Trace Reference Materials and Recommended Analytical Methods for Global Mercury Monitoring in the Marine Environment

The primary goal of the International Atomic Energy Agency's Environment Laboratories (IAEA EL) in Monaco is to help Member States understanding, monitoring and protecting the marine environment. Thereby, the major impact exerted by large coastal cities on marine ecosystems is an issue of primary concern for the Agency and its Environment Laboratories in particular. To this extent, it is noteworthy that marine pollution assessments depend on the accurate knowledge of contaminant concentrations in various environmental compartments.

"Mercury is a global pollutant released into the environment from both natural and anthropogenic sources. Mercury is identified as a highly toxic element because of its accumulative and persistent character and the WHO rates mercury as one of the top ten chemicals of major public health concern."

Among its multiple tasks, Marine Environment Study Laboratory (MESL) as a part of IAEA- EL in Monaco acts as the analytical support center for IAEA Member States laboratories and is the pillar of the Quality assurance program for the determination of non-nuclear pollutant – trace elements and organic contaminants in the marine environment.

The laboratory has a research program encompassing analytical methods development and validation. The application of basic metrological concepts (traceability, validation, uncertainty) are the integral part of the analytical work conducted at MESL.

Due to the high toxicity of mercury and requirements coming from the IAEA Member States, particular attention is paid to the development of methods for determination of mercury and methyl mercury in marine samples. Speciation analysis of mercury based on the application of several hyphenated techniques allows the simultaneous determination of inorganic and organometallic (methyl mercury) forms in marine samples.

Development, validation and dissemination of reliable and sensitivity based on the basic metrological principles methods, fosters the application of common methodologies and the generation of comparable monitoring data by IAEA Member States Laboratories. Other relevant activities of MESL to assure production of reliable monitoring data and to build the technical capacity of regional laboratories for assessing marine pollution are: organisation of global and regional inter-laboratory comparisons, production of matrix certified reference materials and training courses.

Certified Reference Materials produced by MESL

Good laboratory practice associated with quality assurance/quality control should be essential components of the analytical process for generation of marine monitoring data. Quality control procedures are commonly based on the analysis of certified reference materials to assess reproducibility and measurement bias. Reference materials are also used for validation of analytical methods, for quality control schemes and estimation of measurement uncertainties, and to establish traceability to internationally agreed stated references. As such they are cornerstones for the proper implementation of relevant regulations in national and international legislations, for quality control and laboratory accreditation.

The present availability of the certified for mercury and methyl mercury matrix reference materials is shown in Table 1.

The guidelines in the production of the matrix certified reference material are ISO Guide 35 "General and Statistical Principles for Certification" and the ISO Guide 34 "Quality system requirements for reference material producers".

MESL matrix reference materials find widespread use in Member States laboratories for quality control, validation of analytical methods, assessment of measurement bias and precision, repeatability and reproducibility studies.

Inter-laboratory Comparisons for Marine Pollution Studies

MESL has conducted more than 20 global inter-laboratory laboratory performance studies in the past ten years.

The goal of inter-laboratory comparisons (ILCs) and proficiency tests (PTs) is to provide information to the participating laboratories on the quality of their analytical results. The results from ILCs or PTs are of crucial interest for laboratories as they provide clear information of their measurement capabilities. The implementation of obtained recommendations generally leads to improvement of quality of their analytical results.

Although such inter-laboratory studies help to improve measurement

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IAEA – Code	Sample Type	Analyte Groups	Year	Availability
IAEA- 461	Marine biota-Clams	Trace metals & MeHg	2013	On-going
IAEA-456	Marine Sediment	Trace metals & MeHg	2012	Yes
IAEA-452	Marine biota-scallop	Trace metals & MeHg	2010	Yes
IAEA-436	Tuna Tissue	Trace metals & MeHg	2006	Yes
IAEA-433	Marine Sediment	Trace metals & MeHg	2004	Yes

Table 1: CRMs for Trace Metals including Mercury and Methyl Mercury (MeHg) produced in MESL

performance, the results obtained from the last word wide inter-laboratory studies highlighted that there are still remaining problems particularly in the determination of mercury and methyl mercury in marine matrices: sediment and biota. These problems are related with the lack of reference methods for monitoring studies, insufficient knowledge on the basic metrological principles (validation of measurement methods, uncertainty and traceability of measurement results) and in some cases lack of trained laboratory practitioners. (Figure 1 and Figure 2).

