental Impacts

Few studies in Tanzania have evaluated the impacts of the small-scale gold mining operations on the environment and human health. The first environmental survey of the gold mining areas was conducted by the National Environment and Management Council in collaboration with the University of Dar es Salaam between 1992 and 1994 (Ikingura, 1994). The Department of Geology of the University of Dar es Salaam conducted a broad environmental study of the mining areas from 1993 to 1997 with the sponsorship of the Swedish Agency for Research Co-operation with Developing countries (SAREC). These two studies dealt with the effects of mining on the quality of the physical and chemical environment.

The DHV Consultants BV of Netherlands (1998) conducted a study of the impacts of mining on the environment and human health in the Shinyanga region of northern-central Tanzania. Their technical report gave a qualitative and quantitative assessment of the environmental impacts.

Three studies have been published on the monitoring of mercury exposure in fish and human population in the Tanzanian gold fields (Harada et al., 1999, Kahatano et al., 1997, Ikingura and Akagi, 1996).

The results from different studies revealed a number of environmental and health impacts that are caused largely by poor small-scale mining practices, population pressure in the mining areas, and gold extraction using mercury. The major impacts are discussed below.

3.1. Impacts on the physical environment

The impacts of small-scale gold mining operations on the physical environment range from land degradation by deforestation, pitting, trenching, and enhanced soil erosion, through water pollution

by gold panning along river banks and discharge of tailings into the rivers, to air pollution and loss of habitats.

Deforestation in the mining areas is largely due to the high demand of wood for firewood, charcoal burning and construction of shelters and to a lesser extent timbering of mine pits and shafts.

Abandoned pits, trenches and shafts in the mine areas are usually left unattended. This causes loss of land for agriculture and other uses. Abandoned mine excavations also pose danger to people and animals, especially when the excavations are obscured by vegetation.

The physical pollution of rivers is manifested by the reduction of water quality through coloration and increase in turbidity due to suspended particulate matter or colloidal material from the panning and washing of gold ore. The siltation of rivers by ore tailings changes river courses or drainage systems. Brokerage of river channels by the tailings cause ponding of water and creates a breeding ground for vectors of various diseases.

Physical air pollution in the mining areas is due to the emissions of particulate matter during crushing and grinding of gold ore. Thick deposits of dust are commonly found on vegetation cover and on roofs of houses or huts around ore processing sites.

Loss of tree and grass cover due to over population and mining activities enhances soil erosion and as a result decreases soil fertility.

The influx of thousands of people in newly discovered mine areas generally exerts tremendous pressure on local natural resources and results in land degradation and loss of animal and plant habitats.

3.2. Impacts on the biological environment

The impacts of the mining operations on the biological environment have not been studied. It is however likely that the deforestation taking place within and around the mining areas could cause loss of rare tree species or induce changes in animal habitats.

Physical pollution is reportedly to have caused decrease or disappearance of some fish species in the affected rivers.

As no environmental impact assessment studies are undertaken prior to the commencement of the mining operations it will always be difficult to make an accurate evaluation of the impacts of the mining activities on the biological diversity. In spite of this short-coming it is important to conduct studies on the biological impacts before devising any counter measures.

3.3 Impacts on the chemical environment

Mining and mineral processing operations generally enhance release of heavy metals into the environment because of oxidation of rocks and ores brought at the surface from underground and increased chemical reactivity of crushed mineral compounds in mine tailings. Discharge of waste and process water contaminated with chemicals is another source of heavy metal pollution.

The results of aqua regia leachable heavy metal analysis of Pb, Cu, Cr, Cd, and Zn in river and stream sediments from the three principal gold fields (LVFG, MMF, LGF) indicated that the contents of these metals were generally within the range of concentrations reported from uncontaminated sediments in other parts of the world (SAREC Project, 1997). There were however a 1.5 to 16-fold increase in the concentrations of these metals in sediment samples collected from sites of active gold ore washing and panning in comparison with the background concentrations in samples collected upstream away from the processing sites. This indicated enhanced increase of the heavy metal load in the sediments because of gold ore processing activities.

Mercury concentrations in the river sediments ranged from 0.02 to 136 mg/kg (average 1.91 mg/kg). These mercury concentrations are already high in comparison with an average of <0.30 mg/kg from world non-contaminated river sediments. Mercury contamination factors (relative to background mercury levels) ranged from 1.1 to 138 in the river sediments and from 93 to 612 in highly contaminated soils around amalgam firing places.

As there are no known rocks with high Hg content that could be the source for elevated Hg levels in the river sediments, the Hg contamination in the sediments is certainly of anthropogenic origin, directly related with gold extraction by amalgamation in the gold fields.

The mercury contamination in the gold mining areas is attributed to: (i) mercury vapour produced from firing of gold-mercury amalgams, (ii) metallic mercury left behind in the mine tailings during amalgamation and, (iii) spillage of metallic mercury through poor handling by artisanal miners.

Estimates of mercury releases into the environment in the gold fields are in the order of 4 to 6 tonnes annually based on the gold production of 3 to 4.5 tonnes per year from the small-scale mining. According to Pffeifer and Larceda (1988), about 1.3 kg of mercury is lost to the environment for every 1kg of gold produced by amalgamation. About 40% of the mercury losses are thought to occur during the amalgamation process whereas the remaining 60% occur during the burning of the amalgams.

3.4. Impacts on human health

The potential impacts of the small-scale mining operations on human health are related to: (i) poor mining conditions including inhalation of air containing high concentrations of silica dust, (ii) occupational exposure to mercury during amalgamation and burning of amalgams, (iii) public exposure to mercury through consumption of mercury contaminated fish from rivers, ponds and swamps in the mining areas, (iv) domestic use of mercury contaminated water, and (v) poor sanitary conditions.

No studies of health impacts due to mining and sanitary conditions in the gold fields have been published. There is a potential risk for silicosis and other dust-related ailments in the miners who are frequently exposed to high levels of dust during mining and grinding of the gold ore.

Poor sanitary conditions, excessive alcoholism and promiscuous sexual acts have caused epidemics such as diarrhoea and sexually transmitted diseases (STD) according to health officials in the clinics close to the mining areas.

The occupational exposure to mercury in gold miners in Tanzania has been documented in studies conducted by Ikingura and Akagi (1996), Kahatano et al (1997), DHV Consultants BV (1998) and Harada et al. (1999).

Ikingura and Akagi (1996) found abnormally high concentrations of total mercury in urine (average 241 μ g/litre) from workers who were occupationally exposed to Hg vapour during amalgamation and burning of gold-mercury amalgams at the Mugusu gold mine in the Lake Victoria gold fields. The mine inhabitants who were not occupationally exposed to mercury had low urinary mercury levels (average: 2.6 μ g/litre). Subsequent studies by other researchers (Kahatano et al., 1997) reported similar findings.

In a recent study by DHV Consultants BV (1998), 36% of the occupationally exposed group exceeded the WHO guideline (1980) concentration of 50 μ g Hg/g creatinine in urine. Incidences of mercury toxic effects increase when urinary Hg concentration exceeds 50 μ g Hg/g creatinine.

Harada et al. (1999) have reported clinical symptoms of inorganic mercury poisoning in the gold miners who are frequently exposed to mercury in the Lake Victoria gold fields.

Low total mercury (T-Hg) and methylmercury (MeHg) concentrations have been found in the human hair (T-Hg < 5,400 μ g/kg, MeHg < 7-82%) and fish (T-Hg < 25 μ g/kg, MeHg 55-95%) samples from the Lake Victoria gold fields (Ikingura and Akagi, 1996, Kahatano et al., 1997, DHV Consultants BV, 1998). This suggests that the risk of environmental methylmercury exposure in fish and the inhabitants of the gold mining areas is still very low at present. The risk however may increase in future due to the biogeochemical transformation of inorganic mercury accumulated in river and lake sediments to methylmercury. Periodic monitoring of total mercury and methylmercury in fish and other aquatic biota is therefore still important in the gold mining areas in order to evaluate the potential risk of human exposure to methylmercury through fish consumption.

4. Discussion and Recommendations

The existing information and data on the environmental impacts of the small-scale mining operations in Tanzania have been reviewed in this presentation. Areas where there is scarcity of data and which need research efforts are pointed out.

Our knowledge of the impacts of the mining operations on the biological environment is generally still poor. Also the dynamics of mercury transformation and distribution in the aquatic environment of our tropical region are not understood. For example we do not understand why fish from the Lake Victoria areas contain extremely low total mercury concentrations, mostly in the 10-20 μ g/kg range, despite of the large mercury input into the environment from the mining operations.

Further research efforts should be directed towards understanding the pathways and accumulation mechanisms of mercury in the local tropical environments. Environmental factors which favour the transformation of inorgarnic mercury to methylmercury and the partitioning of the methylmercury in aquatic systems need to be identified and evaluated both in field and laboratory experiments in order to gain better understanding of mercury cycling in the tropical region of Eastern and Southern Africa.

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Atmospheric Mercury in Northern Europe-Concentrations and Time Trends

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Atmospheric concentration of mercury have decreased significantly in northern Europe over the last decade. Gas-phase concentrations and wet deposition fluxes have decreased by at least 40% on the Swedish West coast. A significant geographical trend with higher wet deposition in southern Sweden and lower in the north still exists indicating that European source areas are still influencing the mercury levels in Sweden. Recent data suggests that this gradient is mainly caused by emissions of oxidized and particulate phase mercury since these species are more readily removed from the atmosphere by wet deposition. Total gaseous mercury in air is more evenly distributed which reflects the long atmospheric lifetime and global transport scale of this species.

Investigation into Suspected Mercury Contamination at Sihanoukville, Cambodia

National Institute for Minamata Disease

(This Mission Report was prepared in collaboration with the following Study Group) Study Group for WPRO-Project Proposal (1998)

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1. Background

On 28 November, 1998, a ship arrived in Sihanoukville with 2,779 tons of waste from Taiwan. According to information obtained from the Ministry of Environment in Cambodia the waste was old byproducts from battery production. The English language newspaper 'Cambodia Daily' described it as "waste which was left over from treating alkaline with mercury and had been encased in cement in Taiwan for two decades." It had been impossible for the Ministry of Environment to obtain any more exact information on the content since the Taiwanese Company, Formosa Plastic, has refused to cooperate.

The ship was unloaded on 4 December, and the contents rather huge, solid plastic bags were transported to a site approximately 15 kilometers outside of Sihanoukville, and dumped on a field 500 meters from Road No.4. About 200 workers were involved in the transportation and unloading of the cargo. In the process the plastic bags were opened and purloined as valuable items, and much of the solid content broken into pieces of various sizes, creating much dust and sand-like particles.

According to the Director of Health in Sihanoukville, 10 patients admitted to the provincial hospital with symptoms of poisoning and 1 has died. The main symptoms were vomiting, diarrhea and dyspnea. The patients were all among the workers who

transported the waste or local people who took part in the looting of the plastic bags.

It seems plausible that the process of opening the bags created dust particles that were inhaled by the workers, and this may have caused acute poisoning. The symptoms are compatible with mercury poisoning, but more detailed patient histories should be obtained.

The blocks are of all different sizes, from approximately 1/2 cubic meter down to brick size. The waste covered approximately 500 square meter and an earth wall is being constructed around it. The site has a slight slope, and is situated in a hilly area with lower lying land surrounding it. The workers constructing the earth wall with heavy machinery used plastic gloves and paper face masks (some in a rather invalid way). The soldiers and policeman guarding the area had no protection, and just a few hundred meters away there were children playing and cattle grazing.

The main purpose of this investigation was to make preliminary recommendations to contain possible risks to the environment, population in Sihanoukville and workers at the site. Five samples of the waste, and 3 samples of water from well, and tap water supplied to the people around the site were collected for further analysis in Japan. The evaluations of the potential hazards of mercury exposure to the port and site workers were performed by analyzing mercury concentrations in red blood cells, serum, urine and hair. As a control, 5 hair samples of villagers working in the center of the Sihanoukville about 5 km away from the site were selected for the purpose of comparison. All the samples were analyzed at the NIMD to determine the mercury concentrations. Waste samples were further analyzed for cadmium, manganese and nickel. In addition, Dr K E Mony and Dr P P Raingsey from the Ministry of Health interviewed the port and site workers regarding health condition. This report summarizes the results of the findings.

2. Mission objectives

The objectives of the mission are as follows:

- I To make preliminary recommendations to contain possible risks from the waste in Sihanoukville.
- II To analyze the waste samples for mercury and some other metals.
- III To carry out analyses of the water samples for mercury.
- W To conduct a health assessment of the port workers and soldiers to ascertain whether they are suffering from mercury poisoning.
- V To make further recommendations to contain possible risks from the waste in

Sihanoukville based on the results analyzed at NIMD.

3. Survey

3-1. Sample collection

Sihanoukville is about 10 km south of the waste dump site. About 3,000 tons of the waste was dumped on the hill. We collected two gray solids, which were the main component, of the waste, two black solids and sand-like waste. Water samples from the wells and tap water near the dumping site were also collected. Hair, urine and blood samples of 9 port workers who have complaints after the work of unloading or cleaning the waste were collected. Hair, urine and blood samples of 5 workers/soldiers involved in the clean-up operation at the site were also collected. Hair samples from 5 villagers at the center of Sihanoukville were collected as a control.

Waste

Although the plastic bags were partially broken most of the waste in the bags looked homogeneous. Most of the waste was fragile and gray solid, which accounts for more than 95% of the entire waste. Also, the fragile and gray solid contained some broken coal-like pieces, which were black and very hard. The remaining 5% or so of the whole waste was sand-like brown waste.

About 100 g of waste samples of was collected into pre-cleaned plastic bottles and stored in an icebox with ice cubes.

The property and color of the samples taken were:

- ① Fragile and gray solid (A part of main component of the waste)
- ② Fragile and gray solid (A part of main component of the waste)
- ③ Hard and back solid (Pieces found in the main waste)
- ④ Hard and back solid (Pieces found in the main waste)
- (5) Sand-like brown waste (A small part of the waste)

(1) and (2) account for more than 95% of the waste.

Water

Samples were collected in a 100 ml pre-cleaned bottle, then stored in an icebox, avoiding sunlight exposure until analysis.

Water samples were taken from:

① Well water 200-300 m below the site (for both drinking and washing by

woodcutters).

- ⁽²⁾ Tap water in a Pagoda (supplies both drinking and washing for priests and soldiers working in the site).
- 3 Well water 200 m below the Pagoda (for both drinking and washing by a small village)
- ④ Tap water for inhabitants living 1.5 km away from the site; another side across Road No.4. (A control without the waste contamination).

Blood

About 10 ml of blood sample was collected by syringe and transferred into sample tubes including heparin. All the samples were stored in icebox with ice cubes.

Urine

Urine was collected in a paper cup. From that cup, 5 ml of the urine was transferred into sample tubes. All the samples were stored in an icebox with ice cubes.

Hair

About 0.5 g of hair was cut with scissors from the root and taken into small sealed bags as samples.

Health conditions

Two doctors from Ministry of Health of Cambodia also interviewed workers at the port and site for their health conditions.

3-2 Mercury analyses

Total mercury

(a) Biological samples

A known amount of samples (1 - 10 mg of human hair, 0.5 g or less blood, fish) is placed in a 50 ml volumetric flask, to which 2 ml of nitric acid-perchloric acid (1+1), 5 ml of sulfuric acid 1 ml of water are added and heated at 230 - 250°C on a hot plate for 20 minutes. In the case of urine sample, the mixture of these acids are placed first in the 50 ml volumetric flask, to which 1 - 5 ml of urine sample is added dropwise with stirring the mixture and then digested in the same manner as described above. After cooling, the digest samples is made up to 50 ml with mercury free water. An aliquot of the sample solution is introduced into automated circulating airflow system with addition of 10% stannous chloride solution. After air was circulated for 30 seconds, the circulating air is measured with cold vapor atomic absorption spectrometry. The detection limit is around 1 ng/g for 0.5 g samples.

(b) Water

For water analysis, preconcentration is carried out by dithizone extraction. Mess 100 ml of a water sample in a separately funnel is mixed with 0.5 ml of 20 N sulfuric acid and 0.25 ml of 0.5% potassium permanganate and allowed to stand for 5 minutes. The treated sample is then neutralized with 1 ml of 10 N sodium hydroxide and 0.25 ml of 10% hydroxylamine hydrochloride and allowed to stand for 20 minutes. After addition of 5 ml of 10% ethylenediamine tetraacetic acid tetrasodium salt, the sample is extracted with 0.5 ml of purified 0.01% dithizone in benzene. The sample was left until it separated and the water layer was discarded. A known volume of benzene layer is transferred into a 10 ml volumetric flask and evaporated to dryness using a rotary evaporator. The residue in the volumetric flask is digested with nitric acid - sulfuric acid -perchloric acid system, diluted and measured by cold vapor atomic absorption spectrometry in the same manner as described in above.

(c) Other

Total mercury in other environmental materials (0.5 g or less of biota, 1 - 2 g of sediment) is measured by the same method as outlined for biological samples.

(2) Methylmercury

The procedure for methylmercury analysis developed in our laboratory is based on the combined techniques of dithizone extraction and ECD gas chromatography. For hair methylmercury, we have developed a simple and rapid technique by the combination of hydrochloric acid - benzene extraction and ECD gas chromatography.

Gas chromatographic conditions for the methylmercury measurement are as follows. Column: Glass column (100 cm \times 3 mm I.D.) packed with Hg-20A (Gasukuro Kogyo Inc., Tokyo, 60 - 80 mesh) and about 0.2 g of NaCl crystal on the top of the column, Column temperature: 150 - 155°C; injection port temperature: 180°C; detector oven temperature: 200°C; carrier gas (N₂): 35 - 40 ml/min.

