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# Determination of total mercury and monomethylmercury compounds in water samples from Minamata Bay, Japan: an interlaboratory comparative study of different analytical techniques

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**Concentration levels of mercury and its organic compounds in natural water samples are very low, so accurate analysis is still a major problem. To ensure the validity of such measurements a proper quality control/quality assurance programme should be implemented. In the absence of reference materials, the best way to check the accuracy of the results is through interlaboratory comparisons and comparisons of the results obtained by different analytical methods. The purpose of the present work was to compare independent analytical techniques used by two different laboratories for determination of total mercury (Hg-T) and monomethylmercury compounds (MeHg) in seawater samples from Minamata Bay, Japan. The interlaboratory study was carried out between the Jožef Stefan Institute, Ljubljana, Slovenia and the National Institute for Minamata Disease, Minamata, Japan. Copyright © 2001 John Wiley & Sons, Ltd.**

**Keywords: Minamata Bay; mercury; monomethylmercury; sea water; fresh water effluents; intercomparison exercise**

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## INTRODUCTION

Concentration levels of mercury and its organic compounds in water samples are very low, so accurate analysis is still a major problem and represents a special challenge for analytical chemists.[1] To ensure the validity of such measurements a strict and appropriate quality control/quality assurance (QC/QA) programme should be implemented, particularly for monomethylmercury (MeHg), which is the most toxic mercury compound.

The most common way to control the accuracy of measurement results is by analysing suitable certified reference materials (CRMs). Unfortunately, only a few reference materials are certified for MeHg in biological and environmental samples.[2] So, for the case of water samples with low mercury content, no reference materials are available, and the best way to check the accuracy of the results is through participation in interlaboratory studies and/or comparison of the results obtained by various methodologies.

Such exercises have been organized for determination of total mercury (Hg-T) and MeHg in natural lake water. The results obtained showed excellent comparability of results for MeHg, whereas for Hg-T some outliers were recorded, which can be attributed to contamination problems during the measurement procedure.[3] One of the important observations of the above intercomparison was that '*no fundamentally different methods for mercury analysis were used, which means that the relatively narrow confidence intervals still remain unverified as to a true value*'. In the present interlaboratory study, two fundamentally different analytical

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procedures were employed by two different laboratories performing the analysis under good QC. Seawater samples were jointly sampled at two occasions during 1999 and subsequently analysed by the Jožef Stefan Institute (IJS), Ljubljana, Slovenia, and the National Institute for Minamata Disease (NIMD), Minamata, Japan.

Minamata Bay was chosen as a case study owing to its well-known history of mercury pollution. It was severely contaminated with mercury and MeHg from the Chisso acetaldehyde and vinyl chloride plant in Minamata City until 1968.[4] After extensive clean-up actions, Minamata Bay was again open for fishing in August 1998. In order to control the environmental behaviour of mercury in the bay, continuous environmental monitoring is necessary. This should include determination of Hg-T and MeHg in marine organisms for the purpose of health monitoring, whereas spatial changes and trends of mercury pollution require accurate measurements of mercury and its species in air and in sediments and water samples. This would allow an assessment of the current status and model the future trends of mercury pollution in this coastal environment. Reliable results for Hg-T and MeHg are, therefore, essential for further biogeochemical studies in Minamata Bay, the assessment of Hg-T and MeHg fluxes from sediment into the water column and the mass balance of mercury in the bay and its exchange with the Yatsushiro Sea.

Analytical methods for determination of Hg-T and MeHg in water samples used by the NIMD consisted of acid leaching and simultaneous extraction of $Hg^{2+}$ and $CH_3Hg^+$ dithizonates into toluene. An aliquot of this extract was used for the determination of Hg-T by cold vapour atomic absorption spectrometry (CV AAS) after solvent evaporation and acid digestion. The remaining part of the organic solvent was used for the determination of MeHg. The procedure consisted of a clean-up step, back extraction into toluene, separation by packed column gas chromatography (GC) and detection of MeHgCl by an electron capture detector (ECD). Determination of Hg-T in suspended particles was performed by CV AAS after acid digestion and $SnCl_2$ reduction. Analytical methods used by the IJS were based on the following principles: (1) MeHg in water samples and suspended particles was determined by acid leaching, solvent extraction, aqueous-phase ethylation, gas chromatographic separation and cold vapour atomic fluorescence spectrometric (CV AFS) detection, (2) determination of Hg-T was performed by acid digestion, $SnCl_2$ reduction followed by gold amalgamation CV AAS. The results obtained showed good agreement between the participating laboratories, especially for MeHg and Hg-T on suspended solids. Notable disagreements for some samples were occasionally observed for dissolved Hg-T, which is mainly due to contamination during sampling and sample transport and/or losses during the sample preparation procedures.

The results obtained for Hg-T and MeHg in water samples of Minamata Bay show that mercury concentrations are still elevated in this coastal environment. Mercury originates mainly from two effluents and resuspension of mercury from sediments. Tidal forcing is probably the most important mechanism affecting transport of mercury within Minamata Bay and its exchange with the Yatsushiro Sea.

## EXPERIMENTAL

### Cleaning procedures

Owing to the very low concentrations of Hg-T and MeHg in seawater it is essential to avoid any possible contamination prior to and during the sampling procedures. One of the most common sources of contamination is contaminated glassware and Teflon-ware.

Containers at NIMD consisted mainly of glassware (bottles, pipettes, filtration units, etc.), which were pre-cleaned by rinsing with 2% $KMnO_4$ aqueous solution, followed by tap and deionized water washing. Glassware was cleaned each time just before use in analysis.

Containers for the analysis of water samples at IJS consisted mainly of Teflon-ware, which requires a more rigorous cleaning procedure. After soaking the Teflon in 50% $HNO_3$ (w/w) at 70 °C overnight, the ware was rinsed with Milli-Q water and then soaked in 10% HCl for 3 days, followed by thorough rinsing with Milli-Q, then filled with 1% HCl and stored in hermetically closed polyethylene bags in a mercury-free environment.

### Sample collection

Sampling of seawater samples from Minamata Bay, Japan, was performed on two occasions: on 1 February 1999 from 14:00 to 18:00 and 19 October 1999 from 15:00 to 16:30. The sampling locations are presented in Fig. 1.