Recommended methods for Mercury and Methyl Mercury

Mercury is a global pollutant released into the environment from both natural and anthropogenic sources. Mercury is identified as a highly toxic element because of its accumulative and persistent character and the WHO rates mercury as one of the top ten chemicals of major public health concern. Therefore good quality of data on mercury in marine environment is of particular importance for monitoring studies.

To achieve SI-traceable values with small combined uncertainties, several measurement procedures for total mercury, based on the application of Atomic Absorption Spectrometry (AAS), Atomic Fluorescence Spectrometry (AFS) and Inductively Coupled Mass Spectrometry (ICP-MS) have been developed and validated in MESL. Following acidic digestion of samples cold vapour AAS or cold vapour AFS has been widely applied for the determination of total mercury in marine samples: sea water, biota and sediments. The main advantages of the cold vapour (CV) technique are the separation of the analyte from the potentially interfering sample matrix and its comparatively low cost.

Similar limits of quantification are obtained by CV-AFS (LOQ 0.01 - 1 µg.kg-1) and CV-AAS (0.1 ng.L-1 in sea water and 0.1 - 10 µg.kg-1 in biota and sediment samples). The correct assessment of the measurement uncertainty is of crucial importance for obtaining reliable results. In order to achieve the lowest possible procedural blank, all sample-processing steps were accomplished in a class-100 clean chemical laboratory.

After pressure digestion of the samples, inductively coupled plasma-mass spectrometry (ICP-MS) is increasingly being applied for mercury determination, due to its good sensitivity (LOQ of 0.1 µg.kg-1). Memory effects of mercury in the sample delivery system may influence the results of samples analysed after measurement of high concentrations and the need for prolonged washout times and addition of substances stabilising mercury in the solution. The calibration approach used in above described analytical procedure was external calibration or standard addition and expanded uncertainty for all of them was 12%-14 % (k=2).

Another recommended method, for determination of total mercury in marine samples developed in MESL is the Isotope Dilution Inductively Coupled Plasma Mass Spectrometry (ID ICP-MS). Among many analytical methods ID ICP-MS has a well established position as potentially the primary method of measurements and provide independent and SI-traceable values. ID ICP-MS methodology is systematically applied for the production of the assigned values in the frame of IAEA inter-laboratory comparisons and for reference measurements in the framework of the production process of IAEA's certified marine reference material for mercury and methyl mercury.

The estimation of the total uncertainty associated to each measurement result is a fundamental tool for sorting the main sources of measurement biases. Preliminary forecast of the uncertainty budgets is used as a strategy to ensure that the determination of mercury in marine samples could be achieved with demonstrated traceability to a stated system of reference within less than 3% uncertainty (k=2).

The most critical step in almost all commonly used analytical methods for mercury determination is the sample preparation because of its extreme volatility.

One of the possible solutions of this problem is the application of methods for direct analysis mercury and methyl mercury in solid samples, using high resolution continuum source atomic absorption spectrometer (HR-CS-AAS) equipped with solid sampling. Analytical procedure for solid sampling HR-CS-AAS determination of inorganic mercury in various marine samples has been recently developed in MESL. Various measurement options (peak height, peak area and peak volume measurement options) have been optimised. The possible calibration methods using liquid standards and/or solid CRMs were investigated in detail. The limits of quantification obtained by HR-CS-AAS (is about 2 µg.kg-1in sea water and 2 - 5 µg.kg-1 in biota and