(a) Human hair

Hair samples (10 - 20 mg) were put into 10 ml test tube with a screw cap, to which 2 drops of ethanol, 5 ml of 2 N hydrochloric acid and a small amount of cotton are added to prevent the floating of hair sample. The test tube is capped, and then heated 100° C for five minutes. After cooling, 1 ml of the 2 N hydrochloric acid extract is transferred to another 10 ml test tube with screw cap and extracted with 4 ml of benzene. Methylmercury in the final benzene extract is measured by ECD - gas chromatography.

(b) Other biological samples (fish, blood, urine etc)

A known amount of blood or fish (usually 0.5 g or less) is digested with 10 ml of 1 N potassium hydroxide in ethanol in a 50-ml screw-capped centrifuge tube at 100°C on a water bath for 1 hour. The digested sample is mixed with 1 ml of 20% ethylenediamine tetraacetic acid tetrasodium salt (EDTA) and then slightly acidified with 10 ml of 1N hydrochloric acid. After washing with 5 ml of n - hexane, the methylmercury in the sample is extracted by 5 ml of 0.05% dithizone - benzene. Urine sample (usually 20 ml) is simply shaken with 10 ml of 1 N potassium hydroxide for 15 minutes using a mechanical shaker. The sample is mixed with 1 ml of 20% EDTA and then slightly acidified with 10 ml of 1 N hydrochloric acid, followed by the extraction with 0.05% dithizone benzene. Each benzene layer is then washed twice with 3 ml of 1 N sodium hydroxide to remove excess dithizone in the benzene layer. An aliquot of the benzene layer (usually 3 ml) is transferred to 10 ml test tube with cap and back - extracted with 2 ml of 5 ppm sodium sulfide in 0.2 N NaOH - ethanol (1+1). After centrifugation, the benzene layer is removed, 1 N hydroxide acid is added dropwise until a blue color appears with bubbling nitrogen gas thorough the solution, and bubbling is continued for three more minutes to eliminate the excess sulfide ions as H₂S gas. To the sample solution, 2 ml of Walpole's buffer (pH 3.0) is added and the mixture is re-extracted with 0.05% dithizone in benzene purified with an equal volume of 0.2 N sodium hydroxide just before use. The benzene layer is washed twice with 2 ml of 1 N NaOH and subsequently with 4 ml of distilled water. After acidifying with a few drops of 1 N hydrochloric acid, methylmercury in the benzene layer is measured by ECD - gas chromatography.

(c) Sediment (waste)

Sediment samples (1 - 5 g) were simply shaken with 10 ml of 1 N potassium hydroxide for 15 minutes using a mechanical shaker. The treated sediment sample with 1 N potassium hydroxide in ethanol was bubbled with nitrogen gas through the solution for 5 minutes at a flow rate of 100 ml/min., after acidifying with hydrochloric acid. The sample was then mixed with 2 ml of 20% hydroxylamine hydrochloride and 2 ml of 20% ethylenediamine tetraacetic acid tetrasodium salt and extracted with 5 ml of purified 0.05% dithizone in benzene followed by a cleanup procedure using sodium sulfide solution, re-extraction with purified dithizone in benzene, and ECD gas chromatography.

Determination of other metals

Powdered samples of 0.200 g were exactly measured and placed in sample tubes. Samples were digested with 3 ml of HCl and 1 ml of HNO_3 . The digested samples were diluted to 1/500. Manganese, lithium, zinc, lead, iron and copper were measured by the method of ICP-emission spectrometry (ICP-Emission Spectoscopy Optima 3000XL, Perkin-Elmer Co. Ltd.). Cadmium d and nickel were measured by the flame-less atomic absorption method (Atomic Absorption Spectroscopy, Z-5100, Perkin-Elmer Co. Ltd.). Background absorbance was corrected by Zeeman effect.

Conversion factors

1 ppm (parts per million) = $1 \mu g/g$ 1 ppb (parts per billion) = 1 ng/g

4. Results and discussion

4-1 Total and methylmercury concentrations in waste samples

Total mercury concentrations in the two gray samples were 496 μ g/g and 726 μ g/g, respectively. Total mercury concentrations in the two black samples were 2497 μ g/g and 3984 μ g/g, respectively. Total mercury concentrations in the brown sample was 97 μ g/g.

Mercury concentrations in soils and sediments are summarized in Mercury Contamination in Man and his Environment (International Atomic Energy Agency, Vienna, 1972) as follows. In Sweden, the mercury concentration in the soil ranged from 20 to 920 ng/g, with a mean of 70 ng/g (Anderson 1967). Stock and Cucuel (1934) regarded 100 ng/g as normal but gave ranges 100-290 ng/g for forest soils, 140-1000 ng/g for cultivated soil, 30-34 ng/g for clay soil and 1-29 μ g/kg for sand. In the vicinity of gold, molybdenum and base-metal deposits, soils were found to contain 50-250 ng/g but sometimes 2000 ng/g. Thus, mercury concentrations in the waste were very high compared with the environmental levels. The average mercury concentration of the whole waste may be around 500 to 700 ppm, since the gray solids were predominant.

Methylmercury concentrations in all but the sand-like waste samles were lower than detective level (0.01 ng/g). Methylmercury concentration in the sand-like sample was 33.7 ng/g, suggesting the contamination of surface soil or some other organic matters.

4-2 Metals in waste samples

Cadmium concentrations in black samples were below 1.0 ng/g, and 9-26 ng/g in the other samples. The other metal concentrations were as follows: manganese 83.3-1779.6 μ g/g; nickel 10.6 -76.7 μ g/g; lithium 7.5-147.6 μ g/g; zinc 101.8-698 μ g/g. lead 35.1-499 μ g/g; iron 23058-87525 μ g/g; and copper 35.1-323.3 μ g/g.

Cadmium, manganese and nickel concentrations in natural soils and sediments are summarized in Trace Elements in Biochemistry (Bowen 1966) as follows: cadmium 0.01-0.7 μ g/g; manganese 100-4000 μ g/g; nickel 10-1000 μ g/g; lithium 7-200 μ g/g; zinc 100-300 μ g/g; lead 2-200 μ g/g; iron 7000-550000 μ g/g; and copper 2-100 μ g/g. Thus, almost all concentrations of these metals in the waste samples were within the natural soil levels. However, lead concentration in sample 2 was slightly higher than natural soil and sediments.

4-3 Total mercury concentrations in water samples

The mercury concentrations in water samples in wells and tap water were from 4.2 to 6.3 ng/l. Even the water of the well near the site was 6.3 ng/l. Tap water from 1.5 km from the site and another side across Road No.4 was 5.5 ng/l, which was considered as a control.

Representative values for dissolved total mercury are: open ocean, 0.5-3 ng/l; coastal seawater, 2-15 ng/l; and fresh water rivers and lakes, 1-3 ng/l (WHO, 1990). The concentration range for mercury in drinking water is the same as in rain, with an average of about 25 ng/l (Lindqvist *et al.* 1984, in WHO, 1990). All the water samples around the site showed natural levels of total mercury suggesting no leakage of mercury from the site. The dry weather prevailing in Cambodia at present may be a redeeming feature in a tragic affair.

On the other hand, the whole water near the site was 153.2 ng/l, suggesting the contamination of the well water with the dust containing mercury. The well water was suspended with some small particles, which should be the dust of the waste carried by the wind from the site. In mercury analysis, the mercury must be extracted from the waste particle under the acid condition. However, mercury can not easily dissolve from the waste under the natural condition. As a matter of fact, the mercury concentration of water itself was still low, suggesting the stability of the mercury in the waste.

4-4 Total and/or methylmercury in red blood cells and serum

Total mercury concentrations in red blood cells were 13.1-25.5 ng/g for port workers and 10.5-17.0 ng/g for site workers. Methylmercury concentrations in red blood cells were 11.8-22.4 ng/g for port workers and 7.5-14.0 ng/g for site workers. Total mercury concentrations in serum were 1.96-3.59 ng/g for port workers and 3.26-4.58 ng/g for site workers.

The methylmercury concentration in red blood cells is one of the best indicators of the actual methylmercury pollution. It is said that certain groups with a high fish consumption may attain a blood methylmercury level (about 400 ng/g) associated with a low (5%) risk of neurological damage to adults (WH0, 1990). The methylmercury level of non-exposed populations is about 16 ng/g for blood cells. The methylmercury concentrations of port workers and site workers were similar to the control level. The methylmercury concentrations in the port workers looks higher than the in the site workers, suggesting that inhabitants of Sihanoukville consume fish often. However, the methylmercury levels of port workers and site workers were lower than about 1/20 of the critical level.

Serum mercury concentration is a good indicator of inorganic mercury. In inorganic mercury exposure, the serum total mercury increases. In the present result, the serum mercury percentage against methylmercury in red blood cells is slightly higher, suggesting slight inorganic mercury exposure to waste. The mercury concentration in

serum of persons that never used to eat fish was about 2.7 ng/ml (Birke, 1972). The serum mercury concentration of the port and site workers were almost control level, but about 1.5 times higher than the people who never used to eat fish.

4-5 Total mercury in urine

Urine mercury concentrations were 0.82-3.43 ng/ml for the port workers, and 3.83- 6.53 ng/ml for the site workers.

Mercury concentration in urine is one of the best indicators of inorganic mercury pollution. Mercury levels in urine correlate with mercury vapor only after long-term exposure (Cherian *et al.*, 1978 in WHO, 1990). The urine mercury concentrations of the port workers and site workers were similar to the normal level (approximately 4 ng/ml for non-exposed populations, in WHO, 1990). The urine mercury level was higher in site workers than port workers, suggesting that there was more mercury exposure during removal of the waste.

According to WHO (1976), the recommended maximum individual urine mercury concentration is 50 μ g/g creatinine, corresponding to about 25 ng/ml of urine. The urine mercury concentration in site workers with the highest value is about 1/4 the recommended level.

4-6 Total mercury in hair

In this mission, mercury concentrations in hair were analyzed to determine the possible adhesion of mercury-contaminated waste.

Mercury concentrations in hair were 2.17-5.08 μ g/g for the port workers, 1.29-4.32 μ g /g for the site workers and 1.68-3.55 μ g/g for the control.

The mercury concentration in hair is also one of the best indicators of methylmercury pollution. It is said that certain population groups with a high fish consumption may attain a blood methylmercury level (about 50 μ g/g of hair) but it associated with a low (5%) risk of neurological damage to adults (WHO, 1990). Fish consumption is the main source of mercury accumulation in human. Since the people of this area eat fish daily, the methylmercury accumulation of these populations was similar to that of Japanese. The mean mercury concentrations corresponds to fish consumption patterns as follows: once or less a month, 1.4 μ g/g; once every 2 weeks, 1.9 μ g/g; once a week, 2.5 μ g/g; and once or more a day, 11.6 μ g/g (Airey, 1983 in WHO, IPCS, Environmental Criteria 101: Methylmercury, 1990). The mercury concentrations were similar to normal levels (0.8-2.5 μ g/g) among people in the Southern Hemisphere.

4-7 Complaints of port and site workers

According to the two local doctors, the present health conditions of most site workers

were better than at that time of the accident.

Since the symptoms developed soon after work began, it is quite conceivable the cause was environmental. However, the cause of the symptoms in port stevedores and/or engaged in wastes disposal operations must be found in other than mercury.

Symptoms such as dizziness and visual trouble, symptoms that suggest a disturbance of consciousness, together with headaches and weakness, are common among the patients. Judging from the working environment, heat stroke and hypoxia must be considered as the differential diagnosis. If so, it would be only reasonable that patients would complain of abdominal pain, diarrhea and chest pain. It would have helped had data been available on body temperature, blood pressure, pulse, findings from electrocardiogram and chest X rays, hematocrit, urine and serum electrolytes. But such would now be impossible. Therefore, one can only say at this juncture that the above symptoms would have occurred due to the heavy physical workload undertaken in such a dusty and hot environment.

However, the above differential diagnosis is no more than an analogy. Since urine and blood samples, not to mention clear data on body conditions, are presently unavailable, we consider it impossible to pinpoint the exact causes of the symptoms even were a thorough investigation conducted.

5. Conclusions

- 1 Total mercury concentration in the waste sample was very high. The other metal concentrations in the waste were within the normal levels in natural soils.
- 2 The total mercury concentrations in water around the site were normal natural levels. It was suggested that the well water near the site had been contaminated with the dust of the waste. However, the elution of the mercury from the waste would appear to be very limited.
- **3** The total mercury concentrations in red blood cells, serum urine and hair of port workers and site workers were normal. They were similar to those of the general population in Asia.
- 4 Since the typical health effects due to mercury exposure were not specified in the complaints of port and site workers and since none of the human samples showed a high mercury concentration, it is unlikely that they suffer from mercury poisoning.
6. Preliminary recommendations from the investigation of the waste site in Sihanoukville by Dr M Sakamoto, WHO temporary adviser, National Institute for Minamata Disease, Japan and Dr G Petersen, WHO Representative to Cambodia, 25th - 26th December, 1998.

Samples were taken of various parts of the waste for further analysis in Japan. The site was tested for radioactivity (alpha- and beta rays). A test was also done for mercury vapor. Water samples from water sources in neighboring village and a well used by woodcutters just 200 meters from the site were taken and tested. The preliminary tests of airs and water showed no abnormal level of radioactivity or mercury. The results of tests on the soil samples will not be available until Dr Sakamoto returns.

Results from analysis of soil sampled previously and sent to Singapore for testing was given to the team. It showed a high level of mercury (675 μ g/g) but did not specify if is inorganic mercury and/or methyl-mercury.

Samples of hair, blood and urine were collected from port workers complaining of sickness after handing the waste and of soldiers engaged in the clean up operation.

The available data are not sufficient to make any firm conclusion as to the toxicity of the waste. It may contain toxic organic material or toxic metals.

However, based on the size and location of the site, our observations and tests so far along with the information given to us by the Department of Health and the Singaporean test results, we make the following preliminary recommendations:

- 1. The waste site as it is today does not pose any short-term threat to the population of Sihanoukville, and there is no need for any special precautions directed towards the general population. There is no need to evacuate anybody. Neither does the waste pose any threat to the water supply of Sihanoukville. There is no danger of contamination of any food produced in the province or any fish or other seafood from the waters outside the province.
- 2. As the waste may pose a long-term risk for the population in the area, it should be removed as soon as possible in a safe way. To speed up the process, more heavy machinery should be employed and the waste stored in larger containers. This will reduce the number of people exposed.
- 3. The local authorities should do its utmost to calm the population and dispel unfounded rumors about the danger of the waste.
- 4. The dumpsite must be carefully scaled off against enter by possible scavengers, children and other unauthorized persons.
- 5. Workers/soldiers employed in the removal of the waste must wear protective clothing: long sleeves, gloves and facemasks. Any solid gloves protecting against direct contact and dust will do. Paper masks plus a tight krama (a kind of muffler) are recommended. After work thorough body cleaning is needed. Work clothes should be washed every day after use.
- 6. No special precautions are needed for the population in the closest villages. Their water source seems safe.

- 7. The well in the woodcutter's camp very close to the site should be closed.
- 8. As there may be long-term health risks from the waste, anyone possessing ascertained parts of the waste should notify local authorities so that it can be collected and disposed of properly.
- 9. Workers and soldiers exposed to the waste should be followed up medically if they develop symptoms of poisoning. Guidelines for this should be developed.
- 10. Domestic animals should be kept outside the waste area.
- 11. Fish in the pond next to the site should not be consumed.
- 12. After removal of the waste, a layer of approximately 5 cm of topsoil should also be removed.

7. Further recommendations after the sample analysis

- 1 The mercury concentrations in the waste were very high and it was dumped on the hilly area surrounded by a pagoda and some villages in lower lying land. It should therefore be removed as soon as possible in a safe way. Waste should not be heated in order to avoid possible mercury vapor exposure.
- 2 Workers/soldiers employed in the removal of the waste must wear protective clothing: long sleeves, gloves and facemasks in order to avoid dust inhalation and direct skin contact.
- **3** The contamination of the environment of Sihanoukville should not be a concerns as long as the site clean-up is properly carried out. No special precautions are needed for the population in Sihanoukville and in the closest villages, since their water sources close to the site were not contaminated with mercury. The well near the site should be closed, since the water was contaminated with the dust of waste.
- 4 After removal of the waste a layer of approximately 5-10 cm of topsoil should also be removed. Further checks on the mercury contamination are necessary to know whether the clean-up was done properly or not.

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Levels of Mercury Contamination in Minamata Bay and Kagoshima Bay, Japan:Mussel Adductor Muscle as a Bioindicator for Methylmercury Contamination

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Concentrations of total mercury and methylmercury in five organs of the mussel *Mytilus* galloprovincialis collected from Minamata City and Kagoshima City in 1995 were determined. Methylmercury deposited in the adductor muscle, mantle, and gonads constituted 78-100% of the total mercury detected, except for a few samples (41-66%), while methylmercury deposited in the gills and digestive gland constituted less than 50 % of the total mercury detected. The concentration of total mercury in the adductor muscle may be used for roughly assessing the level of methylmercury contamination in local environments because it is less affected by the inorganic-mercury contamination.

We compared concentrations of total mercury in the mussel adductor muscle among 4 sites around Minamata City in 1993 to 1995 and 4 sites in Kagoshima Bay in 1997 to 1998. Around Minamata City, though input of mercury from the chemical plant had stopped by around 1970, concentrations of total mercury in the mussel adductor muscle were higher in 2 sites (26-121 ng/g, n=135) near the main fallout of wastewater from the chemical plant than in the other sites, i.e., 2 sites 1 to 5 km apart from the former sites in Minamata City (6-28 ng/g, n=52), and all sites in Kagoshima Bay (2-30 ng/g, n=287).