In February 1999, about 5 l of water was taken with a Niskin sampler in 5 l glass bottles from each

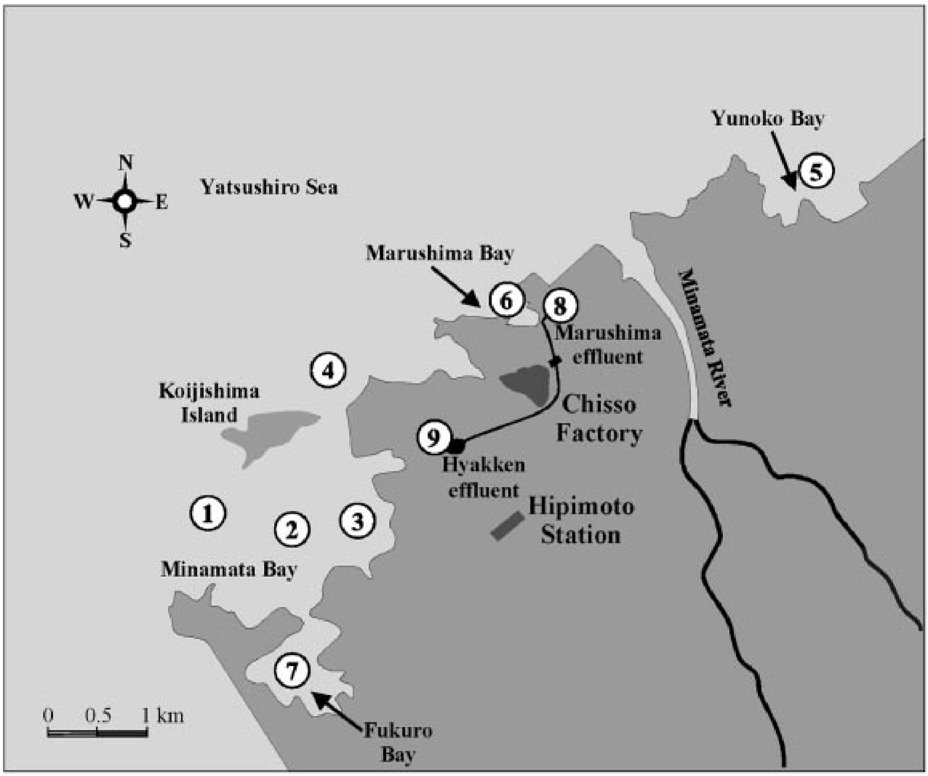

**Figure 1** Sampling locations in Minamata Bay: (1) Entrance to Minamata Bay; (2) central part of Minamata Bay; (3) Minamata Harbour; (4) outside Minamata Bay; (5) Yunoko Bay; (6) Marushima Bay; (7) Fukuro Bay; (8) Marushima effluent; (9) Hyakken effluent.

sampling location from two or three depths. In addition, freshwater samples from two effluent streams (viz. the Marushima and Hyakken) that empty into Minamata Bay were taken (Fig. 1). For clarity, the preparation procedure prior to analysis is presented in Fig. 2. About 500 ml of water was transferred into a pre-cleaned Teflon bottle and stored deep frozen until shipped to Slovenia. These water samples were used for the determination of Hg-T and MeHg in non-filtered water samples. The remaining water was immediately filtered (~0.75 μm Whatman GF/C glass filter) to prepare water samples for the determination of dissolved fractions of Hg-T (Hg-$T_{diss}$) and MeHg ($MeHg_{diss}$). In addition, the mass of total suspended solids was also determined on the filters through which 2 l of water sample were filtered. About 0.5 l of water sample was stored in a Teflon bottle and stored in a deep freezer until shipped to Slovenia. The remaining filtered water samples were used for the determination of Hg-$T_{diss}$ and $MeHg_{diss}$ at NIMD. Filter papers through which 2.5 l aliquots of water were filtered were used for the determination of total Hg-T bound to particulates (Hg-$T_{ss}$). During the sampling in Februry 1999 the amount of water sample taken (i.e. 5 l) was insufficient to perform analysis of $MeHg_{ss}$ at NIMD.

In October 1999 about 10 l of water sample was collected at the surface, in order to provide enough samples for comparative analysis of all parameters. The sample preparation procedure during this period was slightly different from the previous exercise. The whole amount of the water samples was filtered (~0.75 μm Whatman GF/C glass filter). Filters with suspended solids were used for the determination of Hg-$T_{ss}$ and $MeHg_{ss}$ at both institutions and for the determination of the mass of total suspended solids (TSS). Aliquots of 2 and 1 l were filtered for NIMD and IJS, subsequentially. Filtered water samples were then used for the determination of Hg-$T_{diss}$ and $MeHg_{diss}$. Samples at NIMD were analysed immediately, whereas samples analysed at IJS were transported frozen and analysed within 1 month of sampling.