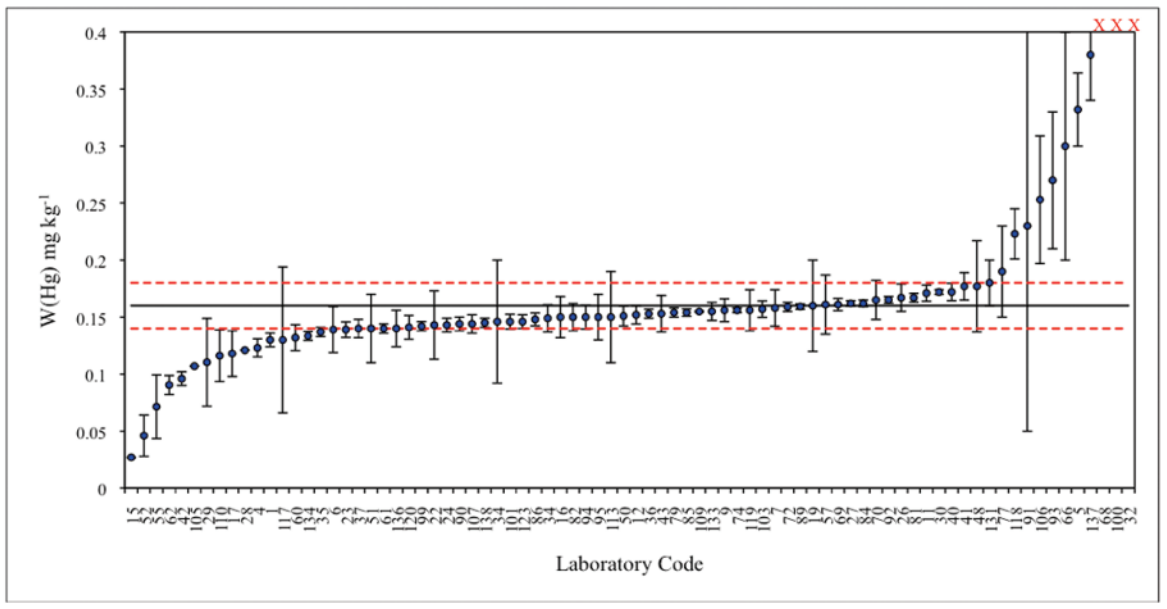

Figure 1: Results for amount content of mercury in marine biota sample reported by IAEA Member States laboratories in the frame of inter-laboratory comparison IAEA 452. The solid line represents the reference value Xref for mercury, dotted lines represent expanded uncertainty of the reference value (k=2). The results outside of the scale are introduced with X.

