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WORKING METHODS PAPER

Interlaboratory Study to Improve the Quality Control of Methylmercury Determination in Sediment

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The results are presented of an interlaboratory study on methylmercury (MeHg) in sediment carried out by a group of European laboratories within the framework of a project managed by the EC Standards, Measurements and Testing Programme (formerly BCR). The aim of this exercise was to evaluate the performance of current methods used for MeHg determination in sediment in order to improve the state-of-the-art prior to the certification of a candidate reference material. The paper describes the organization of the interlaboratory study, the preparation of the sediment material used, the techniques evaluated and the results obtained by the participating laboratories. The outcome of the collaborative project showed that certification could be contemplated, providing that certain analytical techniques were optimized, especially with regard to extraction methods.

Keywords: Methylmercury; sediment; interlaboratory study; performance evaluation; improvement

INTRODUCTION

Methylmercury (MeHg) is extremely toxic, which is of particular concern since this compound is widespread in the environment; it may originate either from direct release or from the biomethylation of inorganic mercury in biological tissues,[1,2] or it may be produced by abiotic routes.[3] Due to a very effective bioaccumulation mechanism, methylmercury enrichment occurs in food chains which results in high levels in top predators (e.g. tuna fish).[4] The lack of knowledge of the toxic impact of MeHg in sediment (e.g. on filter-feeding organisms) and the need to understand better the environmental pathways stimulates the monitoring of this compound in various matrices (biota, water and sediment). Legislation on MeHg within the European Union, e.g. in food (national regulations) or water (EC Directives), requires that the determinations are of proven quality. The quality control of MeHg determination was hardly possible some five years ago, which prompted the organization of several interlaboratory studies to evaluate and improve the state of the analytical art.[5,6] These improvements allowed certified reference materials (CRMs) of fish to be produced, e.g. by the National Research Council of Canada[7] and the Community Bureau of Reference, BCR (now renamed the Standards, Measurements and Testing Programme),[8] which in turn offered to laboratories the means to verify the performance of their methods using reliable CRMs. An evaluation of the state-of-the-art of MeHg determination in sediment has recently been organized by the International Atomic Energy Agency (IAEA, Vienna), which showed a reasonable agreement between techniques (coefficient of variation between laboratory means of *ca* 14% for a MeHg

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level of 5.28 μg/kg).[9] Upon the requests of a consortium of laboratories from different EU and EFTA Member States, the Standards, Measurements and Testing Programme decided to organize an interlaboratory study for the evaluation of the method performance for the determination of MeHg in a highly contaminated sediment; this exercise was intended to prepare a group of laboratories for the further certification of a sediment reference material to be conducted in 1996. This paper presents the main results of this collaborative study.

AIM OF THE PROJECT

The EC Standards, Measurements and Testing Programme (formerly BCR) aims to contribute to the harmonization and improvement of methods of measurement and analysis when these methods are not sufficiently accurate, and laboratories obtain differing results. A significant part of this programme (as a continuation of BCR activities) is devoted to the organization of interlaboratory studies and certification exercises with appropriate expert laboratories from the European Union and Associated States. Owing to the difficulties encountered in speciation analysis (mainly due to the multiplicity of analytical steps such as, for example, extraction, derivatization, separation and detection), a series of projects have been organized in the last five years, including one for methylmercury.[10] One of the most powerful tools in detecting and removing sources of error due to a particular technique or a lack of quality control (QC) within a laboratory is to participate in interlaboratory studies.[11, 12] In general, besides the sampling error, the following main sources of error can be detected in speciation analyses.[13]

(a) instability of compounds during storage and sample drying (volatilization, degradation);
(b) sample pretreatment (e.g. incomplete extraction, change of original speciation, losses in clean-up);
(c) derivatization (inhibition, incomplete transformation, decomposition);
(d) separation (decomposition of the species, adsorption on the column, peak overlap);
(e) final measurement (e.g. calibration errors, spectral interferences, background correction);
(f) the laboratory conditions (e.g. training and educational level of workers, care applied to the work, awareness of pitfalls, management, clean bench facilities).

When different laboratories participate in an interlaboratory study, different sample pretreatment methods and different techniques of final determination are compared and discussed as well as the laboratory's performance. If the results of such an intercomparison are in good statistical agreement, the collaboratively obtained value is likely to be the best approximation of the true content of the analyte. Therefore, an interlaboratory study can be held (i) to detect the pitfalls of a commonly applied method and to ascertain its performance in practice, (ii) to measure the quality of a laboratory or a part of a laboratory (e.g. audits for accreditation of laboratories), (iii) to improve the quality of a laboratory in collaborative work in a mutual learning process and (iv) to certify the contents of a reference material. The programme described here is of the type (iii).