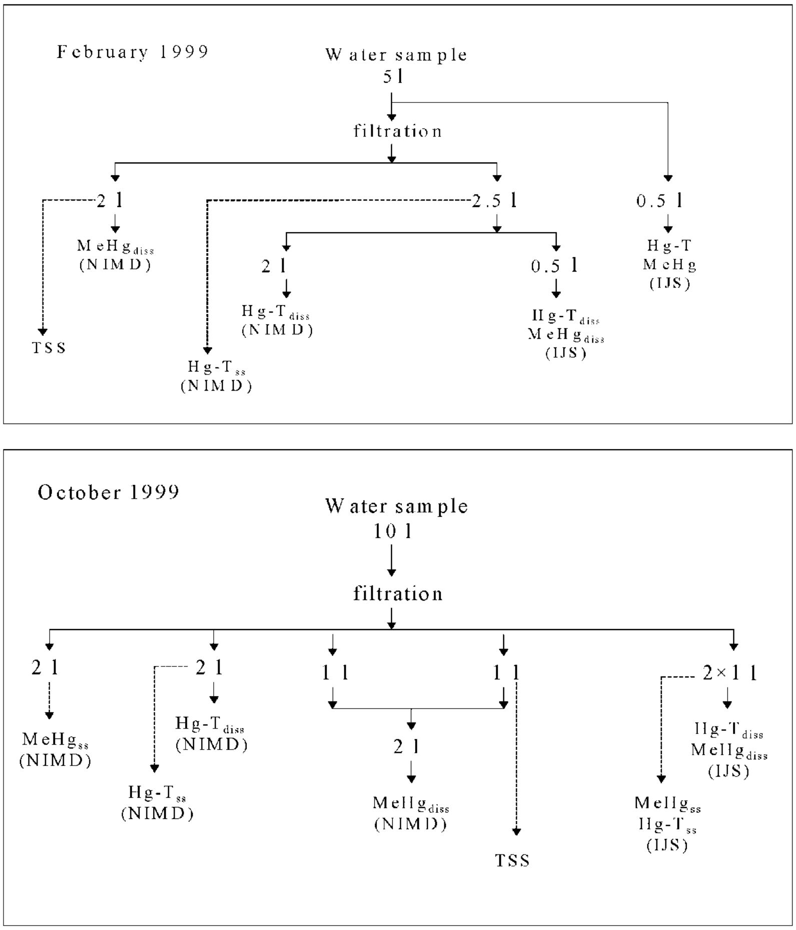

**Figure 2** Sampling schemes. IJS: Jožef Stefan Institute; NIMD: National Institute for Minamata Disease; Hg-T$_{diss}$: dissolved total mercury; MeHg$_{diss}$: dissolved methyl mercury; Hg-T$_{ss}$: particulate total mercury; MeHg$_{ss}$: particulate methyl mercury; Hg-T = Hg-T$_{diss}$ + Hg-T$_{ss}$; MeHg = MeHg$_{diss}$ + MeHg$_{ss}$; TSS: total suspended solids.

## Analytical methods

A detailed description of the analytical methods used is presented elsewhere;[5–12] therefore, only a brief description is provided in the present paper. Schematic representations of the analytical methods used by NIMD and IJS are given in Fig. 3 for the determination of Hg-T and MeHg in water samples, and in Fig. 4 for the determination of Hg-T and MeHg in suspended solids.

### Hg–T

Methods for the determination of Hg–T at NIMD and IJS were based on CV AAS detection; however, the preparation of the sample prior to final measurement was different.

At NIMD the determination of Hg-T was based on preconcentration of mercury from about 2 l of water sample by solvent extraction of mercury dithizonates (Hg-Dz). In the first step, simultaneous extraction of $Hg^{2+}$ and $CH_3Hg^+$ dithizonates into toluene was performed. An aliquot of this extract was used for the determination of Hg-T and the remaining part of the organic solvent used for the determination of MeHg (see below). After removal of the organic solvent by evaporation in a rotavapour, Hg-Dz was decomposed by acid digestion and measured by CV AAS using $SnCl_2$ reduction. The measurement was performed without gold amalgamation. The limit of detection (LOD) was 0.01 ng $l^{-1}$, calculated on the basis of three standard deviations of the blank. The system was calibrated by the use of MeHg standard solution prepared in aqueous cysteine solution. Recoveries obtained on