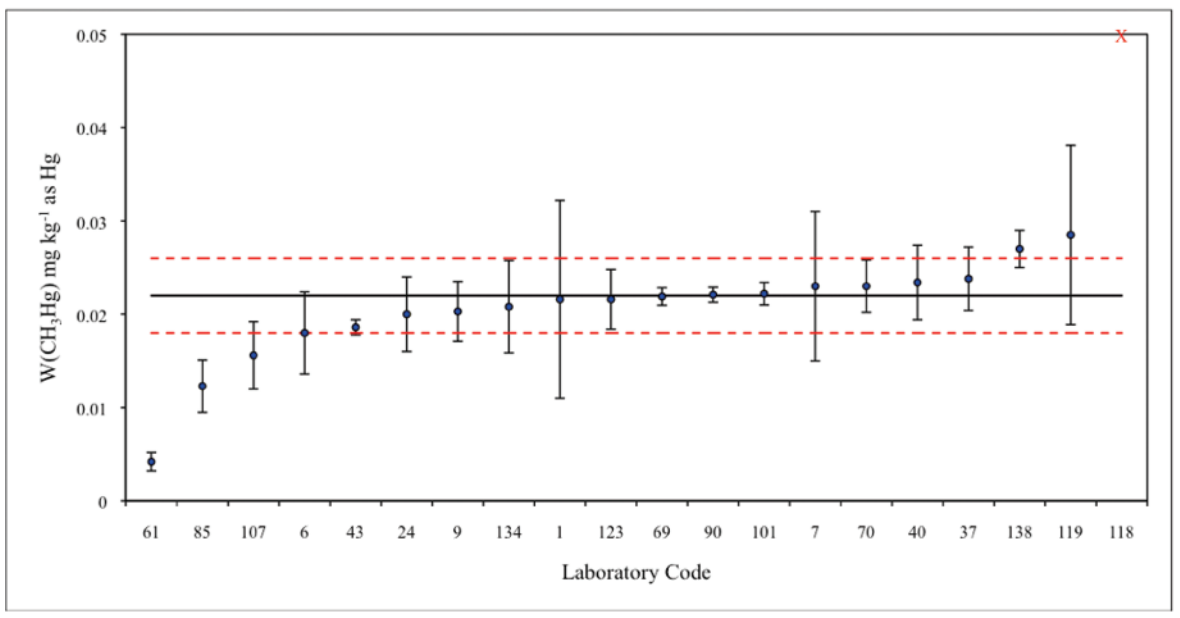

Figure 2. Results for amount content of methyl mercury in marine biota sample reported by IAEA Member States laboratories in the frame of inter-laboratory comparison IAEA 452. The solid line represents the reference value Xref for methyl mercury, dotted lines represent expanded uncertainty of the reference value (k=2). X is a result outside of the scale.

sediment samples. The calibration approach used is external calibration and the obtained expanded uncertainty for this type of calibration was 10%-12 % (k=2).

Elemental mercury analysers, also known as automated or direct mercury analysers, with atomic absorption spectrometry (AAS) or atomic fluorescence spectrometry (AFS) detection are also commonly used in monitoring studies undertaken by MESL. Their main advantages is that they are designed for the direct mercury determination in solid and liquid samples without the need for sample chemical pre-treatment and have a high sensitivity (LOQ < 1 µg.kg-1). The typical uncertainty was estimated 12% (k=2). This technique was also recently used for the estimation of organic mercury content, using "specific" extraction of organic mercury species. An extraction protocol and validated method for methyl mercury in a wide range of marine biological samples has been developed in MESL's laboratories. The typical quantification limit was calculated to be 5µg kg-1 and the expanded uncertainty 16% (k=2).

Various mercury species differ greatly in their bio-physico-chemical properties such as toxicity and rate of bioaccumulation by organisms. Therefore the analysis of samples only for total mercury is no longer acceptable, because it provides only partial information about the element's impact on human health and the environment. Future regulations on methyl mercury will require standardised procedures for quantitative determination of alkyl-mercury species; therefore there is a strong need for development of robust analytical procedures providing reliable data on both, total mercury and its chemical species in complex marine matrices. As a consequence, considerable efforts have been invested in MESL for the development of analytical procedures, which are capable of quantifying various mercury species.

Mercury speciation analysis in marine samples is influenced by the nature of the matrix and the analytical method used. Therefore, the main difficulty is to preserve the initial distribution of mercury species in the sample avoiding losses and/or cross-species transformations that may occur during sampling handling and sample preparation process. Extraction is one of the most critical steps, because two conflicting issues need to be addressed: obtaining high extraction efficiency and minimising losses. Speciation of organo-mercury compounds is performed by Gas Chromatography with packed columns, coupled to AFS as a detector. The important advantages of this hyphenated technique for mercury speciation analysis in marine matrices is the low absolute detection and quantification limits (4 - 6 pg), therefore this is the most cost-effective alternative.

To avoid all problems related with quantitative recovery and account properly for cross-species transformations of methyl mercury, second measurement method based on species specific isotope dilution inductively coupled plasma mass spectrometry was developed in MESL. Separation of methyl mercury was performed after addition of species specific methyl mercury spike solution, according to preliminary validated column separation procedure, based on ion-exchange of [HgCl4]2- ions. The estimation of the total uncertainty associated to each measurement result was fundamental tool for sorting the main sources of measurement biases. Preliminary forecast of the uncertainty budgets was used as a strategy to ensure that determination of methyl mercury in biota samples could be achieved with demonstrated traceability to a stated system of reference within less than 4 % expanded uncertainty (k=2).

Conclusions

The IAEA's Marine Environmental Studies Laboratory, supports the improvement of the worldwide performance of monitoring laboratories and the availability of reliable analytical data for global mercury monitoring through the following activities: development of criteria for generating high-quality data for monitoring programmes, validation and distribution of recommended analytical procedures for determination of mercury and methyl mercury at different concentration and uncertainty levels, production of matrix reference materials, organisation of global inter-laboratory comparisons, training and capacity building of laboratory practitioners form Member States laboratories.