Occurrence of the localized methylmercury contamination around the fallout of wastewater from the chemical plant was supported by the results of our sensitive analysis of mercury concentrations in seawater and sediment samples: Concentrations of both total mercury and methylmercury in seawater and sediment samples were higher in the Minamata Bay than the outside of the bay, though the difference in seawater and sediment samples was not so conspicuous as that in the mussel adductor muscle. In Minamata Bay, 0.03 to 0.5% of total mercury occurred as methylmercury (the concentrations of methylmercury: 0.4 to 20 ng/g) in the sediment samples, and 3 to 12% of total mercury occurred as methylmercury (the concentrations of methylmercury: 0.05 to 0.15 ng/l) in water-soluble fractions of seawater.

The survey of levels of concentrations of total mercury in the mussel adductor muscle may be useful for convenient monitoring the methylmercury contamination, because they seem to represent the amplified and moving time-averaged values of the localized methylmercury contamination in a narrow coastal area, and the analysis of concentrations of total mercury in the mussel organ is much easier than that of methylmercury in the sediments and seawater there.

Study on Macrobenthos in Minamata Bay

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Introduction

Minamata Bay has been polluted with methyl mercury in factory waste. The area where the mercury concentration in sediment exceeds 25 ppm had dredged off until 1990. And it is said the marine product is safe from mercury pollution now. Unfortunately, however, the coastal area in Minamata Bay has never been treated throughout the pollution control measurement. This study was carried on the intertidal macrobenthos in Minamata Bay and around the Yatsushiro Sea. They live in the coastal area and are important in food chain, because those are the main food for coastal fishes.

Materials and Methods



The sampling stations located in Minamata Bay (inside and outside), the coast of the Yatsushiro Sea (Goshonoura island and Kawaura town in Amakusa island), and in control area (Goto islands and Kitaura town) (Fig. 1).



The

Fig. 1 The location of sampling stations Nereis pelagica and Gaetice depressus. Thais clavigera is carnivore snail and ranked high in food chain. Nereis pelagica is carnivore

annelid living in sand and important as fish food. *Gaetice depressus* is omnivore crab which is recentry evidenced one of the main foods of scorpionfish.

Samples were dissolved in 1 N sodium hydroxide at 60 °C for overnight just before methyl mercury extraction. Crustacean samples, which is hard to dissolve in NaOH, owing to their chitin shell, were freeze-dried and crushed prior to dissolving in acid (for total Hg analysis) or in KOH-Ethanol (for methyl Hg analysis). Total mercury concentration was determined by flameless atomic absorption spectrometry or cold vapor atomic absorption spectrometry. Methyl mercury was extracted twice with dithizone-toluene, and quantified by ECD-gas chromatography.

A quantitative survey was also carried out in the same stations. In every station, samples were collected in 3 tidal zones, low (L), middle (M) and high (H). In some stations, samples in the highest tidal zone (HH) were collected additionally. 4 quadrates that are 50 cm square were placed randomly in every tidal zone in boulder shore and the macrobenthos inside them were collected. We used 6 quadrates that are 25 cm square in rocky shore. The samples were fixed with formalin, sorted by species and counted. They were then dried in 60 °C for 3 days and weighed. The data of Goto Islands is now under analysis.

Results

The mercury concentration in *Thais clavigera* was maximal inside of Minamata Bay, and decreased with distance. Mercury concentration in *Nereis pelagica* or *Gaetice depressus*, there was same tendency (Fig. 2).





Nereis pelagica

Fig. 2 The mercury concentration in 3 benthos species





Number of	Minam	Minamata Bay					awaura Ki	itaura
Species	A	В	С	D		ura		
Boulder shore	(33	53	81	51	36	113	89
Rocky shore	6	53	64	81	54	38	64	68
Total	12	6 1	.17	162	105	74	177	157

Number	Minamata	Bay			Goshono-	Kawaura	Kitaura
	A	3 C		D	ura		
Boulder shore	1567	1098	2295	944	1423	2879	1845
Rocky shore	9059	5600	4145	4733	42290	13518	26597
Total	10626	6698	6440	5677	43713	16 <mark>39</mark> 7	28442

Dry Weight	Minamat	Minamata Bay				Kawaura	Kitaura
	A	В	C	D	ura		
Boulder shore	1633.03	449.676	1968.71	476.674	435.702	1037.82	1658.57
Rocky shore	1474.78	1411.25	1582.24	271.394	120.127	319.09	285.716
Total	3107.8	1860.9	3550.9	748.07	555.83	1356.9	1944.3

Shannon-Wiener	Minamat	Minamata Bay				waura K	litaura
index	Α	В	C	D	ura		
Boulder shore	1.110	1.178	1.270	1.214	0.900	1.015	0.959
Rocky shore	0.634	1.088	1.186	0.761	0.138	0.254	0.272
Total	0.841	1.251	1.348	0.832	0.222 (0.471	0.370

Diversity	Minamat	ta Bay		Goshono-	Kawaura	Kitaura	
	Α	В	C	D	ura		
Boulder shore	18.78	17.43	21.72	16.81	11.42	32.67	23.88
Rocky shore	14.66	16.27	21.56	13.60	8.21	15.49	13.56
Total	20.37	22.22	27.57	19.71	12.28	32.27	24.25

Table 1 The amount and the community diversity

Table 1 shows the amount and the community diversity of the sample. In Minamata Bay, numbers of individuals are smaller. It reflects that *Chthamalus challengeri*; a kind of small barnacle was fewer than in other stations. And dry weight was heavier. There was large quantity of oysters (*Crassostrea gigas*) in Minamata Bay. But they were deleted from following analyses since their amount varies every year. Community diversity was richer in Minamata Bay.

The major part of community in boulder shore was gastropods. Minamata Bay had more annelids and pelecypods, and fewer arthropods. In rocky shore of Goshonoura, Kawaura and Kitaura, the grater part of macrobenthos was arthropods and the majority of them were barnacle. On the other hand, the community of Minamata Bay, especially station C concluded few barnacles. The dominant in station C was attached pelecypods instead of barnacles. They are competitive for rock surface.



Fig. 3 The ratio of each taxonomical group (upper) and food habit (lower)



The community in the boulder shore of station **D** includes many carnivores in contrast to Goshonoura. In rocky shore, filter feeders are dominant (Fig. 3).

communities The classified were by percentage difference using group average strategy. In boulder shore, they were similar in every station. But that in the rocky shore of Minamata Bay was different from each other or in other stations (Fig. 4).

Discussion

The maximum concentration of total mercury was over 0.4 ppm, which is Japanese regulatory standard for fishes. But it is not sure whether this concentration is hazardous or not, because the ratio of methyl mercury for total mercury is lower than in fish. The maximum concentration was over 0.4 ppm also. Although human don't eat them directly, it is not clear the effect through food chain.

The mercury level in Minamata Bay is still higher than natural level. But the effect of that on human population is not clear.

The community of Minamata Bay is more diverse and contains fewer barnacles in rocky shore. It may due to a reduction of disturbance caused by the restricted fishing within the seclusion net. It is still subject to be studied the cause of these phenomenon or the effect of higher mercury concentration on biological community.

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Certification of Total Mercury and Methylmercury Concentrations in Estuarine Sediment Reference Material, IAEA-405

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In order to improve the quality of determination of trace contaminants in marine samples, the IAEA Marine Environment Laboratory in Monaco has been organizing several analytical intercomparison exercises that result in the preparation of reference materials certified for their content of trace elements and methylmercury. The intercomparison exercises aim at enabling individual laboratories to monitor their performance and are also designed to test the intercomparability of current analytical methods used. Due to the increased demand for new reference materials certified for total and methyl mercury (MeHg), a sample of estuarine sediment was prepared. A large quantity of sediment was collected from the Tagus estuary, Portugal. This sediment was deep-frozen, freeze-dried, ground and passed through a 125 μ m sieve and then thoroughly homogenized.

Certification of total mercury in this material was achieved as an outcome of an international analytical intercomparison study in which 73 laboratories reported results. Most of the laboratories used acid digestion followed either by CV-AAS or CV-AFS. Three laboratories used neutron activation analyses, three used ICP-MS and one used ICP emission spectroscopy. The data received were in good agreement. All certification criteria were fulfilled and therefore the value for total Hg was certified to 0.816 μ g kg⁻¹ with a 95% confidence interval from 0.779 to 0.853 μ g kg⁻¹.

An invitation to participate in the intercomparison exercise for methylmercury analyses was sent to several laboratories that had reported MeHg values in sediment. Thirteen laboratories reported results for MeHg using various isolation procedures and detection systems. In the very first step, when MeHg was released from the binding sites, three approached were used: distillation, saponification and acid leaching. Further processing included additional separation using anion exchange separation of organic and inorganic mercury, solvent extraction with or without a clean up step, and derivatization by aqueous phase ethylation. The detection systems included cold vapour atomic absorption spectrophotometry (CV-AAS), gas chromatography (GC) combined with cold vapour atomic fluorescence spectrophotometry, GC with electron capture detector and HPLC with CV-AAS. Three results were clearly outliers and they correspond to laboratories that did not report results for reference value for MeHg of 5.60 μ g kg⁻¹ with a 95% confidence interval from 5.02 to 6.18 μ g kg⁻¹. Comparison of the data obtained by various methodologies will be discussed.

THE ROLE OF MERCURY AIR/SURFACE EXCHANGE PROCESSES IN THE GLOBAL BIOGEOCHEMICAL CYCLE: A Brief Summary of Research by the ORNL Mercury Group

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Introduction

Atmospheric sources are significant in the cycling of Hg in the biosphere, but there have been few reliable data on air/surface exchange of Hg in terrestrial or aquatic systems until recently. Since the first Global Hg Conference in Gavle, Sweden, there have been significant developments in the areas of automated field analysis, flux chamber enclosure, and tower-based micrometeorological gradient methods for measuring gas-phase Hg fluxes over waters, soils, and vegetation. Numerous groups have now applied these methods in flux campaigns around the world, and the data base on Hg fluxes has increased significantly. An important milestone was reached in 1997 when scientists from several countries collaborated in an EPRI-sponsored field intercomparison of Hg flux measurements using seven field flux chamber designs and four micrometeorological approaches at the Steamboat Springs Geothermal Area, Reno, Nevada. This study led to important improvements in flux measurement methods. Another major recent advance was the development of methodologies for determining the speciation of The discovery of measurable levels of water-soluble Hg compounds (reactive atmospheric Hg. gaseous mercury, RGM) in both flue gas, and, more recently, ambient air has significant implications for modeling the fate of airborne Hg. All these advances and their recent application have provided important clues to the behavior of Hg in the global bigeochemical

cycle.

There is no longer any doubt that Hg, once deposited, has the capability to be re-emitted from environmental surfaces, and that re-emission is significantly enhanced by green plants via a transpiration-like process. There is also no doubt that Hg associated with geological sources demonstrates a similar capacity. What is in doubt is the relative role of these so-called natural emissions in the global cycle, and to what extent Anatural \cong emissions include re-emitted Hg.

On the other end of the Hg Abehavior scaleA, recently measured levels of RGM compounds support the hypothesis that the dry and wet deposition of Hg may be strongly influenced by the behavior of RGM and that elevated regional exposure may be possible near major point sources of RGM compounds. This appears to be true even though RGM may represent only a few percent of total mercury in air. Source measurements have indicated that RGM is formed in combustion processes, and the recent discovery of so-called Hg-depletion events in the Arctic suggests that there may be atmospheric reaction pathways for the production of RGM from Hg^o.

¹Research sponsored by EPRI, Florida DEP, US EPA, SFWMD, NOAA, Lake Superior Trust, and the U.S. Department of Energy under contract with ORNL ORNL is managed by Lockheed-Martin Energy Research, Inc., for the U.S. Department of Energy.

Our research attempts to summarize the state of our understanding of these processes and how they influence the global Hg cycle, based on studies largely completed since the 1990 Mercury Conference.

ORNL Methods for Automated Measurements of Environmental Mercury Speciation and Fluxes

Two of the most critical measurements needed to understand and predict the behavior of environmental pollutants are chemical speciation and ecosystem flux. This is particularly true for mercury which exhibits markedly different mobility and toxicity among species. Geochemists in ESD and their collaborators recently developed and published several methods for automated, nearreal-time analysis of these parameters in the field using commercially available instrumentation. These methods are now in widespread use, and are undergoing extensive intercomparison studies.

Reactive gaseous mercury (RGM) refers to one or more oxidized (Hg²⁺) forms of airborne mercury. Reliable measurement of these RGM species is a critical need in understanding the biogeochemical mercury cycle because the speciation of atmospheric mercury controls its deposition. Measurement of RGM is a difficult challenge because of its low concentration (in the pg/m³ range), and because of its inherent instability and reactivity. We developed a method for collection and analysis of RGM in air using refluxing mist chambers (MC) combined with a Tekran automated field mercury analyzer. The MC method efficiently extracts water soluble gases from short-term ambient air samples, and exhibits excellent precision and blanks. Our data show that RGM represents a few percent of Hg in air, exhibits a strong daytime maximum, and is strongly removed by wet and dry deposition.

Dissolved gaseous mercury (DGM) refers to the species of reduced mercury in water which exists in the gas phase. Measurement of this species is critical to predicting the fate of aqueous mercury because formation of DGM from oxidized mercury is thought to compete with the formation of highly toxic methylmercury. Because it is volatile, DGM also represents a mercury removal process from water. We developed a method using the Tekran analyzer to quantify DGM in surface waters using an automated purging system. The system is preferred over the former pressure purge and trap approach because data are generated in near-real-time, and losses are readily detected and minimized. Our data show that DGM is formed from reactions with other metals and organic carbon which are mediated by sunlight, and that it can be oxidized in water prior to removal by evasion.

These same methods have been combined with recently developed chamber and micrometeorological gradient methods to quantify the fluxes of various mercury species between the atmosphere and the Earth=s surface. The Tekran has been fitted with an external solenoid unit which allows rapid sequential sampling at two heights to quantify concentration gradients, or to allow similar sampling of inlet and outlet concentrations from flux chambers, both of which yield near-real-time air/surface exchange rates for mercury vapor. We have used these methods to measure mercury fluxes in diverse ecosystems from the Florida Everglades to Pt. Barrow, Alaska, and from the California Coastal Range to Lake Gardsjon, Sweden. Mercury vapor is strongly

emitted from these ecosystems to the atmosphere, indicating that industry regulations must also consider the role of mercury re-emission from natural surfaces in order to understand emissioncontrol benefits.

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Steps to Improve Our Assessment of the Health Impacts of Environmental Levels of Mercury Obtained from Fish Consumption

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Past accidental exposures to mercury have clearly demonstrated that mercury is a The contamination of estuarine waters from industrial developmental neurotoxin. discharge led to the accumulation of methylmercury (MeHg) compounds in fish in Minamata Bay. As individuals consumed fish products, severe health consequences were observed, and the first linkage between neurological effects and environmental mercury levels was observed. (Kutsuna, 1968; Tamashiro et al., 1986; Igata, 1993) Subsequently, MeHg-treated grain meant for planting was consumed in Iraq; again neurological consequences were observed in those eating the grain as well as in the offspring of women who consumed grain while pregnant. (Bakir et al., 1973) Attempts were made to estimate the exposures of pregnant women to derive a dose-response estimate of the effects of environmental mercury. (Cox et al., 1979)The unfortunate occurrences in Iraq and Minamata clearly indicate the effects of high accidental exposures to MeHg, but it is difficult to know how to extrapolate from these events to any effects that might be associated with fish consumption per se. Several studies have been undertaken to derive an understanding of the health risks from mercury associated with fish consumption, but these studies have generally been small, and their results have been ambiguous or negative. (e.g., Marsh et al, 1995; Crump et al., 1998) Two large studies have recently reported results of in utero exposure to MeHg on neurological development of offspring; these studies were undertaken in the Faroes Islands and in the Seychelle Islands. The Seychelle Island was essentially a negative study, finding no statistically significant association between MeHg exposures and the results of several developmental tests. (Davidson et al., 1998; Marsh et. al, 1995) The Faroes study reported statistically significant associations between MeHg exposure and the results from several tests. (Grandjean et al., 1997; Grandjean et al., 1998; Weihe et al., 1996.)

Both studies were well conducted. Relatively accurate estimates were made of fetal and maternal exposure to MeHg, and the neurological tests were carefully designed and conducted. There are, however, several differences between these two studies, and an understanding (or resolution) of these differences may help reconcile the different findings. This paper explores the differences between these two studies and reports upon research sponsored by EPRI to aid in resolving these differences.

One of the major differences between these two studies is the study design; that of the Seychelle Islands is a longitudinal prospective study whereas that in the Faroes was a cross-sectional study. These differences in design resulted in differences in the age of the children who were tested. The Seychelles Islands study examined children at 6, 19, 29,

and 66 months with plans to test these children again at older ages. The Faroes Islands study examined children once at 7 years of age. It is possible that effects may not be easily observed at younger ages since children are developing more rapidly neurologically at younger ages; hence there is more variability in neurological development, and changes may be more difficult to observe. Follow-on testing among the children in the Seychelles cohort can help resolve the issue of whether age at testing per se explains the difference in the two studies. Neurological tests must be designed for the ages of the children that are being tested. There were differences in the tests used in both studies. The test battery in the Seychelles obtained standardized measures of global neurological function. The test battery in the Faroes examined multifocal, domain-related effects. Tests of memory, language, and attention all vielded results suggesting adverse response to MeHg exposure. It is conceivable that there is no overall global response to MeHg exposure at the levels to which the children were exposed, but that specific domains demonstrate response. For that reason, it will be necessary to examine specific domains in the Seychellois children when tested at older ages to determine whether such a response is observed in the Sevchelles Islands. To that end EPRI has supported work to develop appropriate and sensitive test methods which can be applied to older children in the Seychelles cohort.

The specific aim of this work was to develop a test battery to measure subtle changes in 11-year-old Seychellois children's motor, sensory, cognitive, and neurophysiological functions. First, an extensive battery of tests believed to sensitively reflect endpoints that are likely to be affected by MeHg exposure in children, experimental animals and adult humans were selected. A study of each of the tests' characteristics and then the feasibility of administering each test -- first in a small group of children in Rochester and then in a subset of approximately 50 children in the Pilot Cohort in the Seychelles -- was carried out. The pilot test battery contained several types of assessments (Table 1).