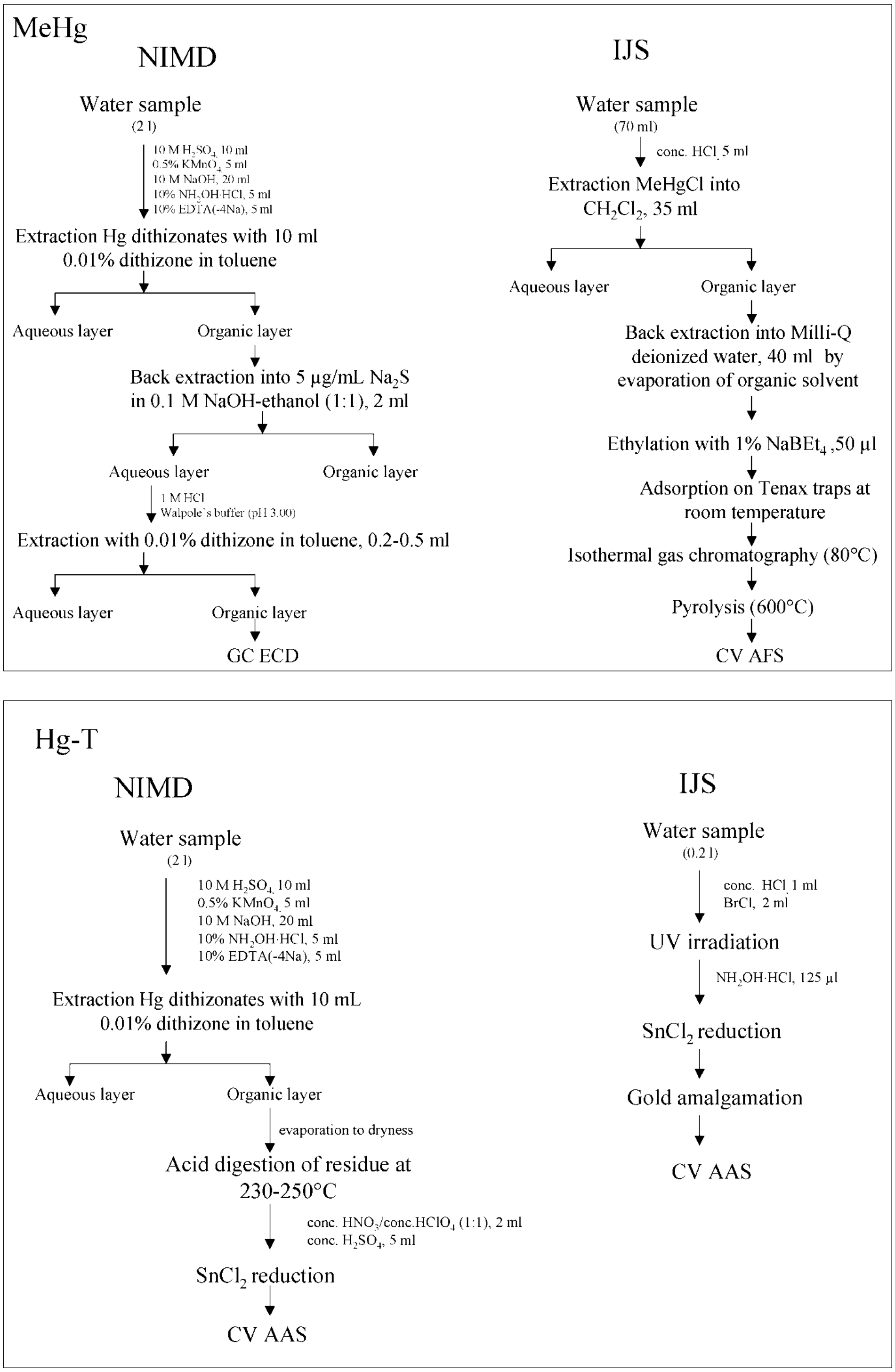

**Figure 3** Schematic presentation of analytical procedures for determination of MeHg and Hg-T in water samples by NIMD and the IJS.

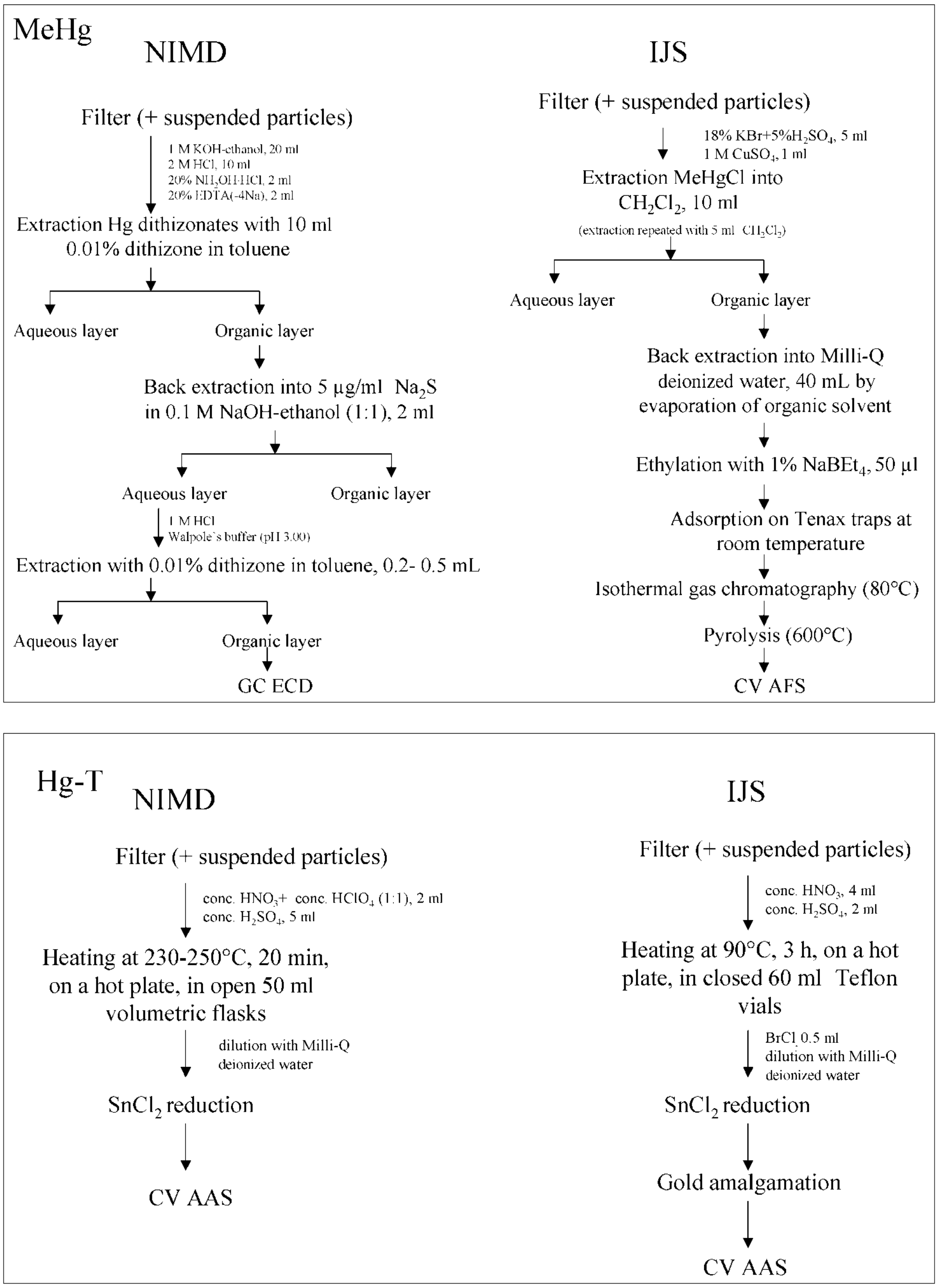

**Figure 4** Schematic presentation of analytical procedures for determination of MeHg and Hg-T in suspended particles by analysis of the filters by NIMD and the IJS.

the basis of the standard addition method were nearly quantitative, so no recovery factors were necessary for the calculation of final results.[5] Repeatability of measurement is about 3–5 %.

The method for determination of Hg-T in water used at IJS was based on acidification and oxidation with BrCl in combination with UV irradiation. For each measurement only about 100–200 ml of water sample was needed. The final measurement step included the reduction of $Hg^{2+}$ with $SnCl_2$, preconcentration by gold amalgamation and CV AAS detection. The LOD was also 0.01 ng $l^{-1}$, calculated on the basis of three standard deviation of the reagent blanks. The system was calibrated by the use of $Hg^{2+}$ solution in 1% $HNO_3$, which was prepared on a daily basis. Recoveries were quantitative and no recovery corrections were necessary.[6–9] The repeatability and reproducibility of the method were 5% and 10%, respectively.

### MeHg

Completely different analytical techniques for determination of MeHg were used by NIMD and IJS in terms of extraction and detection.