PARTICIPATING LABORATORIES

The preparation of the sediment reference material (*not* certified) used in this study was carried out by Ecoconsult (Gavirate, Italy) and the Environment Institute of the Joint Research Centre of Ispra (Italy). The homogeneity and stability were verified at the Presidio Multizonale di Prevenzione (La Spezia, Italy). The material characterization (with regard to bacterial flora) was performed at the University of Siena (Italy).

The following laboratories participated in the interlaboratory study:

De Montfort University, Leicester (United Kingdom)
ENEA, Rome (Italy)
GKSS Forschungszentrum, Geesthacht (Germany)
IAEA, Marine Environment Laboratory (Monaco)
Institut für Angewandte Physikalische Chemie, Jülich (Germany)
Universiteit Amsterdam, Inst. Milieuvraagstukken, Amsterdam (The Netherlands)
Presidio Multizonale di Prevenzione, La

Spezia (Italy)
Presidio Multizonale di Prevenzione, Venezia (Italy)
Rivo-dlo, IJmuiden (The Netherlands)
Sheffield Hallam University, Sheffield (United Kingdom)
Swedish Environmental Research Institute, Göteborg (Sweden)
Universidad de Oviedo (Spain)
Universidad de Santiago de Compostella (Spain)
Universität Heidelberg, Inst. Sedimentforschung, Heidelberg (Germany)
Université de Bordeaux, Talence (France)
University of Umeå, Department of Chemistry (Sweden)
Vrije Universiteit Brussel, Lab. voor Anal. Scheikunde (Belgium).

PREPARATION OF THE MATERIAL

Sample collection and treatment

Sediment samples were collected in the Ravenna Lagoon (Italy) at two locations, namely close to a petrochemical plant water discharge (high mercury levels) and downstream. Two batches of *ca* 250 kg each were made available. The wet materials were dried at ambient temperature under an air stream. The two batches of dry sediment were then mixed and homogenized in order to obtain around 80 kg of dry homogenized sediment to be used as a candidate CRM. A composite sediment was prepared for the purpose of the interlaboratory study by mixing two parts of contaminated sediment with one part of sediment containing low levels of MeHg; around 100 bottles, each containing *ca* 50 g, were prepared and distributed to the participating laboratories.

Stabilization of the material: effect of gamma irradiation on MeHg content

The effects of various gamma doses on the stabilization of MeHg were studied by determining the content in five bottles submitted to doses of, respectively, 4, 8, 12.5, 25 and 50 kGy. No significant differences were observed in the results, which varied from (54.3±3.3) to (56.4±1.8) μg/kg as MeHg. It was hence concluded that methylmercury is not affected by gamma irradiation and that this procedure could be used safely for the stabilization of the material used in this study and, at a later stage, for the candidate reference material.

Homogeneity and stability tests

The homogeneity of the sediment material was verified by determining the methylmercury content of 200-mg levels of sample five-fold in each of five bottles randomly selected during the bottling procedure; five replicate determinations were also performed in each of four bottles irradiated at, respectively, 8 kGy, 12.5 kGy, 25 kGy and 50 kGy. Stability tests were performed at +20 °C by determining MeHg five-fold at regular intervals over a period of 12 months (after 1, 2, 6, 7, 8 and 12 months). For methylmercury determination, 0.2 g of sediment was digested in 5 ml of H_2SO_4 by heating for 30 min in a water bath at 100 °C; this was followed by toluene extraction and back-extraction into sodium thiosulphate. After $NaBH_4$ derivatization, methylmercury hydrides were cryogenically trapped and purged for GC/FTIR separation. The final detection was by cold vapor atomic absorption spectrometry (CVAAS).[14]

The coefficients of variation (CVs) obtained from within-bottle samples ranged from 3.3 to 8.6%, whereas the between-bottle samples CV (calculation made on the basis of the mean values found in 10 separate bottles) was about 5%; the reproducibility of the method was of the same order and it was hence considered that the material was homogeneous. The variability of the MeHg content over 12 months was also small (CV of 4.8%), which demonstrates the stability of this material.