Table 1

Domain	Neuropsychological and Behavioral	Physiological	Experimental
Cognitive	 Tower of London Wisconsin Card Sorting K-BIT Matrices (Caregiver) 	 P-300 during CPT 	 Visual Motor Learning Delayed Matching Repeated Acquisition Progressive Ratio Fixed Ratio & Interval Fixed Ratio/Wait
Visual	 Visual system response 	 Pattern Reversal Evoked Potentials 	 Scotopic Form Discrimination Spatial contrast Sensitivity
Auditory	 Pure Tone Audiogram Screening Test for 	Evoked PotentialsOtoacoustic Emissions	

Tests Administered to Seychelles Pilot Cohort during Test Battery Development

	Auditory Perceptual	
	Disorders	
	 Test of Auditory 	
	Perceptual Skills	
	 Pitch pattern 	
	Sequence Test	
	 Competing 	
	Environmental Sounds	
	Test	
	 Dichotic Digit Test 	
Intersensory	 Complex Form 	
•	Matching	
Motor	 Fine motor control 	 Coordination, Tremor
		and Positioning RT,
		МТ
Sensory-Motor	 Alertness 	 Monitoring and
-		Coordination Multi-
		tasking
Somatosensory	Tactile sensitivity	 Spatial contrast
·		sensitivity on skin
Social	• PSI	<i>,</i>
	-	

Deficits in motor, cognitive, sensory, and social functions following exposure to relatively low concentrations (those that do not produce overt toxicity) of MeHg, have been reported in the human epidemiological and animal toxicology literature. The test battery was based on what is known from the literature in both humans and animals and also included newly developed tests for which there is a theoretical basis. The test battery contained neuropsychological and behavioral, physiological, and experimental tests that assessed cognitive, visual, visuospatial, motor, sensory-motor, and somatosensory endpoints. Children tested in the Pilot Cohort were those whose mother's hair mercury was in the highest and lowest quartile during pregnancy ($\leq 3 \text{ or} > 12 \text{ ppm}$). Each test listed in Table 1 was administered to the children in the Pilot Cohort at age 11 and these results are being evaluated for psychometric properties and validity. Distribution of test scores is being examined; validity is being assessed using intraclass correlation coefficients among other methods. It is planned that tests meeting a priori established criteria for inclusion in the final battery will be combined with certain of the tests being administered at 96 months of age to constitute a final test battery for the 11 year old Seychellois children in the main cohort.

There are two important differences in the two cohorts associated with the exposures of the two populations and the way in which they were measured. The source of the MeHg exposure in the Seychelles study is ocean fish; in the Faroes, the most important source of MeHg was pilot whale although fish were also eaten. In the Seychelles, families reported eating fish an average of 12 times per week. Fish had an average mercury concentration of ≤ 0.3 ppm. In the Faroes, pilot whale meat had a much higher concentration of mercury than fish. Fish was eaten there an average of 1-3 times per week. The consumption patterns for the two sources of MeHg may be quite different. In the Faroes, fish is eaten more regularly; pilot whale may be eaten more episodically.

The significance of these patterns suggests that even if the total or average mercury consumption is similar for the two studies, episodic consumption could lead to temporally higher exposure levels. There is a need to examine whether such pattern differences can lead to substantially different concentrations during relatively short time periods in the brains of developing fetuses (and of nursing infants). One tool to undertake this examination is the application of physiologically-based pharmacokinetic (PBPK) models.

These models can also be used to assess the difference in the exposure measures for the two studies. The Faroes study reported that cord blood levels of MeHg were the best indicator of exposure; that is, these levels were more highly associated with neurological response than measure of MeHg in the hair of mothers. The Seychelles study used the accepted exposure metric of maternal hair mercury concentration measured at birth. The use of PBPK models could examine the association between these two MeHg exposure methods and indicate the extent to which they may represent different estimates of fetal brain mercury level. To what extent, for example, does the timing of exposure impact a measure.

EPRI has overseen the development of a pharmacokinetic model (PBPK model) for mercury that includes the fetus. (Gearhart et al., 1995). This model describes MeHg kinetics in the pregnant human and fetus. The structure of the model is shown in Figure 1.



Figure 1

This model consists of an adult with eleven compartments representing both organspecific and combined tissues and a fetal sub-model for MeHg which consists of four compartments (fetal plasma, RBCs, brain and the remaining fetal body) which grow during the time of gestation. The model includes a description of enterohepatic recirculation of MeHg, conversion to inorganic mercury in tissues and intestinal flora, slowly reversible incorporation of mercury in tissues, and excretion of both organic and inorganic mercury into urine, feces, and hair. This model has been validated and provides a coherent description of MeHg kinetics for a variety of dosing scenarios in both monkeys and humans. Figure 2 illustrates the time course of MeHg in whole blood from a human subject in a controlled study of chronic ingestion of MeHg in fish over a three month period. The curves depict the computer model simulation at each dose illustrating the ability of the model to accurately predict the rise and fall of blood MeHg levels during and after fish ingestion.



Figure 2

The model has been applied to estimate uncertainty in an oral reference dose for MeHg due to interindividual variability in pharmacokinetics using a Monte Carlo approach. (Clewell et al., 1999). In this study, two ingestion rates were considered: 0.1 $\mu g/kg/day$ and 0.5 $\mu g/kg/day$. In order to evaluate the degree of uncertainty in converting hair concentration of mercury to an oral chronic MeHg ingestion rate, Monte Carlo analyses were performed in which distributions for each of the parameters in the PBPK model were randomly sampled 1000 times. The resulting distributions of conversion factors were a factor of 1.8 and 1.5 below the median for the 1st and 5th percentiles of the distribution. Thus, interindividual pharmacokinetic variability in relating hair Hg levels to ingestion rates is relatively low compared with the usual default assumption of 10 to account for uncertainty between individual humans. Utilizing this PBPK model, work is underway to assess potential differences in relatively short-term fetal brain MeHg levels that might occur from differing consumption patterns during various periods of pregnancy focusing on those thought to be most sensitive for neurological development. The associations between predicted fetal brain Hg concentrations and exposure as estimated by either maternal hair mercury concentration or mercury concentration in cord blood may also be examined using the PBPK model.

The different sources of MeHg in the two studies introduce a number of potential confounders that may not have been fully accounted for in the various analyses. For example, the presence of selenium in ocean fish may serve to modulate effects of MeHg; likewise, 3-omega fatty acids, also present in fish. are known to have a beneficial effect on neural development and activity. Hence the co-exposure to other compounds in fish may explain why the response to MeHg in fish is different from that in pilot whale where these compounds are not present in the same quantities. Additional studies, such as controlled exposure studies in animals, could be undertaken to determine whether the biological availability and metabolism of MeHg is impacted by the presence of these other compounds in fish.

Another difference is the presence of PCBs in the blubber of the pilot whales consumed in the Faroes. PCBs are known neurotoxicants and may act synergistically with McHg in that capacity. The potential influence of PCBs on MeHg neurotoxicity is controversial. At a recent workshop (NIEHS, 1998) where this issue was discussed, there were mixed opinions on the likelihood of whether the presence of PCBs in pilot whale might modify the neurological responses to MeHg.

Another way to resolve this issue is to undertake a new third study that tries to anticipate and address all of these issues before the study is completed. EPRI is helping to participate in such a study now which recently began in the Northern Adriatic Sea. Present plans call for a relatively small cohort to be studied; it is hoped that this cohort can be expanded. Unfortunately it will take several years before older children can be tested for potentially adverse neurological response. Uncertainty remains about the adverse nature of MeHg exposure from eating fish. We issue fish advisories suggesting that citizens, especially pregnant women, limit their fish consumption because of its MeHg content. In the research to be undertaken in the near future, we may be able to increase our understanding of these risks, but it is unlikely that they will be definitively defined until more studies are undertaken.

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A Simple and Sensitive Radiochemical Technique for Evaluating Mercury Transformation and Distribution in the Aquatic Environments

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Abstract

A sensitive radiochemical technique for evaluating environmental factors influencing the methylation and partitioning of mercury in aquatic systems has been developed and tested in laboratory experiments. Sediment-water systems are spiked with 203 HgCl₂ and incubated for a few days under various environmental conditions. After incubation, samples of sediment, biota and water from experimental cells are extracted with 0.1% dithizone solution in benzene (Dz-Bz) to recover the incorporated mercury. The separation of inorganic- and methyl-mercury from the Dz-Bz extracts is achieved by thin-layer chromatography (TLC) before the activity of these mercury species is measured using a gamma counter.

The proportion and concentration of the mercury species in the original samples are determined from the specific activity of the mercury spike and gamma activity measurements of the samples and the mercury species. The technique is sensitive enough even at very low mercury concentrations in a wide range of environmental materials.

Key words: Radiochemical technique; Methylmercury; Gamma activity; Environmental factors

1. Introduction

Although radio-tracer techniques are potentially powerful for monitoring the transformations and pathways of chemical pollutants in aquatic environments, only few studies have used such techniques to investigate the environmental behaviour of mercury. Early studies include that of Beckert et al (1974) who used ²⁰³Hg radio-tracer to study the formation of organo-mercury compounds in soils contaminated with inorganic mercury. Cappon and Smith (1978) estimated methylmercury recovery from biological and sediment samples by measuring ²⁰³Hg beta-activity in the sample extracts. Kudo et al (1977) and Akagi et al (1979) investigated the generation and distribution of methylmecrury in river sediments spiked with radioactive mercuric chloride. Furutani and Rudd (1980) used radioisotopic assays to measure mercury methylation in lake water and sediment. Czuba et al (1981) studied the quantitative separation of inorganic mercury and methylmecrury from animal and plant tissues using a radiochemical method. More recently, Guimareas et al (1995) described a simplified radiochemical technique for the measurement of mercury methylation rates in water determined by using a ²⁰³Hg radio-tracer technique. However, no detailed radiochemical procedures were published in most of these studies.

Advancements in analytical instrumentation and mercury extraction techniques have made it possible to overcome shortcomings encountered previously, such as poor extraction efficiency and low sensitivity. The radiochemical method presented here represents a significant advancement over the previous methods. It can be used to study the methylation and distribution of mercury in wide range of environmental materials including those containing very low mercury concentrations close to ambient levels.

2. Material and methods

2.1 Laboratory facilities

A laboratory equipped with a gamma counter and essential facilities for handling radioactive reagents and materials is required.

Basic safety standards for radiation protection have to be observed according to the Radiation Commission health and safety standards and regulations.

2.2 Reagents

Analytical grade reagents are recommended in the preparation of various chemicals for sample treatment.

Basic reagents

Radioactive mercuric chloride (²⁰³HgCl₂) diluted HCl (1:4), potassium hydroxide solution in ethanol (1N KOH-EtOH), diluted ammonium hydroxide (1:9), benzene (C6H6), hexane (C₄H₁₄), toluene (C₆H₅CH₃), acetone ((CH₃)₂CO), 0.1% dithizone-benzene solution (0.1% Dz-Bz), sodium sulphate (Na₂SO₄, anhydrous).

Other consumable materials

Florisil (60-100 mesh), 20 ml glass vials with caps, 50-100 ml volumetric flasks, disposable measuring pipettes (1-20 ml), pasteur pipettes (1 cm long x 5 mm diameter, Corning co. Ltd.), glass wool, 0.2 mm silica gel-coated TLC paper (Polygram SIL N-HR, Macherey-Nagel, Germany), TLC chamber, gloves.

Preparation of standards

Cold (i.e. non-radioactive) mercuric dithizonate and methylmercury dithizonate standards are required in the recognition of mercury species separated by thin-layer chromatography (TLC). To prepare these standards, the mixture of 2 ml of 1ppm HgCl₂ in aqueous solution and 2 ml of 1 ppm CH₃HgCl in benzene is slightly acidified with 2 drops of HCl and extracted with 1 ml of 0.1% dithizone-benzene. Wash the Dz-Bz extract with 2-3 ml NH₄OH solution (1:9) to remove excess dithizone. Evaporate to dryness the Dz-Bz extract by purging with N₂ gas. Then dissolve the residue in 0.2-0.5 ml acetone by adding acetone dropwise. The solution of cold mercuric dithizonate and methylmercury dithizonate in acetone is used as a reference standard on the TLC paper.

Preparation of Florisil column for the clean-up of Dz-Bz extract

Insert a cotton wool plug into a pasteur pipette (12 cm long x 5 mm diameter) and add 0.5 g Florisil and then 0.5 g of sodium sulphate (Na₂SO₄, anhydrous) with gentle tapping. Place the pipette in a suitable holder so that the neck points vertically downwards.

TLC Paper

Cut the TLC paper into 5 cm x 20 cm strips. Use the strips in the separation of inorganic- and organic-mercury dithizonates by thin-layer chromatography as described under the sample treatment section.

2.3 Sample Treatment

Samples of sediment, water or biota, are collected from experimental cells (glass cylinders, 8 cm diameter, 30 cm height) containing sediment-water previously spiked with 203 HgCl₂ and incubated for several days or weeks under different environmental conditions. The following sample treatment procedures are then followed for different sample materials.

Sediment and biological samples

Weigh about 1 g of the sediment or biological sample in a 20 ml counting vial. Cap the vial, load the vial in a gamma counter and count for 5-10 minutes to measure 203 Hg total activity in the sample. Unload the vial from the counter, add 8 ml of 1N KOH-EtOH and shake for 20 minutes in a reciprocal shaker. Slightly acidify the sample by adding 5 ml of HCl (1:4) and shake briefly by hand. Add 5 ml of 0.1% Dz-Bz and shake for 5 minutes in the reciprocal shaker to extract mercury from aquoeus acid solution into Dz-Bz organic layer. Centrifuge the sample for 5 minutes at 1000 rpm.

After centrifuging, pipette 2 ml of the organic extract onto a Florisil column and collect the eluate in a clean centrifuge tube. Add 10 ml of diluted NH₄OH (1:9), shake for 5 minutes and then centrifuge for 5 minutes. Discard the aqueous layer after centrifuging. Repeat the ammonium hydroxide washing step (Fig. 1). After the washing, pipette 0.5 ml of the organic extract into a clean 20 ml glass vial. Cap the vial and load it in a gamma counter and count for 5-10 minutes for mercury recovery check.. Unload the vial, open the cap and dry the extract under reduced pressure in a glass dissector or by blowing with nitrogen gas (N₂). The drying process takes about 10 to 15 minutes.

After drying the extract, add 3 to 4 drops of acetone into the vial to dissolve the residue. Carefully apply the acetone solution of the sample on a TLC paper using hair-like capillary glass tube, making sure that you apply the sample slowly in a series of uniform smears along a straight line on the TLC paper without scratching the paper. The whole sample should be applied to the TLC paper step by step. If necessary, rinse the sample bottle with 1-3 drops of acetone and add again the sample to the TLC paper. The sample should be applied along a straight line about 5 cm from the bottom of the TLC paper, and parallel to the short dimension of the paper, leaving at least 5 mm on either side of the vertical margins of the paper.

Let the sample on the TLC paper to dry up for 1-2 minutes. Then add a mixture of non-radioactive methylmercury and inorganic merucry dithizonate reference standard to the TLC paper in two or three spots along the sample application position. Place the TLC paper in a TLC chamber containing the developing solvent (benzene-hexane, 1:1). Make sure the chamber is in a horizontal position on a flat table before immersing the TLC paper into the solvent chamber. A height of 1 cm of the solvent in the chamber is adequate. The TLC paper should be kept nearly vertical in the chamber. Cover the chamber with a lid to maintain uniform conditions in the chamber and to reduce evaporation of the solvent.

The chromatographic separation of mercury species on the TLC paper takes about 60 to 75 minutes. When the separation process is completed the solvent front on the TLC paper will be about 2-3 cm from the top of the TLC paper.

Remove the TLC paper from the chamber when the separation process is completed and mark the level of the solvent front on the paper using a pencil. Then hang the TCL paper to dry up in air at room temperature for 5 minutes.

The positions of methylmercury and mercuric dithizonates on the TLC paper are recognized from the colours formed by the reference standards, usually yellow for methylmercury dithizonate and orange for mercuric dithizonate.

Measure the distance of the solvent front from the sample application line as well as the distances of the inorganic and organic mercury dithizonate standards from the same line. The ratio of the distance of the mercury dithizonate standards to the distance of the solvent front give the estimates of the rate of flow (R_f) for the respective mercury dithizonates on the TLC paper. The Rf value for methylmercury dithizonate is bout 0.65 whereas for mercuric dithizonate the value is about 0.35.

The TLC paper is then cut into four strips corresponding to the sample origin or application line, mercuric dithizonate, methylmercuric dithizonate and the solvent front (Fig. 2). The strips are loaded separately but in similar position into clean glass vials (i.e. free of radioactive contaminants) for gamma activity counting. The counting time will depend on the intensity of gamma activity in the sample relative to the background level. Samples with low activity will normally require longer counting times and vice-versa. When the sample activity is at least three times the background level, then 30-60 minutes counting would be adequate. For very low activity samples, counting overnight may be necessary to accumulate enough gamma counts above the background level.

Water samples

Pipette 20 ml of the water sample into a counting vial. Cap and load the vial into the gamma counter. Count for 5-10 minutes for measuring the total activity in the sample. Measure 50 ml of the water sample, add to it 5 drops of 0.5% KMnO₄ solution in a volumetric flask and shake briefly. Acidify the sample with 1 ml of HCl (1:4) and then add 5 ml of 0.1% Dz-Bz and shake for 5 minutes to extract mercury into the organic layer. Centrifuge for 5 minutes at 1000 rpm and discard the aqueous layer after centrifugation. Wash the organic extract twice with NH₄OH solution (1:9) as described earlier for the sediment and biological samples. After washing, pipette 1 ml of the extract into a clean glass vial and do gamma counting for 5-10 minutes for mercury recovery check. After counting, follow the same procedure as described for the sediment and biological samples (Fig. 1).