Determination of MeHg in water samples by NIMD was based on simultaneous extraction of $Hg^{2+}$ and MeHg dithizonates into toluene after acidification of the water samples. After removal of an aliquot for Hg-T determination, the remaining part was used for determination of MeHg, a procedure consisting of clean-up steps in which MeHg was stripped from the organic solvent into aqueous $Na_2S$ solution, removal of $H_2S$ by evaporation and back extraction of MeHg-Dz into a small volume of organic solvent and detection by gas chromatography coupled with electron capture detection (GC–ECD). Recoveries obtained by spiking with aqueous MeHg–cysteine solution were almost quantitative, so no recovery corrections were necessary. Calibration was performed by the standard addition method, spiking the samples with two different MeHg solutions. The repeatability of the whole procedure is about 3%. The LOD of the method was 0.05 ng $l^{-1}$ when 2 l of water sample was taken for analysis.[5] The LOD was calculated on the basis of three times the peak height of the signal noise. Determination of MeHg in suspended solids was also performed with the same procedure, except that in the first step alkaline dissolution was used instead of acid leaching. The same performance characteristics as for water samples were obtained.

At the IJS, MeHg in water samples and suspended particles was determined by acid leaching, solvent extraction, aqueous-phase ethylation, gas chromatographie separation and CV AFS detection. The LOD, calculated on the basis of three times the standard deviation of the blank, was about 0.01 ng $l^{-1}$ when 70 ml of water sample was taken for analysis.[8,10,11] Recoveries for MeHg in water samples were about 75–85%. In each batch of samples analysed, recovery was checked by spiking about 10% of samples with a known amount of MeHg in aqueous solution. All results were then corrected for the recovery factor. Calibration of the final measurement/detection step was performed by an aqueous MeHg standard solution. The repeatability of the procedure is 5% and the reproducibility is 7%.

Determination of MeHg in suspended matter was performed by a similar procedure, except that the volume and concentration of reagents in the preparation/isolation step were different. The recoveries for this procedure were almost quantitative, so no correction of the final results was necessary.[9,11,12] The repeatability and reproducibility of the procedure were 7% and 12% respectively.

## RESULTS AND DISCUSSION

The main aim of this study was to compare the results for Hg-T and MeHg in water samples from Minamata Bay obtained by independent analytical techniques used by two laboratories. The results for Hg-T and MeHg obtained during sampling campaigns in February and October 1999 are given in Tables 1 and 2. For better appreciation, data are also presented graphically in Figs 5 and 6.

Comparison of the results for total mercury is presented in Fig. 5. The results obtained for dissolved mercury ($Hg–T_{diss}$) (Fig. 5a) show that in a number of samples excellent agreement was obtained, but not in some other cases; this can be explained either by contamination and/or losses during the sample preparation procedures. Data with significant deviations are given in bold and italic in Tables 1 and 2. Surprisingly large disagreement for $Hg–T_{diss}$ was obtained for the two effluent samples, namely Marushima and Hyakken, during both campaigns. It was suspected that mercury was either (1) not sufficiently extracted by the NIMD procedure due to the presence of interfering compounds discharged into this very contaminated effluents or (2) positive spectroscopic interference was present during the method employed at IJS. After thorough discussion, the first reason was believed to be more probable.

Comparison of the results obtained for $Hg-T_{ss}$ and Hg-T (Fig. 5b and c) shows excellent agreement, with no significant outlying results.

Comparison of data for MeHg are presented in Fig. 6. Data obtained for $MeHg_{diss}$ are in excellent agreement for both sampling periods (Fig. 6a), even though the concentration of MeHg is much smaller than Hg-T. Comparison of data for MeHg in suspended solids ($MeHg_{ss}$) was only carried out for samples taken in October 1999 (Fig. 6b). Slightly higher results were obtained by the NIMD; however, closer observation of

**Table 1** Comparison of Hg-T and MeHg results in water samples taken in February 1999. Results are given in nanograms of mercury per litre

| Sampling locations | Depth (m) | IJS Hg-T | IJS Hg-T$_{diss}$ | IJS Hg-T$_{ss}$$^{a}$ | IJS MeHg | IJS MeHg$_{diss}$ | IJS MeHg$_{ss}$$^{b}$ | NIMD Hg-T$^{c}$ | NIMD Hg-T$_{diss}$ | NIMD Hg-T$_{ss}$ | NIMD MeHg$_{diss}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Entrance to Minamata Bay | Surface | 3.01 | – | – | 0.080 | – | – | 2.30 | 0.88 | 1.42 | 0.064 |
| | 10 | 2.21 | 0.63 | 1.58 | 0.100 | 0.055 | 0.045 | 2.91 | 0.58 | 2.33 | 0.058 |
| | 17 | 3.23 | ***1.29*** | 1.94 | 0.052 | 0.047 | 0.005 | 3.87 | ***0.58*** | 3.29 | 0.055 |
| Central part of Minamata Bay | Surface | 2.63 | ***1.19*** | 1.44 | 0.123 | 0.056 | 0.067 | 5.23 | ***2.27*** | 2.96 | 0.061 |
| | 10 | 2.82 | 0.74 | 2.08 | 0.091 | 0.046 | 0.045 | 3.01 | 0.51 | 2.50 | 0.055 |
| | 17 | 4.81 | 0.56 | 4.25 | 0.070 | 0.065 | 0.005 | 4.21 | 0.51 | 3.70 | 0.061 |
| Minamata Harbour | Surface | 5.66 | ***1.08*** | 4.58 | 0.110 | 0.048 | 0.062 | 4.77 | ***0.51*** | 4.24 | 0.051 |
| | 10 | 6.24 | 0.57 | 5.67 | 0.082 | 0.054 | 0.028 | 4.69 | 0.45 | 4.24 | 0.051 |
| Outside Minamata Bay | Surface | 1.99 | 0.41 | 1.58 | 0.058 | 0.041 | 0.017 | 2.03 | 0.57 | 1.46 | 0.039 |
| | 10 | 2.35 | 0.44 | 1.91 | 0.098 | 0.045 | 0.053 | 2.80 | 0.45 | 2.35 | 0.041 |
| Yunoko Bay | Surface | 0.98 | 0.54 | 0.44 | 0.071 | 0.043 | 0.028 | 1.23 | 0.45 | 0.78 | 0.051 |
| | 7 | 1.35 | 0.37 | 0.97 | 0.096 | 0.056 | 0.040 | 1.36 | 0.51 | 0.85 | 0.055 |
| Marushima Bay | Surface | 10.1 | 1.64 | 8.46 | 0.120 | 0.085 | 0.035 | 10.1 | 1.02 | 9.08 | 0.082 |
| Marushima effluent | Surface | 3.75 | ***2.02*** | 1.73 | 0.300 | 0.267 | 0.033 | 3.17 | ***0.51*** | 2.66 | 0.285 |
| Hyakken effluent | Surface | 19.3 | 7.53 | 9.77 | 0.330 | 0.243 | 0.087 | 17.1 | 5.79 | 11.3 | 0.246 |

For abbreviations see Fig. 2; (–) sample lost; results in bold and italic: disagreement due to contamination/losses.