Characterization of the bacterial flora

The main difficulty in preparing a sediment reference material for interlaboratory studies on chemical species is to achieve the stability of the relevant compounds.[10] With respect to MeHg, the main source of instability is due to bacteria, either by demethylation[15,16] or formation of volatile dimethylmercury.[17] The conversion is indirectly provoked by the biological activity of various types of bacteria such as, e.g. (i) aerobic mesophilic heterotrophic microorganisms, (ii) anaerobic sulphate-reducing bacteria and (iii) anaerobic spore-forming bacteria. In order to control the remaining bacteria present after

different irradiation treatments, a bacterial enumeration was performed on samples which were dehydrated after homogenization and irradiated at various γ-ray doses (0, 4, 8, 12, 25 and 50 kGy). The determination of the sulphate-reducing bacteria was performed using the Most Probable Number (MPN) technique,[18] anaerobic spore-forming bacteria were enumerated by plate count on nutrient agar; and the enumeration of aerobic mesophilic heterotrophic microorganisms was performed by a plate count method.

The cultivable bacteria of the three groups were high in sediment samples analysed immediately after drying and homogenization processes (non-irradiated samples). In the sample irradiated with a 4-kGy dose the bacteria content was significant for the heterotrophic and the spore-forming bacterial group, whereas the sulphate reducers were inhibited. The γ-irradiation of sediment at 8 kGy was found to be able to sterilize the sediment completely.

ANALYTICAL METHODS

Seventeen laboratories from nine European countries participated in the programme (see above). The extraction techniques were based on solvent or acid/solvent extraction (e.g. HCl/toluene, H_2SO_4/toluene, toluene/cysteine/toluene). Separation was generally performed by gas chromatography (packed-column or capillary) or HPLC. The final determination was made by electron capture detection (ECD), cold-vapour atomic absorption spectrometry (CVAAS) or atomic fluorescence spectrometry (CVAFS). The Laboratory Numbers below do *not* correspond in order to the named laboratories above. Technique abbreviations are given in Table 1.

Laboratory 01

MeHg was separated from the matrix as MeHgCl by water–steam distillation (H_2SO_4/NaCl mixture) and inorganic mercury was removed from the distillate by an anion-exchange resin (Dowex 1X8, 100–200 mesh) after HCl addition. MeHgCl was then decomposed to ionic mercury by UV irradiation and detected by CVAAS after reduction by $SnCl_2$ and preconcentration on gold wool.

Laboratory 02

The method was based on distillation in aqueous acidic media (H_2SO_4/KCl), followed by aqueous-phase ethylation in an acetate buffer solution. MeHgEt was separated by gas chromatography and detected by CVAAS after pyrolysis.

Laboratory 03

MeHg was extracted by n-hexane after HCl addition. A second extraction was carried out after centrifugation. The extract was derivatized by sodium tetraphenylborate addition and the organic phase (containing MeHgPh) was measured by gas chromatography–microwave–induced plasma atomic emission spectrometry (GC/MIP–AES).

Laboratory 04

The method was similar to the one used by Laboratory 02.

Laboratory 05

Extraction was by toluene after acidification, followed by re-extraction with cysteine acetate

Table 1 Summary of techniques used in the intercomparison

AE	Anion exchange
CVAAS	Cold vapour atomic absorption spectrometry
CVAFS	Cold vapour atomic fluorescence spectrometry
GC	Gas chromatography
GC/ECD	Gas chromatography/electron capture detection
GC/FPD	Gas chromatography/flame photometric detection
GC/FTIR	Gas chromatography/Fourier transform infrared spectroscopy
GC/MIP	Gas chromatography/microwave-induced plasma atomic emission spectrometry
HPLC	High-performance liquid chromatography
SFE	Supercritical fluid extraction

and back-extraction in toluene. MeHg was separated by capillary gas chromatography and detected by electron capture detection (GC–ECD).

Laboratory 06

This laboratory used two different techniques. The first one (06a) was based on toluene extraction after addition of acetate buffer and diethyldithiocarbamate (DDTC); derivatization was performed by addition of butylmagnesium chloride in tetrahydrofuran. The separation was by capillary gas chromatography and detection was carried out by microwave-induced plasma atomic emission spectrometry. The second method was similar, except that the extraction was performed by supercritical fluid extraction (SFE) (elution with toluene from the C_{18} adsorbent in the SFE system).

Laboratory 07

MeHg was extracted with toluene after HCl addition, followed by a clean-up into aqueous thiosulphate solution and back-extraction into toluene. The separation was performed by high-performance liquid chromatography and the absorbance of MeHg in the eluates was measured at 230 μm (UV detection).

Laboratory 08

The extraction was by acetic acid addition, followed by $NaBH_4$ derivatization, cryogenic trapping, packed-column gas chromatography and detection by quartz furnace atomic absorption spectrometry.

Laboratory 09

The method used was similar to the one applied by Laboratory 01, except that no anion exchange was carried out.

Laboratory 10

The technique used was similar to that of Laboratory 07.

Laboratory 12

Extraction was by addition of HCl/NaCl. The extracts were separated by high-performance liquid chromatography and the detection was by CVAAS.