2.4 Mercury recovery check

For sediment and biological samples the mercury recovery is calculated using the following equation:

Hg recovery (%)	=	<u>100 x (gamma counts for 0,5 ml Dz-Bz extract) x 10</u>
		Total gamma counts of the sample before Hg extraction

Mercury recovery from 50 ml of water sample is calculated from the equation:

Hg recovery (%)	=	100 x (gamma counts for 1ml Dz-Bz extract) x 5
		(gamma counts for 20 ml of water) x 50 ml/20 ml

Mercury recoveries by this radiochemical method are usually 95-100%. Recovery values higher than 100% could be obtained from recovery calculations if there had been appreciable loss of benzene by evaporation before the recovery check aliquot of the Dz-Bz extract is collected for gamma counting. The decrease of benzene volume would make mercury concentration higher in the final extract than in the initial volume of 5 ml. In the mercury recovery calculations it is assumed that the mercury concentration in the recovery check aliquot is the same as in the 5 ml extract of the sample.

2.5 Methylmercury to total mercury ratio

The ratio of methylmercury to total mercury in the sample is determined from gamma counts obtained from the TLC strips after the chromatographic separation of inorganic mercury dithizonate and methylmercury dithizonate.

Methylmercury (%) = <u>100 x (gamma counts of methylmercury TLC strip)</u> (Total gamma counts of all 4 TLC strips)

The amount of active total mercury (T-Hg^{*}) in the sample is calculated from the total gamma activity (A μ Ci) of the sample, the specific activity of ²⁰³Hg spike (B μ Ci/ml) and the mercury concentration in the spike (C μ g Hg/ml) using the equation:

T-Hg* (μ g) = A x (C/B)

2.6 Detection limit

The dectecion limit of this technique depends on the specific activity of the ²⁰³Hg –carrier compound, the mercury extraction procedure and the background level of the gamma detector.

For the background of 60 counts per minute (cpm), the detection limit would be 3 times the background and hence 180 cpm. This would correspond to the detection limit of about 0.1 ng Hg in 203 HgCl₂ with specific activity of 32.4 KBq/µg Hg (Amersham International plc., MBS1 Lot 102A). Back calculation through mercury extraction procedure yields a detection limit of about 1 ng Hg/g in the sediment or biological sample. Lower detection limits could be achieved using a 203 Hg-carrier compound with high specific activity.

3. Experiment set-up

Sediment-water systems were spiked with 203 Hg as HgCl₂ at a level of 700 Hg µg/kg (dry weight) in the sediment and incubated for 21-38 days in glass cylinders (8 cm diameter, 32 cm height). The sediment height was about 4 cm and the water column 16 cm. At the end of the incubation period, samples of sediment, water and biota (sediment invertebrates) were analyzed for methylmercury and total mercury using the radiochemical procedure described in this paper.

4. Results and discussion

Some of the results obtained using the present radiochemical method are shown in Figures 3 to 5. Maximum net methylmercury production occurred generally in the uppermost 1-centimetre layer of the sediment columns (Fig. 3). This was consistent with previous studies reporting maximum levels of methylmercury production at or near the sediment-water interface or redox front (Gilmour and Riedel, 1995).

Methylmercury concentrations in water were as high as 422 ng/L in some of the test cells (Fig. 4) after 21 days of incubation. These methylmercury levels in whole water are among the highest values reported in the literature from mercury methylation experiments. High organic matter content (16%) in the sediment used in the experiment probably favored more methylmercury production and its partitioning to the water column. The presence of methylmercury bound on colloidal organic matter in whole water could also account for unusually high methylmercury content of the water.

The distribution of methylmercury in the sediment-biota-water system is shown in Figure 5. The ratio of methylmercury to total mercury was highest in the biota (sediment macro-invertebrates) and lowest in the sediment. The tendency of methylmercury to bioaccumulate in aquatic organisms is well documented in the literature and our results confirm the same.

Because of high sensitivity the technique can be used for accurate evaluation of mercury transformation and partitioning in sediment-water systems. The examples given here dealt with the distribution of total mercury and methylmercury in sediment, sediment-invertebrates and water. The technique is also applicable to other environmental materials such as fish and plant tissue.

5. Conclusion

The radiochemical technique presented here offers rapid and accurate means for evaluating environmental factors influencing the production and distribution of methylmercury in aquatic environments. The technique may find important applications in the assessment of the dynamics of mercury cycling in environmental materials from newly contaminated sites, especially where information on mercury behaviour in a given environment is lacking.

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LEGEND: S4: Sediment-water system 4 T: Top Layer; M: Middle Layer; B: Bottom Layer

Figure 3. Net methylmercury production rates in sediment-water systems during 21 days of incubation.







Figure 4. Total mercury (T-Hg) and methylmercury (MeHg) concentrations and MeHg:T-Hg ratio in water column above the sediment.


Figure 5. MeHg concentration and MeHg:T-Hg ratio in sediment and biota (sediment macro-invertebrates).

Spatial and temporal variability of atmospheric mercury concentrations in northwestern and central Europe

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1 Introduction

At the end of the last decade the annual anthropogenic emission of mercury into the European atmosphere has been estimated to be 726 tons originating from 928 sources. Emissions from the former German Democratic Republic (GDR) accounted for more than 40 % of the European total. The contribution of the GDR originated from several relatively small but in most cases highly industrialized areas. Extremely high amounts of mercury were emitted in the region Halle/Leipzig/Bitterfeld, due to both burning of lignite coal in power plants without flue gas desulfurization equipment and high losses of mercury from chlor-alkali and acetaldehyde factories. As a consequence of the German reunification many of these emission sources were closed between 1990 and 1992. By a combination of field measurements and numerical modeling, emissions from one of these closed and partly demolished facilities have been estimated to be between 2 and 4 tons per year for 1994. Compared with data for 1988 the emissions of mercury into the atmosphere for this particular plant were reduced by a factor of 50 (Helwig and Neske, 1990; Krüger et al., 1999).

The regional distribution of atmospheric mercury in Central Europe before 1990 was characterized by a strong south-to-north decreasing gradient from the main emission area in Germany to Scandinavia. In 1995 and 1997 we have carried out two south-to-north transect measurement campaigns to obtain an up-dated picture on the regional distribution of atmospheric mercury in Central Europe. At four stations, on a 800 km transect line between Berlin and Stockholm total gaseous mercury (TGM) was measured simultaneously with recently marketed Tekran Analyzers with a time resolution of 5 minutes. The findings suggest a declining TGM gradient from the south to the north.

The data sets on the horizontal regional distribution of TGM have been supplemented by data derived from aircraft measurements over Germany. Since almost all our knowledge is derived from ground based measurements little is known about the vertical and horizontal distribution of mercury in the troposphere. Level flights have been carried out over a distance of approximately 400 km within the mixing layer and in the free troposphere. Additionally, vertical profiles of TGM have been measured in the vicinity of one of the closed plants between 400 and 4000 meter a.s.l.

On a local scale, changes in atmospheric mercury concentrations caused by changing emission situations have a relatively short response time. However, on a regional scale a long-term monitoring at background locations is necessary when trends in the TGM concentration shall be properly observed. Continuous measurements of TGM are carried out at Mace Head, Ireland since September 1995 and will be continued until December 2000. The site is the most westerly atmospheric research station in Europe and is ideally placed to monitor the western inflow boundary condition for atmospheric mercury in Europe.

This paper presents a synthesis of recently published work on the regional distribution of atmospheric mercury in north-western and central Europe (Schmolke et al., 1999; Ebinghaus and Slemr, 2000; Ebinghaus et al., 1999).

2 Methods

For all field measurements described here a variable number of Tekran gas-phase mercury vapor analysers (Model 2537A) were used, operated with a time resolution between 5 and 15 minutes. The instrument utilises two gold-cartridges in parallel, with alternating sampling and desorption/analysis cycles on a predefined time base of 5 min. (Tekran, 1998). Mercury is released at approximately 700°C is then detected by an atomic fluorescence detector (AFS). The instruments were operated with a sampling flow rate of 1500 cm³ min⁻¹ (Standard Temperature and Pressure, STP), corresponding to a sample volume of 7500 cm³ (STP). A detection limit of roughly 0,3 ng m⁻³ can be achieved under these conditions (Schmolke et al., 1999). A 47 mm diameter Teflon pre-filter protects the sampling cartridges against contamination by particulate matter. The accuracy and precision of the Tekran analyser has been assessed during a field intercomparison at a remote marine location where recently marketed automated analysers have been intercompared among each other and with the commonly used manual methods (Ebinghaus et al., 1999a).

3 Spatial and temporal variability of atmospheric mercury

Since almost all our knowledge on the regional distribution of atmospheric mercury is derived from ground-based measurements at single locations for different time periods, little information is available on the vertical and horizontal distribution in the troposphere. Based on the generally accepted view that elemental mercury with an atmospheric residence time of about 1 yr is by far the dominating component of total atmospheric mercury (Lindqvist and Rodhe, 1985; Slemr et al., 1985; Schroeder and Jackson, 1987), rather even vertical and horizontal distribution of atmospheric mercury in the troposphere of a hemisphere is expected. Since all other gaseous mercury species and those attached to aerosols have a much shorter atmospheric residence time of up to a few days, enhanced mercury concentrations and pronounced concentration gradients are expected only in the vicinity of sources. These general suppositions are supported by model simulations of transport and chemistry of mercury in the atmosphere (Mason et al., 1994; Pai et al., 1997; Petersen et al., 1998).

3.1 Simultaneous ground-based measurements on the regional horizontal TGM distribution on a 800 km transect between Stockholm and Berlin

Due to it's long atmospheric residence time, atmospheric mercury is subject local, regional and global transport. Among the airborne mercury species elemental mercury is the most predominant (approximately 90% of total) form. A distinct pattern in Total Gaseous Mercury (TGM) concentration is not to be expected at remote places unless a local mercury source, or long-range transport has direct influence on the site of observation.

There have been only a few attempts to investigate the spatial distribution of mercury on a regional scale. An 15% increasing gas phase mercury concentration gradient was observed from the southern to northern part of Michigan USA (Keeler et al 1995). In the Scandinavian countries a deposition network of eight sites was installed in the mid nineties. A clear north to south increasing gradient in wet mercury deposition fluxes was found (Iverfeld 1991).

Atmospheric dispersion models are currently used to describe the atmospheric distribution and deposition of mercury on regional scales, for example for Europe and North America. Experimental input data are needed for the parametrization of the chemistry scheme and for the validation of the model results. However, most of the available information is based on measurements at single locations for different time periods from days to several years. Very few attempts have been made to investigate the distribution of atmospheric mercury during field experiments at simultaneously operated measurement sites on a regional scale. Extremely valuable information has been generated during the Nordic Network for Scandinavia in the late 80's. During the Scandinavian study manual methods for the analysis of atmospheric mercury with a time resolution of several days have been applied. A north to south increasing gradient of aproximately 15% in the annual average TGM concentration was established (Iverfeld 1991a). This effect was attributed to an increasing impact of the major atmospheric mercury source areas in eastern Germany.

No experimentally derived information on the regional distribution of atmospheric mercury for Central Europe was available for the time after 1990. In this work we present the results from two field experiments carried out for two weeks in summer 1995 (June/July) and three weeks in winter 1997 (March/April) at four European sites along a 800 km line between Berlin and Stockholm. A total number of more than 30000 individual concentration measurements has been carried out during these two campaigns An overview about the geographical arrangement of the Transect sampling sites is given in Fig. 1. To avoid the influence of local point sources all sites were located in rural areas (Neuglobsow: approximately 100 km north of Berlin; Zingst: 200 km north of Berlin, adjacent to the German Baltic Sea coast; Rörvik: Swedish west coast; Aspvreten: Swedish east coast). Based on the model calculations by Petersen et al. (1995) a decrease in vapor phase mercury concentrations on this transect was expected. A detailed description of the 1995 experiment is published by Schmolke et al. (1999)

3.1.1 Quality assurance

At all four sites TGM was detected with Tekran Gas Phase Mercury Analysers (Model 2537A). To achieve the best possible comparability between the four sites the instruments and internal permeation sources were calibrated before and after the experimental work by manual calibration. The procedure was adopted from Dumarey et al. (1985). Additionally all instruments were subjected to an intensive two days intercalibration exercise in the GKSS laboratory before the beginning and after the completion of the measurements. A maximum bias of less then 5% was detected between the instruments. To foster the direct comparability of the measurements at the four sites, correction factors were computed from the intercalibration exercises and used to correct the systematic differences between the instruments.

3.1.2 Results of the transect measurements

All shown graphs, and discussed statistics are based on one hour average TGMconcentrations. The set of available descriptive graphs is thought to give an overview on the temporal and spatial (short time and long term) variability of the TGM concentrations in northern/central Europe. Fig. 2 displays on one hand the north to south gradient of TGM concentrations by viewing the median concentrations observed at the two German and two Swedish sites. To enable the comparison of the summer 1995 and winter 1997 experiments both experiments are displayed in the same graph. An overview about the short time (1h) variability, the TGM time series are displayed separately for each single site in Fig. 3 (summer 1995) and Fig. 4 (winter 1997). To enable a better insight in temporal TGM pattern and concentration variability each local data set is analysed by it's frequency distribution and a diurnal cycle plot (summer 1995, Fig. 5) (winter 1997, Fig. 6). The basic statistics of the 1h average concentrations of each time series is summarized in Table 1.

During both, the summer and the winter experiment a decreasing variability in the TGM concentration from south to north was obvious. This is depicted by the time series plots Fig. 3 and Fig. 4 and is confirmed by the south to north decreasing standard deviation of the fitted Gaussian function within the frequency plots in Fig. 5 and Fig. 6 (left four graphs). The high variability, at least at the southernmost site (Neuglobsow) is dominated by a significant 24h periodicity (Fig. 5 and Fig. 6 right four graphs). A shift of the daily maximum TGM concentration from 5 a.m. during the summer experiment (Fig. 5) to 9 a.m. during the winter (Fig. 6) supports a correlation between the TGM concentration and meteorological, photochemical and perhaps biological processes as suggested by Lindberg 1996 (biosphere/atmosphere exchange processes).

Just as significant as the differences of the variability on the south to north transect, the TGM concentration levels differ between the four sites. During both experiments a south to north decreasing TGM gradient was found. A Δ TGM between the mean concentrations observed at the southernmost site Neuglobsow and the most northerly site Aspvreten of 0.38 (1997) and 0.60 (1995) ng m⁻³ was calculated. To avoid the influence of single peak events on the mean concentration levels, the more robust median TGM levels were also compared (Fig. 2). During winter 1997 a north to south increasing median TGM concentration gradient of approximately 20% was found. During the summer 1995 experiment the gradient was less pronounced but with 14 % also significant. Comparing the median concentrations between the 1995 and 1997 experiments the systematically elevated levels at all sites during the winter

experiment become obvious. This finding is in accordance to model results which predicts elevated concentrations during the late winter/ early spring (Petersen et al. 1995). During the 1997 experiment a contaminated air mass was simultaneously observed at the four sampling sites (highlighted in Fig. 4).

3.2 Aircraft measurements of the vertical and horizontal TGM distribution between 400 and 4000 m altitude over southern and eastern Germany

The very few attempts to measure mercury concentrations onboard airborne platforms have provided conflicting results so far. Slemr et al. (1985) measured TGM concentration above central Europe at altitudes varying from 6000 to 12000 m in a fairly small range from 1.2 to 3.1 ng m⁻³ without any pronounced vertical gradient. This is roughly in agreement with the suppositions outlined above, although the large scatter of the data precludes a more definitive conclusion. The large scatter of the data points to analytical problems, possibly due to using stainless steel sampling inlet. In contrast to this data set, Ionov et al. (1976), Kvietkus et al. (1985), Brosset (1987) and Kvietkus (1995) report the occurrence of pronounced gradients with decreasing TGM concentrations with increasing altitude. Ionov et al. (1976) reported a decrease in mercury concentrations with increasing altitude which was similar to the concentration decrease of radon decay daughters and from this they estimated an atmospheric residence time of mercury to be about 10 days. Measurements of Brosset (1987) were made above sea west of Göteborg where no local sources are to be expected. They cover altitudes up to 3000 m and the mercury concentrations decreased roughly proportional to the pressure decrease with altitude. Kvietkus et al. (1985) and Kvietkus (1995) reported measurements over different areas of the former Soviet Union. Mercury concentrations varied strongly depending on the location but generally decreased with increasing altitude of up to 3500 m. A more detailed analysis of the vertical profile over the eastern Lithuania in June 1988 (Kvietkus, 1995) revealed the almost exact proportionality of measured mercury concentrations to the pressure at the sampling altitudes. A possible dependency of the detector response on ambient pressure is not discussed in either of these works.

In this study we report on mercury measurements onboard an aircraft during a level flight from Oberpfaffenhofen, southwest of Munich, to Halle and back, made on June 13, 1996. In addition, vertical profiles of TGM concentrations were measured up- and downwind of a former chlor-alkali and acetaldehyde plant at Schkopau in East Germany (former GDR), which with an emission of more than 50 t/yr is supposed to be the largest singular point source of mercury in Europe until 1990 (Helwig and Neske, 1990). Production at three of the four chlor-akaline plants and at the acetaldehyde plant have been terminated since and the emissions in 1994 were estimated to 2 - 4 t/yr (Krüger et al. 1999; Ebinghaus and Krüger, 1996).