[a] Calculated: [Hg-T$_{ss}$] = [Hg-T] − [Hg-T$_{diss}$].

[b] Calculated: [MeHg$_{ss}$] = [MeHg] − [MeHg$_{diss}$].

[c] Calculated: [Hg-T] = [Hg-T$_{diss}$] + [Hg-T$_{ss}$].

**Table 2** Comparison of Hg-T and MeHg results in water samples taken in October 1999. Results are given in nanograms of mercury per litre

| Sampling locations | IJS Hg-T$^{a}$ | IJS Hg-T$_{diss}$ | IJS Hg-T$_{ss}$ | IJS MeHg$^{b}$ | IJS MeHg$_{diss}$ | IJS MeHg$_{ss}$ | NIMD Hg-T$^{a}$ | NIMD Hg-T$_{diss}$ | NIMD Hg-T$_{ss}$ | NIMD MeHg$^{b}$ | NIMD MeHg$_{diss}$ | NIMD MeHg$_{ss}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Entrance to Minamata Bay | 2.96 | ***0.51*** | 2.45 | 0.039 | 0.024 | 0.015 | 3.06 | ***1.10*** | 1.96 | 0.021 | 0.017 | 0.004 |
| Central part of Minamata Bay | 6.17 | 0.59 | 5.57 | 0.021 | 0.014 | 0.007 | 6.64 | 0.46 | 6.18 | 0.029 | 0.017 | 0.012 |
| Minamata Harbour | 6.38 | 0.71 | 5.67 | 0.034 | 0.029 | 0.005 | 7.49 | 0.72 | 6.77 | 0.059 | 0.039 | 0.020 |
| Outside Minamata Bay | 1.23 | ***0.43*** | 0.81 | 0.027 | 0.020 | 0.007 | 2.97 | ***2.28*** | 0.69 | 0.026 | 0.022 | 0.004 |
| Yunoko Bay | 1.28 | 0.55 | 0.73 | 0.018 | 0.015 | 0.003 | 1.43 | 0.65 | 0.78 | 0.021 | 0.017 | 0.004 |
| Fukuro Bay | 5.25 | 0.40 | 4.83 | 0.068 | 0.038 | 0.030 | 5.46 | 0.26 | 5.20 | 0.040 | 0.028 | 0.012 |
| Marushima effluent | 42.6 | ***2.01*** | 40.6 | 0.424 | 0.207 | 0.217 | 42.8 | ***0.39*** | 42.4 | 0.507 | 0.191 | 0.316 |
| Hyakken effluent | 20.6 | ***3.30*** | 17.3 | 0.221 | 0.095 | 0.126 | 22.7 | ***0.59*** | 22.1 | 0.256 | 0.090 | 0.166 |

For abbreviations see Fig. 2; Results in bold and italic: disagreement due to contamination/losses.

[a] Calculated: [Hg-T] = [Hg-T$_{diss}$] + [Hg-T$_{ss}$].

[b] Calculated: [MeHg] = [MeHg$_{diss}$] + [MeHg$_{ss}$].

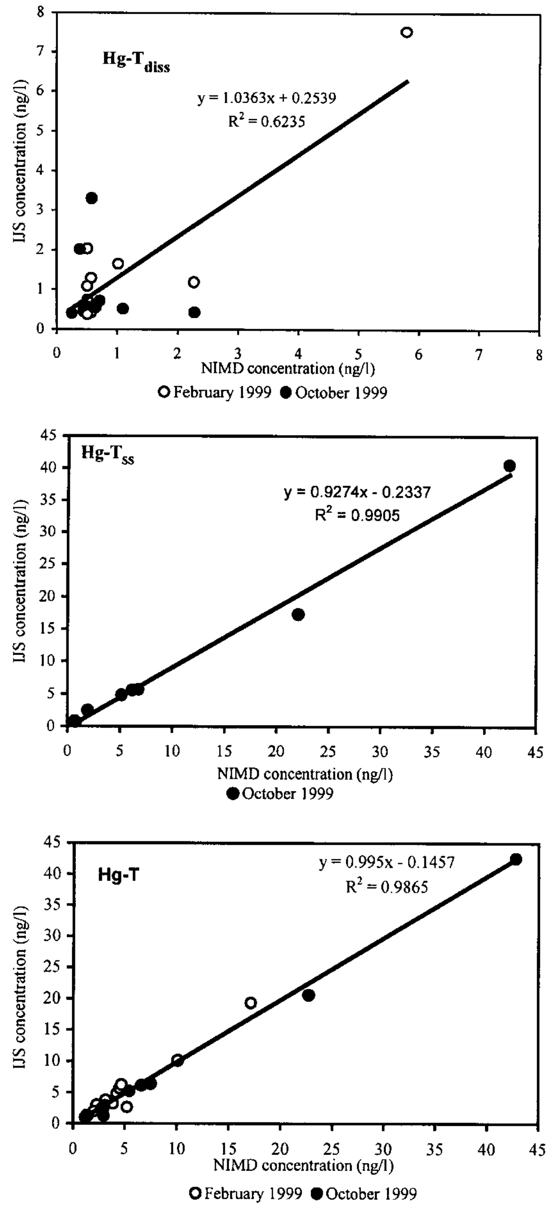

**Figure 5** Comparison of the results for Hg-T$_{diss}$, Hg-T$_{ss}$ and Hg-T obtained by the IJS and the NIMD. For abbreviations see Fig. 2.