Laboratory 13

An alkaline digestion (KOH in CH_3OH) was carried out, followed by acidification with $H_2SO_4/CuSO_4$), addition of KBr and toluene extraction. After centrifugation, the supernatant was separated and extracted once again. Clean-up of the toluene extract consisted in a back-extraction with cysteine solution; after separation of the phases, the cysteine solutions were collected and extracted into a mixture of benzene, $CuSO_4$-saturated solution and KBr. Measurements were performed by capillary gas-chromatographic separation and electron capture detection.

Laboratory 14

The technique applied was similar to those used by Laboratories 02 and 04.

Laboratory 15

An alkaline digestion was performed (KOH in CH_3OH), followed by a toluene extraction. Derivatization of the extract was carried out using $NaBEt_4$ in KOH. This was followed by cryogenic trapping, packed-column gas chromatography and detection by quartz furnace atomic absorption spectrometry.

Laboratory 16

The technique was based on Grignard reaction (butylmagnesium chloride) after toluene extraction. The separation was carried out by capillary gas chromatography, followed by flame photometric detection.

Laboratory 17

A digestion with H_2SO_4 was carried out first, followed by toluene extraction and back-extraction into thiosulphate. After $NaBH_4$ derivatization, methylmercury hydrides were cryogenically trapped and purged for capillary gas-chromatographic separation and Fourier transform infrared spectroscopy (FTIR).

Laboratory 18

The sample was extracted with H_2SO_4, followed by a toluene extraction (repeated twice). Combined extracts were purified by clean-up using an aqueous cysteine solution, and were back-extracted into toluene. Measurements were by capillary gas-chromatographic separation and electron capture detection.

RESULTS OF THE INTERLABORATORY STUDY

The results submitted in the interlaboratory study were discussed amongst all participants at a technical meeting. Each laboratory which participated in the intercomparison was requested to make a minimum of five independent replicate determinations (i.e. five different extractions). The results were presented in the form of bar-graphs indicating the laboratory codes along with the method used, the means of the individual laboratories and the mean of the laboratory means with the corresponding standard deviations.

Figure 1 shows the results selected after technical scrutiny.

Technical discussion

As an introductory remark to the discussion, Laboratory 03 mentioned that very high results submitted at the first attempt (obtained with a technique involving extraction/CVAFS with no separation step) clearly showed its inadequacy for sediment analysis due to the extraction of inorganic mercury at the same time as MeHg; this necessitated the use of a GC/MIP–AES method.

The full data set had three very high outliers (Laboratory 12: 596±48 μg/kg; Laboratory 15: 317±43 μg/kg and Laboratory 16: 184±21 μg/kg) which were discussed first.

Laboratory 12 withdrew its set of data, stressing that its HPLC technique coupled to CVAAS was not optimized for sediment. It had been previously suspected that HCl extraction was not adequate to extract MeHg quantitatively;[9] the very high results of this laboratory

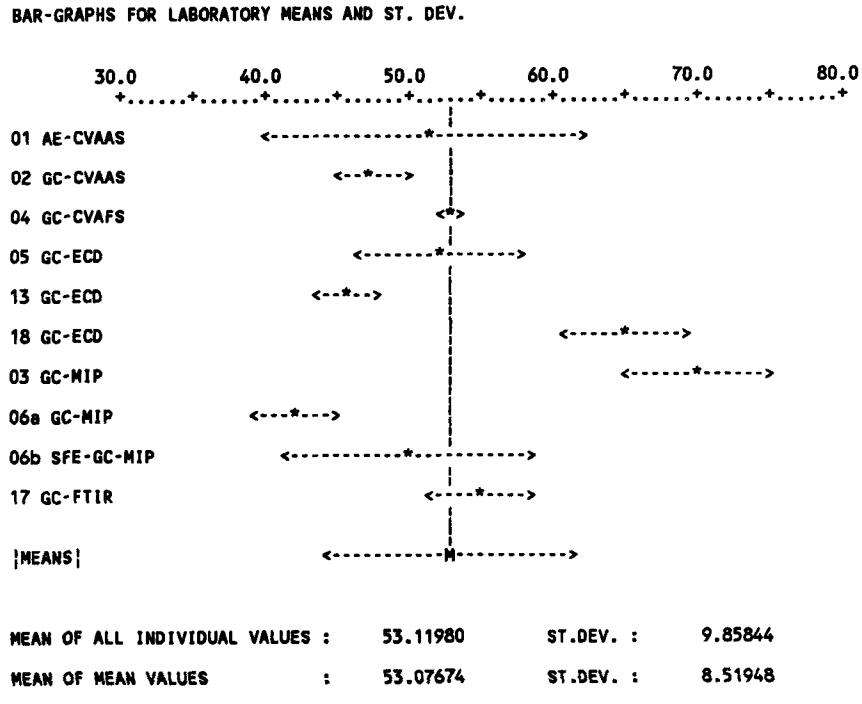

Figure 1 Bar-graph of MeHg in sediment. The laboratory codes are given along with the methods used (abbreviations in Table 1). The results plotted correspond to five replicate determinations. M is the mean of the laboratory means. The results given in this graph correspond to the sets of data selected after technical scrutiny.