3.2.1 Flight path of the research aircraft Dornier 228 CALM and corresponding weather situation

Fig. 7 shows the path of the flight made out on June 13, 1996. The aircraft started at Oberpfaffenhofen southwest of Munich, southern Germany, and turned north-east towards Halle/Leipzig over a distance of approximately 400 km on a constant cruising altitude of 900 m. 5 km away from the former chlor-alkali plant at Schkopau, the aircraft started to ascent from 400 m to a maximum altitude of 3800 m a.s.l. on the upwind side of the plant (51° 26' N; 11° 57' E). The descent from 3800 m to 480 m was carried out 5 km downwind of the factory (51° 20,5' N; 11° 57,8' E). Horizontal sampling was again carried out on the way back to Munich at a constant cruising altitude of 2500 m above the boundary layer.

A PTFE lined stainless steel tubing was used as a sampling inlet. The tubing was placed on top of the cabin about 1 m in front of the propellers. The tubing was bent and its opening faced the rear of the aircraft to prevent sampling of cloud droplets and coarse aerosols. Two Tekran analysers were connected to the inlet by PFA tubing and operated with a time resolution of 5 min. During take-off and landing the instruments were operated with air filtered by an activated carbon cartridge to avoid contamination of the sample lines and gold traps by kerosene vapor or engine exhaust.

The aircraft instruments were operated at ambient pressure which changes with the flight altitude. Therefore, detailed laboratory studies have been carried out in order to obtain information about the pressure dependence of the Tekran analysers (Ebinghaus and Slemr, 2000).

The synoptical weather situation on June 13, 1996, was dominated by a strong high pressure zone "Xaver" over Ireland. At its perimeter cool marine air masses of subpolar origin were transported into Germany and in the evening the corresponding cold front arrived at the northern rim of the Alps. The exchange of warm and humid by cold air masses in northern Alps was accompanied by strong storm activities with plenty of precipitation. Radiosondes from Prague, Wahnsdorf (near Dresden), Meiningen (halfway between Erfurt and Würzburg) at midday showed an inversion at about 2000, 2000, and 1550 m a.s.l., respectively, with well mixed cold humid air mass below and very dry air mass above.

3.2.2 Results of aircraft measurements

TGM concentrations measured during the horizontal cruises at constant altitudes of 900 and 2500 m a.s.l. are shown in Fig. 8. Each bar in this figure represents a 5 minute interval and a sample volume of 7500 cm³ (STP). At an altitude of 900 m a.s.l., TGM concentrations showed a slight gradient with decreasing concentration to the north. This may be due to the incomplete exchange of the air mass with low TGM concentrations replacing from the north the air mass with higher TGM concentration. According to the radiosonde vertical soundings this entire flight leg was within the mixing layer in a humid air mass. During the return level flight at 2500 m a.s.l., free tropospheric extremely dry air mass was encountered with an average TGM concentration of 1.635 ± 0.094 ng m⁻³ (n=22). The average TGM concentration at 900 m a.s.l. was with 1.774 ± 0.101 ng m⁻³ (n=17) slightly higher. We attribute this small difference to the different air masses rather than a systematical vertical gradient.

Two southernmost TGM measurements at the return flight with 2.190 \pm 0.255 ng m⁻³ (n=2) differed substantially from the rest of the flight. But they agreed with the TGM concentration of 2.321 \pm 0.133 ng m⁻³ (n=8) measured at the same time at the summit of the Wank mountain (Ebinghaus and Slemr, 2000).

At the northernmost point of the flight, approximately 5 km upwind and downwind of the chlor-alkali plant at Schkopau a spiral ascent from 400 to 3760 m a.s.l. and descent, respectively, were carried out. The vertical profiles of the TGM concentrations are shown in Fig. 9. Upwind of the plant, the TGM concentration was with 1.762 ± 0.080 ng m⁻³ (n=14) independent of altitude. Downwind of the factory, higher TGM concentrations with 2.016 ± 0.211 ng m⁻³ (n=5) were observed below 1660 m a.s.l. than upwind of the factory. Above this altitude the TGM concentrations with 1.642 ± 0.061 ng m⁻³ (n=10) were comparable with those measured upwind. The vertical distribution of TGM concentrations downwind of the factory was consistent with radiosonde profiles at Meiningen and Wahnsdorf indicating that the mixing layer at Halle may have reached an altitude of about 1650 m a.s.l. (Ebinghaus and Slemr, 2000)

3.3 Highly time resolved measurements of total gaseous mercury at Mace Head, Ireland between September 1995 and December 1999

Since September 1995 continuous measurements of total gaseous mercury (TGM) are carried out at Mace Head, Ireland. The site is the most westerly European atmospheric research

station and is operated by the Atmospheric Physics Research Group at the National University of Ireland, Galway. The site is located 88 km west of Galway city, near Carna, Co. Galway (53°, 19' N; 9°, 54' W). The station has a clean sector zone between 180° and 300°, with open access to the ocean and is ideally placed to study trace gases in air masses that have travelled several thousand kilometers over the North Atlantic ocean.

A Tekran Model 2537A Mercury Vapour Analyzer was installed at Mace Head in September 1995. Sampling is continuous. A 15 minute sampling frequency was used in this work. The instrument has an autocalibration cycle which was set to 25 hours using an internal permeation source. Additionally, the instrument was calibrated manually every two to three months by injecting 20 μ l of Hg-saturated air with a gas-tight syringe. Before continuous measurements at Mace Head were started, the instrument was intercalibrated with other automated analysers and manual methods as well during an international field intercomparison exercise in September 1995 which was carried out at this site (Ebinghaus et al., 1999a).

3.3.1 Results of long-term TGM measurements at Mace Head

Average TGM-concentrations measured at Mace Head between September 1995 and December 1999 are of value 1.75 ng m⁻³. High concentrations of up to 8 ng m⁻³ have been recorded during relatively short (i.e. typically 1 to 4 hours) pollution events and may be related to local or regional sources as indicated by back-trajectory analysis and supporting measurements such as aerosol black carbon mass concentrations. Highest daily average concentration are of value 4.3 ng m⁻³, highest monthly averages 2.6 ng m⁻³.

Atmospheric background conditions can be expected at Mace Head when the air masses are arriving from the clean sector and aerosol black carbon concentrations of < 75 ng m⁻³ are measured. The combination of both parameters has shown to be a successful filter for the analysis of TGM background conditions (Ebinghaus et al., 1999b). These conditions were observed for approximately 4000 hours during the study period.

Daily average concentration data of TGM depicted in Fig. 10 show that the variability is much less pronounced in the period between April 1998 to December 1999 compared with early measurements between September 1995 and April 1997.

Between 1995 and today no significant trend in the atmospheric mercury concentration level has been observed. Average TGM-concentrations measured at Mace Head over the entire

measurement period are of value 1.75 ng m⁻³. However, on an average basis the winter months show higher concentrations compared with the summer months. Over a period of 5 years lowest TGM-concentrations have been observed in summer (i.e. April to September), with approx. 1.6 ng m⁻³, whereas the average concentrations during wintertimwe (October to March) are around 1.9 ng m⁻³. The seasonal variability of monthly averaged TGM concentrations at Mace Head is shown in Fig. 11.

4 Summary and conclusions

During summer 1995 and winter 1997 Total Gaseous Mercury (TGM) concentration was measured simultaneously and highly time resolved at four sampling sites on a 800 km transect from Berlin to Stockholm. The evaluation of the 1h average TGM concentration time series depicted during both experiments a similar, from north to south increasing gradient of TGM concentrations. TGM concentrations in the range between 1.1 and 4.9 ng/m3 were detected. At the most southerly site an increased mean TGM level in the range between 14 and 20% compared with most northerly site was found. Also the short time variability on an hourly time base showed a from north to south increasing trend during both experiments. These effects are expected to be the influence of the important atmospheric mercury sources in the central part of Europe, in particular in eastern Germany. The comparison of the 1995 and 1997 experiment showed consistently elevated TGM concentration during the winter at all four sampling sites, which is in well agreement with model results.

In summary, aircraft TGM measurements made on June 13, 1996, show that a) TGM is evenly distributed within an air mass over long distances, b) TGM concentration may change with the change of air mass, and c) slight difference between TGM concentration in the mixing layer and the free troposphere may be due to the different air masses rather than a vertical gradient.

The difference between downwind and upwind TGM concentrations can be used to estimate roughly the mercury emissions at the factory (see Fig. 9). The width of the factory area normal to the wind direction was approximately 1.5 km. The factory is located at an altitude of 130 m a.s.l. and the mixing height is assumed to be 1660 m a.s.l. Weather map yields a surface wind speed of approximately 10 m/s and an air temperature of 13°C for the region Leipzig/Halle. Assuming that TGM concentrations are evenly distributed over the width of the factory area and that the entire sounding spiral was within the factory plume, the mean

difference of TGM concentrations in the mixing layer then results in emission of about 0.4 kg of mercury per day.

Long-term measurements of TGM at the atmospheric research station at Mace Head, Ireland have been carried out between September 1995 and December 1999. Measurements will be completed by the end of year 2000. From Fig. 10 it can be seen that the variability of daily average concentrations (scattering) was more pronounced in the beginning of the long-term study. Between September 1995 and July 1997 higher concentrations (i.e. > 2.5 ng m⁻³) have been detected much more frequently compared with the second part of the study from April 1998 to December 1999. However, the regression line based on all TGM daily averages shows that no significant trend in the concentration levels can be derived.

Monthly means for the individual months have been used to evaluate seasonal variations in the TGM background levels. Although the number of data points (i.e. for January, February, March, August: N=3; for April, May June, July, September, October, November, December: N=4) is still fairly limited, a seasonally dependent TGM background concentration can be derived with higher levels in winter compared with the summer months.

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6 Tables

1997 March - April						
Site	Valid N [n]	Mean [ng/m³]	Minimum [ng/m³]	Maximum [ng/m³]	Std.Dev [ng/m³].	
ASP	408	1.75	1.38	4.81	0.21	
ROE	427	1.94	1.53	2.87	0.18	
ZIN	427	2.09	1.62	3.79	0.30	
NEU	425	2.13	1.49	4.03	0.34	
1997 Marc	h - April					
ASP	222	1.51	1.10	1.79	0.11	
ROE	232	1.54	1.20	1.91	0.11	
ZIN	233	1.83	1.45	3.79	0.26	
NEU	250	2.11	1.42	4.66	0.50	

Table 1Descriptive statistics of the 1h average TGM data sets measured during the
1995 and 1997 experiments.

7 Figures

Fig. 1: Location of the four sampling sites used during the TGM Transect experiments in 1995 and 1997.



Fig. 2: Regional distribution of the median TGM observations during Summer 1995 and Winter 1997. In the graph the sampling sites are arranged from the most northerly site on the left hand (Asp) to the most southerly site on the right hand (Neu).



Fig. 3 Time series of the 1h average TGM concentrations observed during the 1995 experiment, separately displayed for each sampling site. The sites are arranged from the most northerly site at the top of the graph to the most southerly at the bottom.





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Fig. 5 Combined graphical representation of the frequency distributions (left four graphs) and diurnal cycle (right four graphs) of the 1h average TGM concentrations measured during the 1995 Experiment. The frequency plot groups the TGM concentration cases into 0.2 ng/m³ classes. Additionally a fitted Gaussian probability plot is superimposed. The diurnal cycle is realized as box and whisker plot. All TGM observations are collected in hourly daytime groups on a 24 hour scale. Each box displays the following basic statistics Min, Max, 25%-75% Quartile, Median.



Fig. 6 Combined graphical representation of the frequency distributions (left four graphs) and diurnal cycle (right four graphs) of the 1h average TGM concentrations measured during the 1997 Experiment.



Fig. 7 Map of Germany showing path of the flight made on 13 June 1996.



Fig. 8 TGM concentration measured during the level flight legs at 900 and 2500 m a.s.l. on 13 June 1996



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Fig. 9 Vertical profiles of the TGM concentrations upwind and downwind of the chlor-alkali plant at Schkopau near Halle on 13 June 1996



TGM gradient in upwind position of the factory:

TGM gradient in downwind position of the factory :



Fig. 10 Daily average concentrations of TGM at Mace Head Ireland between September 1995 and December 1999. Between August 1997 and April 1998 no measurements have been carried out



Fig. 11 Annual cycle monthly means of TGM concentrations measured at Mace Head from 1995 to 1999



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Clinical and Pathologic Studies of Methylmercury Poisoning in Common Marmosets

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ABSTRACT

Neuropathological legions found in chronic human Minamata disease tend to be localized in the calcarine cortex of occipital lobes, the pre- and post- central lobules and the temporal gyri. The mechanism for the selective localization of the lesions is still not clear, though some hypotheses have been proposed. One of the hypotheses is vascular factor, which postulated that the lesions are the result of ischemia secondary to compression of sulcal arteries from methylmercury-induced cerebral edema. The cerebral edema is diffuse but sulcal arteries running in deep fissures and sulci tend to be compressed more severely than those running in shallow sulci resulting in selective cortical lesions along deep sulci. To test this theory we selected common marmosets for experiment as the brain of marmosets has two distinct deep sulci, calcarine and Sylvian fissures. The experimental animals were divided into three groups of acute poisoning, chronic poisoning and control. Cerebral edema is best observed in the animals which were sacrificed early in the stage whereas more classical cortical degeneration will be expected in the animals with long-term exposure. The levels of total mercury in blood and the body weight were recorded every week. MRI (Magnetic resonance imaging) of the head and VTR (videotaperecorder) on each animal were taken prior to autopsy. MRI analysis, mercury assay of tissue specimen, histologic and histochemical studies of the brain are reported and discussed. The brains of animals which were sacrificed early after exposure to methylmercury showed high contents of methylmercury with edematous changes in the cerebral white matter. The brains of animals subjected to chronic methylmercury poisoning showed dominant accumulation of inorganic mercury and atrophy of the calcarine cortex. The results showed the presence of cerebral edema in the early and acute stage of methylmercury poisoning, which may be related to the selective cortical degeneration along the deep cerebral fissures or sulci.

Key words: methylmercury poisoning, common marmosets, pathologic study

INTRODUCTION

Neuropathological lesions have been found in the calcarine cortex of the occipital lobes, preand post-central gyri and temporal transverse gyri in the cerebrum of patients who died Minamata disease.

The following factors have been considered in order to explain such selective localization of the cerebral lesions. They are: 1) the tendency of small neurons to be damaged preferentially cytotoxically as seen in the cerebellar cortex and 2) the possibility that microenvironment in different sites of the cerebrum rather than different types of neurons playing critical roles.

In regard to the latter, we have noticed that there are overall histopathological similarities between Minamata disease and anoxic-ischemic encephalopathy pointing to a possibility of vascular factor in the development of cerebral lesions in Minamata disease(1,5). In this connection, we also noted that predilection sites of the cerebral lesions in experimental methylmercury intoxication are more severe in the cortices along deep sulci and fissures. These cortices are found to be either selectively or more severely involved in anoxic-ischemic encephalopathy as these cortices are supplied by sulcal branches of leptomeningeal arteries which are more vulnerable to compression when they are in deep sulci and fissures than those branches on the surface or in the shallow sulci in cases of diffuse cerebral edema which may be associated with anoxia-ischemia and other types of cerebral insult (4).

Cerebral edema has not been observed in the autopsied brains of human Minamata disease when the patients died months and years after exposure to organic mercury. However, cerebral edema may be appear in earlier stage of and subside in later stage in methylmercury intoxication. To test the above hypothesis we used common marmosets, which have two deep fissures, calcarine and Sylvian, for the experiment.

MATERIALS AND METHODS

Four adult male marmosets weighing 340 to 420 g (4 years) were used for the experiments. The animals were divided into two groups of two each. Methylmercury was daily given from drinking water (5 μ g Hg/ml) Four control animals were given water. The first group of two animals were sacrificed when blood mercury levels reaching 8 μ g Hg/ml before the appearance of any clinical sign of mercury poisoning (Fig. 1). The duration of experiment in this group was about one month. Methylmercury exposure to the second group was ceased when the blood mercury levels attained 10 μ g Hg/ml, and the animals were allowed to live for two and a half years until sacrifice.

The clinical condition was recorded by VTR in animals and MRI scan was obtained in one animal in the Group I (99-03) sacrifice using a 1.5-T clinical MR unit at the Kumamoto University

School of Medicine. T1-weighted images were obtained using a spin-echo sequence with 470/14/8 (TR, TE, NEX), and T2-weighted images were obtained using a fast spin-echo sequence with 3500/96/15/7 (TR, TE, NEX, ETL) in sagittal and coronal planes. The section thickness was 2 mm, field of view was 53×70 mm, and the matrix was 112×256 . On T1- and T2-weighted images, signal intensity of the gray and white matter of the occipital lobe, as well as width of the calcarine fissure, was compared to controls by a radiologist.

The content of total mercury in blood was measured once a week. The two animals in the first group were sacrificed 37 days after the start of the experiment. Blood was solvated from the heart under pentobarbital anesthesia, and then perfused via the heart with 0.9% saline followed by 4% para-formaldehyde in phosphate buffered solution. Pieces of brain, kidney and liver were excised and fixed in 10% neutral formalin. These tissues were processed through ethanol and xylol, and embedded in paraffin. Six micron thick sections were stained with H&E, Klüver-Barrera, Bodian, Masson's trichrome stains as well as photo-emulsion histochemical stain for inorganic mercury (3). Another animal brain was perfused with 100 ml of buffer formalin through ascending aorta, followed by an injection of 40 ml of methacrylate resin (Mercox CL-2R, produced by Dainippon Inc., Tokyo). Half-centimeter thick coronal sections of the brains were obtained, and the sections were immersed in 20% KOH solution for 10 to 14 days to dissolve the brain tissue. After washing with tap water, the blood vessel cast thus obtained was freeze dried. The specimens were coated with gold-palladium in a vacuum evaporator before scanning electron microscopic study.

Total mercury level in each tissue sample for inorganic mercury were prepared by removing methylmercury using benzene-petroleum ether extraction from the tissue homogenate as reported before (7). Determination of methylmercury was carried out by ECD gas-liquid chromatography.