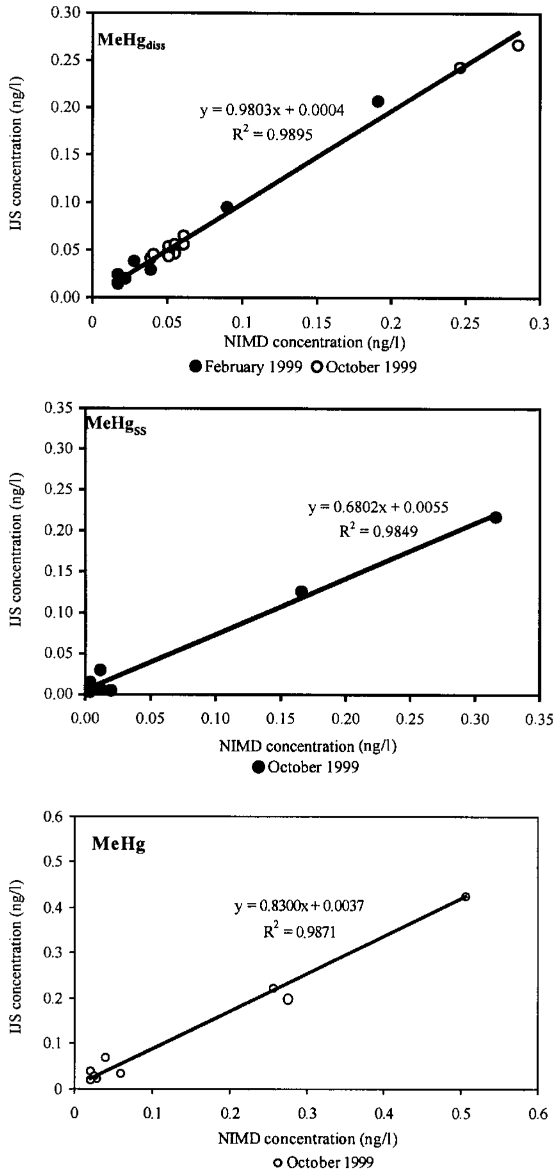

**Figure 6** Comparison of the results for MeHg$_{diss}$, MeHg$_{ss}$ and MeHg obtained by the IJS and the NIMD. For abbreviations see Fig. 2.

the data reveals that the agreement is good, considering the fact that both methods were at their limits of detection. Data obtained for total MeHg (Fig. 6c) were in good agreement.

One of the important differences during the two sampling campaigns was the determination of mercury on suspended solids (Hg-T$_{ss}$), which is an important parameter, in particular for the calculation of the distribution coefficient $K_d$ (the ratio between the mercury concentration in the suspended solids and the concentration in solution expressed in millilitres per gram). The concentrations of Hg-T$_{ss}$ in water samples taken in February 1999 determined by the NIMD were measured by analysing filters containing suspended solids from 2 l of water sample. The concentrations of Hg-$T_{ss}$ and $MeHg_{ss}$ determined by IJS were calculated on the basis of the difference between the results obtained for filtered (Whatman glass filters) and non-filtered water samples (Table 1). The correctness of these values, therefore, is dependent on two measured sets of data (1) Hg-T and Hg-$T_{diss}$ and (2) MeHg and $MeHg_{diss}$. In general, the agreement for Hg-$T_{ss}$ obtained by NIMD and IJS was reasonable (Figs 5 and 6). No comparison for $MeHg_{ss}$ was possible, as results from NIMD were not available. This is further confirmed by the comparison of $K_d$ values calculated from these results. Evidently, differences are notable for samples in which disagreements of the original data mentioned above have occurred: in particular, for the Marushima effluent in February 1999.

In October 1999, both laboratories analysed Hg-$T_{ss}$ and $MeHg_{ss}$ on suspended solids prepared under the same conditions. The agreement for $K_{d_{Hg\text{-}T}}$ and $K_{d_{MeHg}}$ was very good for all seawater samples, expect for $K_{d_{Hg\text{-}T}}$ in effluent samples, which is due to the disagreement of the results obtained for Hg-$T_{diss}$, mentioned above.

Correlation of $K_{d_{Hg\text{-}T}}$, calculated on the basis of NIMD and IJS data, excluding effluent samples and those data where significant differences in Hg-T concentrations were observed, was better for samples taken in October ($r^2 = 0.83$) compared with samples taken in February ($r^2 = 0.75$). This is mainly due to a better and more comparable sample preparation procedure in October, where both institutions analysed filtered water samples and suspended solids on filters. Major disagreements in $K_d$ in October can, therefore, be attributed mainly to the performance characterises of the analytical procedures, rather than differences in sample preparation.

Distribution coefficients for Hg-T and MeHg indicate strong association of both species with particulate matter, and are in agreement with literature data.[13,14] $K_d$ for Hg-T in marine waters varied between $10^5$ and $10^6$, whereas in the effluent waters higher values were occasionally observed, indicating significant differences in binding capacities of mercury during different sampling periods and with the type of sample analysed. $K_d$ values for MeHg are in the range between $10^4$ and $10^5$, indicating that MeHg is less strongly associated with particulates than inorganic mercury. Also, for MeHg, higher values for $K_d$ were observed for effluent samples in October 1999.

Even though Minamata Bay has been subject to severe mercury pollution in the past, only some data on mercury analysis and speciation in environmental samples have been published.[15–19] The data obtained in this study, therefore, are of special importance in order to assess the level of contamination of Minamata Bay waters after more than 30 years of extensive remediation actions. The results presented in Tables 1 and 2 reveal that mercury in the seawater of Minamata Bay is at higher concentrations than at non-affected stations (sampling points 4, Outside Minamata Bay, and 5, Yunoko Bay; Fig. 1). Potential sources of mercury in the area are the Hyakken and Marushima effluents, which empty directly into Minamata and Marushima Bays respectively. The highest concentrations of mercury are found in the central part and in the harbour of Minamata Bay, and in the semi-enclosed Fukuro Bay. Also, mercury levels at the bottom are higher at most stations, indicating that sediment is an important source of mercury in this environment. Interestingly, mercury levels at the entrance to Minamata Bay are also elevated, indicating that mercury is exchanged with the Yatsushiro Sea.