could be due to a general problem related to the hyphenated technique itself, or more simply to a calibration error.

Laboratory 15 suspected that alkaline digestion was not suitable for the analysis of this sediment matrix. However, this digestion was used by Laboratory 13, which did not experience problems, and this procedure was even recommended in the framework of the IAEA interlaboratory study.[9] Here again, problems were likely to be due rather to poor quality control than to the technique itself.

Laboratory 16 could not give clear reasons for explaining the high results except that they lacked experience in this type of analysis; the methods should be considerably improvable and would be considered for certification work.

Laboratory 09 had little experience with sediment analysis. The technique is based on toluene extraction, derivatization and thiosulphate back-extraction followed by CVAAS detection. Since no separation was involved (as carried out by Laboratory 01 using an anion-exchange resin), it was suspected that inorganic mercury might have been detected beside MeHg, thus explaining the high results.

The remaining data showed a better agreement but the spread was still considered to be too high (a CV of 47% between laboratory means). This spread was mainly due to some apparently very low outliers [namely Laboratory 07 (13.9 ± 1.9 μg/kg), Laboratory 08 (19.1 μg/kg, only one replicate), Laboratory 10 (21.5 ± 1.1 μg/kg) and Laboratory 14 (30.9 ± 2.5 μg/kg)].

Laboratory 07 experienced problems with thiosulphate back-extraction followed by diethyldithiocarbamate complexation, which led to low MeHg recoveries. The Laboratory stressed that this problem was not observed for water analysis in which a one-step complexation procedure is used. Similar problems were observed by Laboratory 10, which also obtained low results. The acid leaching procedure followed by solvent extraction could also be responsible for low recoveries, as was stressed in a recent interlaboratory study.[9] Laboratory 08 performed only one replicate determination. It was suspected that the acetic acid extraction was not sufficient to extract MeHg quantitatively.

Low results for Laboratory 14 were suspected to be due to low efficiency of the distillation procedure. The results of the IAEA interlaboratory study have shown that low recoveries could be observed for distillation methods for sample masses exceeding 0.5 g;[9] three laboratories using similar distillation procedures (Laboratories 02, 04 and 14), however, used sample intakes ranging from 0.1 to 0.3 g. It was stressed that the distillation has to be carefully checked by spiking experiments.

Problems at the distillation step were also experienced by Laboratory 01; a rather large standard deviation was suspected to be due to interfering compounds when the distillation was carried out for too long. It was recommended to stop the distillation after 85% recovery of sample volume to avoid this interference which led to higher results (and hence high standard deviations). Furthermore, since the results are based on calculation by difference (measurements before and after UV destruction), it was requested that proof be given that only MeHg is actually determined. Laboratory 18 had experience mainly in fish analysis. It was suspected that some contamination of the capillary column had occurred which might have explained the high results.

Other technical remarks were made on the selected set of data (Fig. 1), e.g. Laboratory 06, using supercritical fluid extraction, mentioned that the precision could be improved by ending the extraction time in order to achieve a better recovery. Finally, interferences from sulphur compounds extracted at the same time as MeHg were noted in MIP–AES, stressing the importance of possible clean-up.

CONCLUSIONS

The technical discussion indicated the techniques which still needed to be improved prior to their use in a possible certification campaign. The outcome of the technical scrutiny is a clear illustration of the effects and importance of participating in interlaboratory studies since most of the laboratories from which the data were selected in the final bar-graph had participated previously in the stepwise interlaboratory programme on MeHg in solutions and biological samples,[5] whereas most of the other laboratories for which sources of error were identified participated in such an exercise for the first time. This interlaboratory study gave encouragement to the latter laboratories to improve their methods which will be further tested in the course of the certification campaign. It clearly stressed, of

course, the importance of method validation, including recovery tests, to obtain accurate results.

A follow-up of this interlaboratory study will be the organization of a certification campaign of another sediment reference material. This candidate CRM was shipped to the laboratories in June 1995; based on the certification results, the CRM could be made available in 1996.

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