The determined ratio of the area in white matter and white and gray matter of occipital lobes was performed in the experimental and control animals by using the comparison of the weight of a piece of paper.

RESULTS

Just before the termination of experiment, the two methylmercury-treated common marmosets appeared restless and irritable, and showed a mild ataxia of the hind limbs compared with a control case. The body weight decreased less than 50 grams in both cases (Fig. 1). Total blood mercury levels of the two methylmercury-treated animals were measured once a week. The levels reached about 7μ g Hg/ml at the time of autopsy (Fig. 1). The levels of total-, inorganic and methylmercury of the cerebrum, cerebellum, liver and kidney of a control animal were shown in Table 1. The levels of mercury in the control case were almost zero whereas, the experimental animal showed high total mercury levels in every organ tissue. And the ratio of methylmercury total mercury was very high (Table 2). Mercury granules were positive in the liver and kidney.

Table 1. Chemical analysis of total-, inorgan	ic and methyl mercury
in the tissues of common marmoset ($\mu g/g$).	Control

Tissue	Total Hg	Inorg Hg	Me Hg	M/T (%)	I/T (%)
Cerebrum	0.009	0.000	0.000	0.000	0.000
Cerebellum	0.012	0.000	0.000	0.000	0.000
Liver	0.021	0.000	0.000	0.000	0.000
Kidney	0.020	0.000	0.000	0.000	0.000

Table 2. Chemical analysis of total-, inorganic and methyl mercury in the tissues of common marmoset ($\mu g/g$). Acute: 99-02

Tissue	Total Hg	Inorg Hg	Me Hg	M/T (%)	I/T (%)
Cerebrum	14.63	0.45	14.39	98.3	3.1
Cerebellum	12.98	0.36	12.42	95.7	2.7
Liver	50.00	4.63	43.39	86.8	9.3
Kidney	64.35	13.58	47.58	73.9	21.1

In MRI study, T2-weighted sagittal image of an acute case of methylmercury-treated (99-02, 35days) showed indistinct images in a calcarine and a Sylvian fissures (Fig. 2) compared with the control (Fig. 3). Macroscopic observation of the medial surface of the right hemisphere in the acute case showed edema of the tissue surrounding the calcarine fissure (Fig. 4) compared with the control (Fig. 5). The calcarine fissures by the T2-weighted coronal image (Figs. 6 and 7) of the acute case were unclear (Fig. 6). Macroscopic observation of coronal sections of the occipital lobe in the acute case showed compression of the calcarine fissures (Fig. 8). Similar change was not observed in the calcarine fissures of the control case. (Fig. 9).

Microscopically the calcarine cortex of the acute case showed gliosis in the second to third layers (Fig. 10), and atrophy of the whole cortex compared with the control case (Fig. 11) which was demonstrated by GFAP stain (Fig. 12). The control showed no gliosis on the GFAP stain (Fig. 13). A white matter of the occipital lobe showed micro-cystic change by Klüver-Barrera stain (Figs. 14 and 16) compared with the control case (Figs. 15 and 17).

In the MRI study of another acute case (99-03), T2-weighted sagittal images showed indistinct images of the calcarine and Sylvian fissures as compared to the same figure of the acute case (99-02) (Fig. 18). T2-weighted coronal image showed also the same image as the another acute case (99-03) (Fig. 19).

The capillary networks of the calcarine cortices were markedly distorted with shrinkage (99-03) (Fig. 20) compared with those of control cases (Fig. 21).

The gray/white matter ratio of occipital lobes was measured in three acute cases of methylmercury-treated animals and two control cases. The gray/white ratio in the acute cases was smaller than that of control cases (Table 3). The difference of the ratio was statistically significant (p<0.001).



Fig. 1. Body weight and blood Hg levels of MeHg-treated common marmosets 99-02 (■) and 99-03 (□). MeHg was given by water (5 ppm Hg).



- Fig.2: T2-weighted sagittal image of an acute case (99-02) of methylmercury poisoning.The calcarine (one arrow) and Sylvian fissures (two arrows) are indistinct compared with a control case.
- Fig,3: T2-weighted sagittal image of a control case. The white lines represent calcarine (one arrow) and Sylvian fissures (two arrows).
- Fig.4: The medial surface of the right hemisphere of an acute case (99-02). The calcarine fissure narrows with edematous change and swelling of the adjacent tissue (arrows)
- Fig.5: The medial surface of the right hemisphere in a control case. The calcarine fissure is very clear (arrows).



- Fig.6: T2-weighted coronal image of an acute case (99-02). Calcarine fissures are swollen and indistinct (arrows).
- Fig.7: T2-weighted coronal image of a control case. The white lines represent the clear calcarine fissures (arrows).
- Fig.8: Coronal sections of the right occipital lobes in an acute case (99-02). The calcarine fissures are not clear (arrows).
- Fig.9: Coronal sections of the right occipital lobes in a control case. The calcarine fissure is open (arrows).



- Fig.10: A photomicrograph of the calcarine cortex and a part of white matter in an acute case (99-02). Gliosis is seen in the second and third layers (Klüver-Barrera stain, $\times 150$).
- Fig.11: A photomicrograph of the calcarine cortex in a control case. (Klüver-Barrera stain, ×150).
- Fig.12: A photomicrograph of the calcarine cortex. Fibrillary astrocytes are increased in number (arrows) (GFAP stain, ×300).
- Fig.13: A photomicrograph of the calcarine cortex in a control case showing a lack of gliosis (GFAP stain, × 300).



- Fig.14: A photomicrograph of the occipital white matter showing micro-cystic change in deep portion in case No. 99-02 (Klüver-Barrera stain, ×150).
- Fig.15: A photomicrograph of the white matter in the occipital lobe in a control case (Klüver-Barrera stain, $\times 150$)
- Fig.16:.High magnification of Fig.14 in the acute case (99-02) Micro-cystic change is clear (Klüver-Barrera stain, ×300).
- Fig. 17: High magnification of Fig. 15 in the control case (Klüver-Barrera stain, \times 300).


- Fig.18: T2-weighted sagittal image of an acute case (99-03). The calcarine (one arrow) and Sylvian fissures (two arrows) are indistinct.
- Fig.19: T2-weighted coronal image of another acute case (99-03). Calcarine fissures are indistinct (arrows).
- Fig.20: The vascular architecture of the calcarine cortex in an acute case. The capillary networks were markedly distorted with shrinkage. (99-03) ($\times 50$).
- Fig.21: The vasculature of the calcarine cortex in a control case, showing smooth arrangement of capillary networks (×50).

				
No. of Cases	White matter (W)	Gray matter (G)	W/W+G (%)	Average & SD
A-①	0.45	1.32	25.4	
A-2	0.33	1.15	22.3	
B-①	0.41	1.14	26.5	
B-②	0.33	0.96	25.6	23.79
99-02-①	0.39	1.30	22.6	±1.95
99-02-②	0.33	1.16	22.1	Ť
99-02-③	0.27	0.96	22.0	p < 0.001
CTL-①	0.33	1.25	20.9	↓
CTL-2	0.26	1.06	19.7	
CTL-4-①	0.33	1.46	18.4	18.62
CTL-4-2	0.26	1.15	18.4	± 1.94
CTL-4-3	0.16	0.86	15.7	

gray matter of occipital lobes in common marmosets

Table 3.

The ratio of areas in white matter to white and

DISCUSSION

There are selective lesions of the cerebrum in human cases of methylmercury poisoning (Minamata Disease). That is, the selective lesions of neuronal loss are found in anterior parts of calcarine cortex, post-and precentral gyri, and temporal transverse gyri. The reason for the occurrence of these selective lesions has not been made clear. The tendency of small neurons to be damaged preferentially may be a clue to the mechanism of the cytotoxic action. But the granule cells of second and fourth layers of the cortices are not always damaged.

Microenvironments in different sites of the brain rather than neurons per se might also explain these observations. For instance, we have noticed an overall similarity between anoxia and Minamata disease with respect to the localization of cerebral lesions, which may be taken to speculate a possible role of circulatory disturbance in the generation of the site specificity observed (1,5). Moreover, a possible damage to the blood-brain barrier may play some role. If this should occur, it might not only aggravate anoxia, but also bring about a decrease in glucose supply, which could be neurotoxic. Therefore Takeuchi and Eto (6) proposed that the selectivity of the lesions of the cerebrum in Minamata Disease was caused by 1) the cytotoxicity of methylmercury to the neurons, 2) special system of the blood-brain barrier.

In this connection, we also noted in the same studies that the preferred sites for methylmercuryinduced damage in the brain roughly coincided with the portion where the arterial branches are particularly complex. The loss of blood supply might also be accounted for by focal compression of arteries due to localized edema in perivascular space.

To demonstrate this hypothesis, common marmosets, which had two deep sulci in the cerebrum, were used for the experiment of methylmercury poisoning. The contents of total mercury in blood of the animals were measured weekly. Methylmercury administration was stopped before the total mercury level reached 10μ g Hg/ml in blood. Over 10μ g Hg/ml in blood of common marmosets showed chinical signs of methylmercury poisoning. Slight ataxia was recognized on the VTR in these two cases. Calcarine and Sylvian fissures of the two animals were poorly visualized in T2-weighted MR image compared with control cases. In the acute phase, T2-weighted images showed decrease in contrast between the gray matter and white matter in the occipital lobe, which reflected increase in the signal intensity of the white matter probably due to white matter edema. There are no apparent decreases in contrast in the anterior areas of the brain. T1-weighted images did not depict any abnormal findings. The calcarine fissure was not dilated on the acute-phase MR images.

The calcarine fissures were compressed by surrounding calcarine cortices at autopsy. The measurement of the gray/white matter ratio of areas in the occipital lobes revealed that the areas of the white matter of experimental animals were greater than for control. Slight gliosis was found in

the calcarine cortex. The vessels on the surface of treated calcarine cortex showed shrinkage and disarrangement compared with control animals. These features of vessel-changes might be explained by the atrophy of calcarine cortex from the loss of neurons and gliosis. Alternatively, secondary compression of subarachnoid arteries and calcarine cortex by edema may have contributed to the change (4). The findings suggest that the localization of selective lesions by methylmercury poisoning may be caused by cerebral edema of the white matter in the calcarine cortex. Mild lesions were found the cortices along the Sylvian fissure, but the histopathological changes were less severe than that of the calcarine cortices.

It is hypothesized that white matter edema occurring during acute methylmercury poisoning leading to compressed sulcal arteries and focal vascular insufficiency in the calcarine cortex. The ratio of methylmercury to total mercury was high in every organ of the cerebrum, cerebellum, liver and kidney. The increased inorganic mercury was due to the mechanism of demethylation in the organ tissues.

SAMMARY

- 1. The most prominent lesion of the cerebrum in a common marmoset by methylmercury poisoning was found in occipital lobes.
- 2. An acute case showed high contents of methylmercury with edematous changes in the white matter of the cerebrum.
- 3. These results suggest that the edematous changes of the white matter near the deep sulci (calcarine fissure) may contribute the selective damage of the calcarine cortex.

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Comparison of neurobehavioral changes in three inbred strains of mice prenatally exposed to methylmercury

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Abstract

Pregnant mice of three inbred strains (BALB/c, C57BL/6J, C57BL/6Cr) were orally given methylmercury (MMC; 3 x 3 mg /kg b.w.) or the equivalent volume of phosphatebuffered saline (PBS) during days 12 - 14 of gestation and allowed to deliver. The behaviors of their male offspring were evaluated in an open field and their home cage and in a Morris water maze. In the open field, the BALB/c and C57BL/6Cr MMC groups exhibited less total locomotor activity than did their respective control groups. In the BALB/c strain, the MMC group exhibited significantly more central locomotion than did the control group. For spontaneous activity in their home cage, all groups except the BALB/c MMC group moved more actively in the dark phase than in the light phase. The BALB/c MMC group moved in the light phase as much as in the dark phase, indicating a disturbance of the nocturnal rhythm of spontaneous activity. In the Morris water maze, the C57BL/6Cr and C57BL/6J control groups performed excellently over 5 consecutive days, but not in BALB/c strain. The prenatal exposure to MMC caused significantly prolonged latency in the C57BL/6Cr and C57BL/6J strains. This result indicated that the performance of mice prenatally exposed to MMC in the Morris water maze depended on the strain of mice. Also, the prenatal MMC exposure caused different neurobehavioral changes in the open field and home cage situations, and in the Morris water maze among three inbred strains of mice.

Introduction

Methylmercury (MMC), a hazardous environmental pollutant, has been shown to be a neurobehavioral toxicant to the developing nervous system of human fetuses (5, 15, 16). In the outbreaks of MMC poisoning in Japan (7, 10) and Iraq (1, 9), many *in utero*-exposed children had signs of severe mental and motor dysfunction, although the mothers of these children exhibited only minor symptoms or none at all. In laboratory animals (such as rats and mice), perinatal exposure to MMC also caused some neurobehavioral changes (for a review, see 15) in reflexive development, locomotor activity, emotionality, cognitive dysfunction (e.g., learning and memory), and other behavioral functions.

There has been increasing concern about several fish-eating populations who have consumed methylmercury at relatively low levels(3). For example, in the Faroe Islands, cognitive deficits (e.g., memory, attention, and language) were found in children that had prenatal methylmercury exposure levels (based on maternal hair mercury concentrations) that are currently considered to be safe (below 10-20 ppm) (5). An important question is whether their cognitive dysfunction becomes worse under a continuous exposure to methylmercury until the adult or senescent age. As a first step in answering this question, we designed a method for evaluating neurobehavioral changes among the three most widely used inbred strains of mice after exposure to methylmercury.

MATERIALS & METHODS

Animals

On days 7 – 10 of gestation (day 0 of gestation was defined by the presence of a vaginal plug), three pregnant inbred strains of mice (BALB/c, 12 mice; C57BL/6Cr, 11 mice; C57BL/6J, 8 mice) were purchased from Nippon SLC Co. Ltd (Hamamatsu, Japan) and Nippon CLEA Co. Ltd (Tokyo, Japan). They were allotted to either a control group or a treatment group. They were kept in a temperature-controlled room $(23 \pm 2^{\circ}C)$ with a 12 h light-dark cycle (light, 0800 - 2000). Mice had free access to food and water. They were orally given MMC (3 x 3 mg/kg b.w.) or an equivalent volume of phosphate-buffered saline (PBS) on days 12 - 14 of gestation and allowed to deliver. On day 3 postpartum, litter size was adjusted to six (three male and three female whenever possible). The litters were weaned 21 days after birth, and only male mice were raised for performance of behavioral tests.

Open field test

At 6 weeks of age, the male offspring were observed in an open field apparatus. The floor of the apparatus measured 50 x 50 cm was divided into 25 evenly spaced squares. The floor was surrounded by a 20-cm-high, opaque wall. The open field apparatus was illuminated by an 80-W fluorescent room light 1.5 m above the apparatus. A CCD camera was mounted directly above the open field apparatus. A fan motor in the room provided constant background noise. Each mouse was moved from its home cage to the center square of the open field, and a box made of opaque black Plexiglas (10 x 10 x 10 cm) was then placed over the mouse. After 20 sec, the box was gently removed, and the behavior of the mouse was observed for 2 min. The behavior of the mouse was recorded and analyzed using an image-analyzer (AXIS 60 video-tracking system, Neuroscience Inc., Tokyo, Japan) capable of counting the frequency of motor movements such as rearings, groomings and preenings. Total locomotor activity was defined as the length (cm) of the path walked in the 25 squares of the open field in 2 min. The squares were classified as either peripheral (the 16 squares adjacent to the wall) or central (the nine remaining squares in the center). Percent of central or peripheral locomotion was defined as following: [the length (cm) of the path of the central or peripheral area / the length (cm) of the total path of the open field] x 100. The defecation score (the number of fecal boli) in the open field was also counted. Before each trial the floor and the wall were cleaned with 70% alcohol followed by wet cotton. All the trials were done in the morning between 1000 and 1200.

Home cage activity

At 12 – 14 weeks of age, the spontaneous activity of the male offspring was determined in their home cage for 24 h. When testing started, all mice were transferred to a new cage each with wooden bedding material and were allowed to acclimatize for 48 h in the cage. The cage was placed 15 cm below an activity sensor (Model NS-AS01, Neuroscience Inc.) connected to a host computer. The 24-h activity of the mice collected at the 5 min-intervals was analyzed using an analysis system (AB system, Neuroscience Inc.).

Morris water maze

At 8 weeks of age, the male offspring were observed in the water maze. The apparatus was based on the design of Grant et al. (6). The water maze was a circular plastic pool 100 cm in diameter, filled with water (at a temperature of approximately 20 °C) thermostatically controlled. The water was made opaque by adding milk or ink to prevent the mice from seeing a submerged platform and to increase the animal-background contrast. A round, 10 cm-diameter platform that was covered with silicon mesh (to provide footing for the mice) was placed at a fixed location, e.g., in the center of one quadrant of the pool, submerged 1 cm below the surface of the water (20 cm depth). Around the water maze were situated curtain walls with various patterns (such as crosses, circles, and lines), a plastic doll, and a picture. All were visible from the

inside of the pool, and served as distant visual cues (11) for the mouse. A mouse was released into the water at a constant position, facing the outer edge of the pool. The mouse was oriented at angle of 135 degrees clockwise from a line from the center of the water maze to the platform. A CCD camera, mounted at the center above the pool, recorded the behavior of the mouse. The movement of mouse were analyzed using an image-analyzer (AXIS 60 video-tracking system, Neuroscience Inc).

Mice of each strain received 3 trials on each of 5 consecutive days. The latency, defined as the time (in sec) from the release of the mouse to the climbing on the platform, was measured. During the 10 min inter-trial interval, the mouse was maintained in a dry holding cage. When a mouse could not find or climb on the platform within 120 sec, it was gently lifted out of the water by hand and placed on the platform for 30 sec. In this case, a latency of 120 sec was recorded.