Concentrations of MeHg also provide some interesting conclusions. The levels of MeHg are higher compared with other mercury-contaminated coastal environments.[13] In the central part of Minamata Bay and in the harbour, MeHg is higher at the surface than close to the bottom, which is the opposite to what was expected. This needs careful consideration in the future in order to identify any potential source of MeHg outside Minamata Bay, such as atmospheric inputs and MeHg carried through effluents and run-off from non-point sources of pollution, taking into account the hydrodynamic circulation. Concentrations of $MeHg_{diss}$ are very similar in all water samples analysed, showing no trends in depth profiles. The percentage of mercury as MeHg in dissolved forms is higher (e.g. the mean percentage was 8% in February and 5% in October) compared with the percentage of MeHg on suspended solids (e.g. the mean percentages were 2% and 0.4% in February and October, respectively).

Comparison of the results obtained for surface water samples in February and October 1999 showed similar values for total mercury, whereas $MeHg_{diss}$ is lower in October, by about a factor of two, even though the water samples contained much more suspended solids in October (13–23 mg $l^{-1}$) than in February 1999 (5–13 mg $l^{-1}$, except for one sample at the bottom that contained 36 mg $l^{-1}$) (Table 3). These data most probably reflect seasonal variations. Further studies are in progress that will allow better interpretation of the data with respect to water quality parameters, water circulation and the biology of the Yatsushiro Sea and Minamata Bay.

**Table 3** Distribution coefficients $K_d$ for Hg-T and MeHg in water samples

| | | | | $10^6 K_{d_{Hg\text{-}T}}$ (ml $g^{-1}$) | | | | $10^5 K_{d_{MeHg}}$ (ml $g^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | TSS (mg $l^{-1}$) | | February 1999 | | October 1999 | | February 1999 | | October 1999 | |
| Sampling location | Depth (m) | February | October | IJS | NIMD | IJS | NIMD | IJS | NIMD | IJS | NIMD |
| Entrance to Minamata Bay | Surface | 12.3 | 13.5 | – | 0.13 | 0.36 | 0.13 | – | – | 0.46 | 0.17 |
| | 10 | 10.6 | – | 0.24 | 0.38 | – | – | 0.77 | – | – | – |
| | 17 | 36.2 | – | 0.04 | 0.16 | – | – | 0.03 | – | – | – |
| Central part of Minamata Bay | Surface | 8.45 | 15.4 | 0.14 | 0.15 | 0.61 | 0.87 | 1.41 | – | 0.32 | 0.46 |
| | 10 | 11.7 | – | 0.24 | 0.38 | – | – | 0.84 | – | – | – |
| | 17 | 9.35 | – | 0.81 | 0.78 | – | – | 0.08 | – | – | – |
| Minamata Harbour | Surface | 12.2 | 14.4 | 0.34 | 0.68 | 0.55 | 0.65 | 1.06 | – | 0.12 | 0.36 |
| | 10 | 10.8 | – | 0.92 | 0.87 | – | – | 0.48 | – | – | – |
| Outside Minamata Bay | Surface | 11.5 | 20.4 | 0.34 | 0.22 | 0.09 | 0.02 | 0.36 | – | 0.17 | 0.09 |
| | 10 | 11.6 | – | 0.37 | 0.45 | – | – | 1.02 | – | – | – |
| Yunoko Bay | Surface | 5.50 | 21.4 | 0.15 | 0.32 | 0.06 | 0.06 | 1.18 | – | 0.09 | 0.11 |
| | 7 | 10.9 | – | 0.24 | 0.15 | – | – | 0.66 | – | – | – |
| Fukuro Bay | Surface | – | 23.4 | – | – | 0.51 | 0.85 | 0.76 | – | 0.33 | 0.18 |
| Marushima Bay | Surface | 5.40 | – | 0.95 | 1.65 | – | – | – | – | – | – |
| Marushima effluent | Surface | 1.95 | 9.2 | 0.44 | 2.67 | 2.19 | 11.8 | 0.63 | – | 1.14 | 1.79 |
| Hyakken effluent | Surface | 6.30 | 8.8 | 0.25 | 0.31 | 0.59 | 4.25 | 0.57 | – | 1.51 | 2.09 |

For abbreviations see the legend of Fig. 2. $K_{d_{Hg\text{-}T}}$ is the distribution coefficient of Hg-T between solid and dissolved phase; $K_{d_{MeHg}}$ is the distribution coefficient of MeHg between solid and dissolved phase.

## CONCLUSION

Comparison of the mercury speciation results obtained by different analytical methods in two laboratories show good agreement in most marine water samples analysed in Minamata Bay. In some samples notable differences occurred for dissolved total mercury (Hg-$T_{diss}$), probably due to contamination of samples during preparation, transport and/or analysis. A similar disagreement for dissolved mercury was observed for freshwater effluent samples, and this needs careful future evaluation. This study also suggests that comparable sample preparation procedures are essential for demonstration of comparability of data. Filtration of water samples through different filters may provide different results. Determination of Hg-$T_{ss}$ should be carried out using well-defined filtration procedures in order to provide comparability of data on a world-wide basis. The analysis of filtered and non-filtered samples and calculation of Hg-$T_{ss}$ from the difference, is less recommended.

In summary, both analytical methods are suitable for mercury analysis and speciation in water samples. The advantage of the method employed by NIMD is its precision and very good recoveries, whereas the methods at IJS are more sensitive and therefore require much less sample for analysis. The generally good agreement of the results obtained provides credibility for the accuracy of the methods used.

Further investigations are in progress in Minamata Bay and the Yatsushiro Sea in order to estimate the potential sources of mercury and MeHg and their relative magnitudes and to calculate a mass balance of mercury in Minamata Bay. Moreover, by combining hydrodynamic and mercury cycling modelling in this coastal environment, meaningful predictions for future behaviour of mercury will be possible. This will also help to assess the effectiveness of remediation actions undertaken more than 30 years ago and to plan future management, if necessary. Accurate and precise analytical data are essential for such an objective. The present paper indicates possible errors in mercury analysis and speciation and, therefore, contributes significantly to the fundamental needs of future research in this area.

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