Statistical analysis

Means of mercury levels in various tissues, activity scores in open field, and spontaneous activity in home cage were calculated, and the differences between the MMC and control groups were analyzed by Student's *t*-test. For body weight, data of each strain were analyzed by repeated measures analysis of variance (ANOVA) with prenatal exposures (MMC or PBS) as a between-subject factor and weeks (3 week to 12 week) as a within-subject factor. For the Morris water maze, the latency to find the platform was averaged per mouse within each trial day. Data of each strain were analyzed by repeated measures ANOVA with prenatal exposures (MMC or PBS) as a between-subject factor and trial days (Day 1 to Day 5) as a within-subject factor. All statistical calculations were performed by the SYSTAT statistical package (version 5.2 edition. Evanston, IL: SYSAT, Inc., 1992), which can handle unbalanced design (i.e., unequal number of data per cell).

RESULTS and DISCUSSIONS

In the present study, we monitored the male offspring of three inbred strains of pregnant mice prenatally exposed to MMC in two different environments: an unfamiliar one (open field) and a familiar one (home cage). Activity scores in the open field situation were considered to reflect both exploration and emotionality (2, 14). Since the open-field situation per se is stressful (4), the spontaneous activity in home cage situation was also measured in the dark and light phases.

In the open field, the BALB/c and C57BL/6Cr MMC groups exhibited less total locomotor activities than did the control groups. However, there was no significant

Table							
TABLE 1							
ACTIVITY SCORES IN THE OPEN FIELD SITUATION							
	Total locomotor Activity (cm)	Rearings (No. of times)	Defecation (No. of fecal boli)	Groomings & Preenings (No. of times)			
BALB/c	· · · · ·						
Control	844 ± 148	0.3 ± 0.8	5.8 ± 2.1	1.0 ± 0.7			
MMC	660 ± 248 *	0.3 ± 0.7	7.0 ± 2.1	0.1 ± 0.4 **			
C57BL/6Cr							
Control	1281 ± 174	10.9 ± 3.7	1.6 ± 1.5	1.6 ± 0.9			
MMC	950 ± 231 ***	8.3 ± 4.3 *	1.2 ± 1.2	1.8 ± 1.0			
C57BL/6J							
Control	1260 ± 303	12.0 ± 4.1	0.9 ± 1.8	0.6 ± 0.7			
MMC	1119 ± 275	10.5 ± 4.9	<u>0.9 ± 1.0</u>	1.5 ± 1.0 *			

All values represent mean \pm SD. The number of mice in each prenatal exposure group: the controls: BALB/c (n = 16), C57BL/6Cr (n = 20), C57BL/6J (n = 11); the MMCs: BALB/c (n = 8), C57BL/6Cr (n = 21), C57BL/6J (n = 16).

The difference between the MMC and control groups in the same strain was analyzed by Student's *t*-test (* p < 0.05, ** p < 0.01, *** p < 0.001).

TABLE 2

SPONTANEOUS ACTIVITY IN HOME CAGE SITUATION					
	Dark phase (count) 2000 – 0800 h	Light phase (count) 0800 – 2000 h	Ratio (Dark phase : Light phase)		
BALB/c					
Control (n=9)	7561 ± 2402	3673 ± 2274	68.6 % : 31.4 %		
MMC (n=8)	6399 ± 4160	7694 ± 7347	47.6 % : 52.4 % †		
C57BL/6Cr					
Control (n=12)	9439 ± 3044	2898 ± 1556	77.2 % : 22.8 %		
MMC (n=13)	7037 ± 3118	1464 ± 746 **	82.3 % : 17.7 %		
C57BL/6J					
Control (n=10)	6680 ± 2170	2025 ± 1086	77.7 % : 22.3 %		
MMC (n=8)	5166 ± 1634	831 ± 466 **	86.0 % : 14.0 %		

Each value represents mean \pm SD. The difference between the MMC and control groups was analyzed by Student's *t*-test (* p < 0.05, ** p < 0.01, *** p < 0.001).

[†] There was no significant difference observed between the dark phase- and the light phase-spontaneous activities in the BALB/c MMC group.

------Table ------

difference observed between the MMC and control C57BL/6J strain (Table 1). The BALB/c MMC group exhibited fewer grooming and preening movements than did the associated control group. The C57BL/6Cr MMC group made fewer rearing movements than did the control group. These results, i.e., less total locomotor activity and a lower frequency of rearing and grooming, indicate that the prenatal exposure to MMC caused significantly a decrease in open-field activity in the BALB/c and C57BL/6Cr MMC groups. These results are consistent with the findings of Su and Okita (12), who observed that offspring of mice prenatally exposed to MMC showed a decrease in activity scores including a reduced frequency of rearings and groomings. For the ratio of the central to peripheral locomotion in the open field, the BALB/c MMC group exhibited significantly more central locomotion and less peripheral locomotion than did the control group (Fig. 1). This result also was consistent with the findings by Su and Okita (12) that the prenatal exposure to MMC resulted in a longer central square latency and a lower number of peripheral squares traversed. It has reported that rats and mice tend to concentrate their ambulation in the peripheral area, where they can physically touch the walls, thus avoiding the more aversive central area (2, 13). Thus, these results, i.e., more frequent central locomotion, were probably due to a change in emotional status.

For spontaneous activity of the dark and light phases, all groups moved more actively in the dark phase than in the light phase except the BALB/c MMC group (Table 2). In the BALB/c strain, the MMC group moved in the light phase as much as in the dark phase. This result indicated that the prenatal exposure to MMC caused a disturbance of the nocturnal rhythm of spontaneous activity in the BALB/c strain. In spontaneous activities of the light phase including the period of the open field test (1000 - 1200 h), the light phase-spontaneous activities were much lower in the C57BL/6Cr and C57BL/6J MMC groups than in their respective control groups. Since the light phase-spontaneous activity may be a background level of open-field activity, it may at least partly account for changes in the level of open-field activity. In the C57BL/6Cr MMC group, a decrease in the light phase-spontaneous activity was accompanied by a decrease in open-field activity. In the BALB/c MMC group, a decrease in open-field activity was accompanied by no change in the light phase-spontaneous activity, which indicates that it was not derived from change in spontaneous activity. Therefore, this finding can explain that the decrease in open-field activity may be due to a change of emotional status with an increase in central locomotion.

For the Morris water maze for 5 consecutive days (Fig. 2), in the C57BL/6Cr strain, the prenatal exposure to MMC caused significantly prolonged latency [F (1, 18)



FIG 1. Central or peripheral locomotion in open field. The percent of central or peripheral locomotion was defined as following: [the length (cm) of the path of the central or peripheral area / the length (cm) of the total path of the open field] x 100. Each histogram represents the mean \pm SD. The number of mice in each prenatal exposure group: the controls: BALB/c (n = 16), C57BL/6Cr (n = 20), C57BL/6J (n = 11); the MMCs: BALB/c (n = 8), C57BL/6Cr (n = 21), C57BL/6J (n = 16). The difference between the MMC and control groups was analyzed by Student's *t* test (* *p* < 0.05).



FIG 2. The performance of three inbred strains in the Morris water maze. Latency is the time (in sec) from the release of the mouse to its climbing on a submerged platform. Data points are daily group means \pm SE for 5 consecutive days. The number of mice in each prenatal exposure group: the controls: BALB/c (n = 10), C57BL/6Cr (n = 10), C57BL/6J (n = 11); the MMCs: BALB/c (n = 8), C57BL/6Cr (n = 10), C57BL/6J (n = 10). Data of each strain were analyzed by repeated measures ANOVA with prenatal exposures (MMC or PBS) as a between-subject factor and trial days (Day 1 to Day 5) as a within-subject factor. The prenatal exposure to MMC caused significantly prolonged latency in the C57BL/6Cr [F (1, 18) = 6.78, p = 0.018] and C57BL/6J strains [F (1, 19) = 8.40, p = 0.009], but not in BALB/c strain mice [F (1, 16) = 3.72, p = 0.072].

-----Fig -----

= 6.78, p = 0.018], but the interaction between the prenatal exposure and trial days was not significant [F (4, 72) = 0.76, p = 0.56]. In the C57BL/6J strain, prenatal MMC exposure caused significantly prolonged latency [F (1, 19) = 8.40, p = 0.009], and the interaction between the prenatal exposure and trial days was also significant [F (4, 76) = 6.66, p < 0.001]. However, in the BALB/c strain, prenatal MMC exposure did not cause significantly prolonged latency [F (1, 16) = 3.72, p = 0.072], and the interaction between prenatal exposures and trial days was not significant [F (4, 64). These results indicated that the prenatal exposure to MMC impaired the performance in the Morris water maze in the C57BL/6Cr and C57BL/6 strains.

In the BALB/c strain, the prenatal exposure to MMC did not cause significantly prolonged latency, showing the marginal level (p = 0.072). Klapdor and Van der Staay (8) has reported that the C57BL strain could readily learn the Morris water maze task, but that the BALB/c strain could not. Considering the finding of Klapdor and Van der Staay (8), it is difficult to evaluate the MMC-induced impairment in the performance of the BALB/c strain in the Morris water maze. Even if the prenatal exposure to MMC impaired spatial learning ability in BALB/c strain, such a poor performance of the BALB/c strain would mask the MMC-induced neurobehavioral change in the Morris water maze. On the other hand, it is likely that the latency in reaching the platform is inversely proportional to the general activity in the light phase. Therefore, we could not exclude the possibility that the decrease in the light-phase spontaneous activity is associated with the prolonged latency in the C57BL/6Cr and C57BL/6J strains.

In summary, we have demonstrated that the prenatal MMC exposure caused different behavioral changes in the open field and home cage situations, and in the Morris water maze among three inbred strains of mice. Therefore, the present study will be useful for the assessment of behavioral changes in an appropriate model animal chronically exposed to MMC. The appropriate model animals might provide valuable tools in the search for a refined biological marker for detecting a possible severe cognitive dysfunction in adult or the elderly of an exposed human population.

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Legends for Illustrations

FIG 1. Central or peripheral locomotion in open field. The percent of central or peripheral locomotion was defined as following: [the length (cm) of the path of the central or peripheral area / the length (cm) of the total path of the open field] x 100. Each histogram represents the mean \pm SD. The number of mice in each prenatal exposure group: the controls: BALB/c (n = 16), C57BL/6Cr (n = 20), C57BL/6J (n = 11); the MMCs: BALB/c (n = 8), C57BL/6Cr (n = 21), C57BL/6J (n = 16). The difference between the MMC and control groups was analyzed by Student's *t* test (* *p* < 0.05).

FIG.2. The performance of three inbred strains in the Morris water maze. Latency is the time (in sec) from the release of the mouse to its climbing on a submerged platform. Data points are daily group means \pm SE for 5 consecutive days. The number of mice in each prenatal exposure group: the controls: BALB/c (n = 10), C57BL/6Cr (n = 10), C57BL/6J (n = 11); the MMCs: BALB/c (n = 8), C57BL/6Cr (n = 10), C57BL/6J (n = 10). Data of each strain were analyzed by repeated measures ANOVA with prenatal exposures (MMC or PBS) as a between-subject factor and trial days (Day 1 to Day 5) as a within-subject factor. The prenatal exposure to MMC caused significantly prolonged latency in the C57BL/6Cr [F (1, 18) = 6.78, *p* = 0.018] and C57BL/6J strains [F (1, 19) = 8.40, *p* = 0.009], but not in BALB/c strain mice [F (1, 16) = 3.72, *p* = 0.072].

Prenatal Methylmercury Exposure in Macaca Fascicularis

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ABSTRACT

The effects of methylmercury on prenatal monkeys were studied. Four macaca fascicularis were used : one was a control animal. and three were methylmercury-treated. Methylmercury was orally administered from a water bottle containing 1 ppm of methylmercury during pregnancies of the 3 treated monkeys. Four male babies were born in normal deliveries. The concentrations of total mercury in the blood of mothers and their babies were 0.76 and 1.62 ppm in No. 3, 0.86 and 1.81 ppm in No. 4, and 1.06 and 3.02 ppm in No. 5 in respectively. The change rates of body weight of methylmercury-treated monkeys were lower than that of the control. As for the growth behavior, that of No. 5 was slower than that of the other monkeys. The spontaneous motor activity (SMA) for 2 hours in the control monkey increased slowly. However, in the methylmercury-treated monkeys, SMA increased for the first two weeks with steep slopes compared with the control, but afterwards decreased gently. The values of SMA decreased with increase of the mercury level 60 days after birth. These results indicate that SMA and the behaviors of crawling, catching, standing, and walking were good indices to estimate the effects of prenatal methylmercury exposure.

Key words: methylmercury, prenatal exposure, behavior, spontaneous motor activity (SMA)

INTRODUTION

Methylmercury is a well-established neurotoxicant that can have serious adverse effects on the development and functioning of the human central nervous system, especially when exposure occurs prenatally. In 1990, when "Environmental Health Criteria 101: Methylmercury" was published, it was known that prenatal exposure could cause fetotoxic effects in human beings. It has been estimated that the risk of fetal brain damage may be increased when the mercury concentration in maternal scalp hair exceeds a level of 10-20 ppm. However, this threshold was not fixed from the Seyshelles and Faroe studies. That is, in the Seyshelles study (Marsh D.O.), there is no effect on child in a level of 10ppm. On the other hand, in the Faroe study (Grandjean P), on 917 children around the age of 7 for mercury poisoning and/or nervous disorder, possibilities were found of some children being handicapped in terms of memory, physical performance and speech disorder at levels of methylmercury less than 10ppm. There is no information about effects of methylmercury on prenatal The effects of methylmercury on prenatal monkeys were studied in this monkeys. experiment.

MATERIALS AND METHODS

The subjects were 4 macaca fascicularis, including one control animal and three methylmercury-treated monkeys, which were orally administered methylmercury from a water bottle containing 1 ppm of glutathione-conjugated methylmercury during their pregnancies. Four male babies were born in normal deliveries. The indices of the effects of methylmercury are body weight, spontaneous motor activity (SMA) measured with AB system (NEUROSCIECE ICN) for 2 hours, and the growth behavior of the babies (crawling, catching, standing, walking, and hanging). These observations were made by video recording with CCD camera.

RESULTS AND DISCUSSION

1) Contents of mercury in macaca fascicularis

The concentrations of total mercury in the blood of mothers and their babies were 0.76 and 1.62 ppm in No. 3, 0.86 and 1.81 ppm in No. 4, and 1.06 and 3.02 in No. 5 respectively.

(2) Body Weight

The four body weights of monkeys were divided. We compared the rates of the body weight change, because body weight of each monkey varied at birth. The change rates of the methylmercury-treated monkeys' body weight were lower than that of the control (Fig. 1).



Fig. 1. The change rates of body weight.



Fig. 2. The development of behavior in macaca fascicularis.

(3) The growth behavior

As for the growth behavior, No. 5 was later in crawling, catching, standing and walking than the other monkeys. No. 3 and No. 4 were slower in standing and walking than the control (Fig. 2).

(4) The spontaneous motor activity (SMA)

It is difficult to know the tendencies of the original data of SMA. Therefore, these data were analyzed with a polynomial regression model (Fig. 3). In the control monkey, SMA increased slowly. In methylmercury-treated monkeys, however, SMA increased for the first two weeks with steep slopes compared with that of the control animal, but afterwards decreased gently. SMA was compared 60 days after the birth. The values of SMA in monkeys were decreased with increase of dose (Fig. 4). The values of SMA were decreased with increase of the mercury level. The trend line was y=-0.844x+5970 (P value was 0.038). Therefore, SMA could be good indices in examination of the effects of low-level methylmercury on prenatal monkeys. There have been no reports about prenatal methylmercury exposure in the monkey. Cheng-Mei Shaw at the University of Washington mentioned in NIMD seminar that when the mercury concentration in blood was below 2ppm, there was no effect on the behavior of prenatal monkeys.

However, we found that the maternal mercury levels of 0.76-1.06 ppm in the blood indeed affect growth and SMA of the babies. This might have been due to difference



Fig. 3. The spontaneous motor activity.



Fig. 4. The relation between mercury and SMA.

CONCLUSION

- 1. The change rates of body weight of the methylmercury-treated monkeys were lower than that of the control.
- 2. In the control, SMA increased slowly. In the methylmercury-treated animals, the SMA increased in the initial stage, and then decreased gradually.
- 3. The appearance of the behaviors of crawling, catching, standing, and walking was delayed in the methylmercury-treated animals.

Therefore, the maternal methylmercury levels of 0.76-1.06 ppm affected the growth and SMA of the babies. SMA and the behaviors of crawling, catching, standing and walking behavior are good indices. For evaluation the effects of low-level methyl mercury-treated animals, standing and walking are especially good indices.

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Research Needed to Support Risk Assessment and Risk Management of Methylmercury Contamination

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In 1997 the United States Environmental Protection Agency released its *Mercury Study Report to Congress* which was required by the Clean Air Act Amendments of 1990. This Report provided a risk assessment for mercury that identified health risks to humans with emphasis on sensitive subpopulations including the maternal/fetal pair and young children. It was estimated that 7% of United States' women of childbearing age have mercury exposures that exceed the Reference Dose(RfD) Which was based on neurodevelopmental deficits in the child following *in utero* exposures secondary to maternal fish consumption. An assessment of the impact of mercury emissions on wildlife health was conducted that identified seriously elevated tissue mercury residues in piscivorus wildlife species. The Report also cited a plausible link between merthylmercury concentrations in freshwater fish and anthropogenic mercury emissions.

To address uncertainties identified by this risk assessment, US EPA has evaluated these research needs and developed a *draft* long-term research strategy. Because of the small margin between current exposure of humans and wildlife, and exposure associated with adverse health effects, most of research strategy focuses on control technologies, non-ambient source sources of mercury pollution, and other methods for limiting release of mercury into the environment. The strategy also identifies research needs in human health, ecological assessment, risk assessment, and risk communication. In this risk major elements of the research strategy will be described.

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