



FINAL PUBLISHABLE JRP REPORT

JRP-Contract number	ENV51	
JRP short name	MeTra	
JRP full title	Traceability for mercury measurements	
Version numbers of latest contracted Annex Ia and Annex Ib against which the assessment will be made	Annex Ia:	V1.1
	Annex Ib:	V1.01
Period covered (dates)	From 1 st October 2014	To 30 September 2017
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Report Status: PU Public





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1 Executive Summary

Introduction

This project has made a significant contribution to the development of an underpinning traceability framework for the measurements of mercury in all environmental compartments, such as air, water and biota, needed to ensure the quality, comparability and traceability of measurement results.

So far, the measurement infrastructure to provide traceable measurements of forms of mercury that are currently regulated and to underpin advanced analytical techniques to support the next generation of environmental mercury measurement is absent in Europe and globally.

The Problem

Mercury in its many chemical forms is highly toxic to human, animal and plant health. Its ability to accumulate in terrestrial and aquatic bio systems and its ability to be transported in air over long distances makes it a particularly insidious threat to environmental sustainability. The increase in the presence of mercury in the environment has been due to human activity over the last one hundred years, and whilst legislation is in place to limit human releases, the assessment of the ongoing effect of mercury on humans and the environment remains of high importance and is critically dependent on accurate measurements to assess concentrations and trends.

Without improved pollution controls or other actions to reduce mercury emissions, mercury emissions are likely to be substantially higher in 2050 than they are today.

The Solution

This project has produced: (i) a calibration infrastructure enabling the traceable assessment of mercury in air, (ii) in-line measurement methods for continuous and semi-continuous Hg(0) and Hg(II) measurement in harsh matrices, (iii) investigation in emerging requirements in mercury science such as the evaluation of mercury concentrations in indoor air from the use of mercury containing compact fluorescent lamps, (iv) reference measurement procedures for dissolved elemental mercury and mercury species in water and in biota, (v) reliable methods for bulk and compound specific isotope signature measurement for Hg(0) and Hg species and (vi) ratio measurement for light isotopes in organo Hg species.

Impact

This project has established the metrological infrastructure for mercury measurements in all environmental media, as required by current and future national and international legislation aimed at controlling mercury emissions and releases.

The new primary measurement standard that has been produced to link the mercury traceability chain to gravimetry will be used by calibration laboratories and instrument manufacturers.

The new digestion method developed for mercury in particulate matter based on an aqua regia digestion will enable routine ICP-MS analysis to be performed with greater repeatability and therefore a lower overall uncertainty. This new digestion method has been included in the revised EN13211 standard for the analysis of mercury in stationary source emissions by the CEN/TC264/WG8.

A novel low-cost prototype passive sensor based on nanostructured sensing materials has been developed. This novel sensor is meant to be used for measuring the most abundant Hg form in ambient air have already been employed within the GMOS network across 5 selected GMOS monitoring sites at different latitude, altitude and thus in different conditions.

The primary reference procedures validated for total mercury and mercury speciation in water and biota have already been, and will be further, used to characterise reference materials (CRMs), in collaboration with CRM producers such as IAEA. These CRMs will be in turn used by testing laboratories and research institutions to validate their measurement procedures.

The pre-concentration method developed for the first time to determine Hg isotopic composition in liquid samples opens up the unique possibility of metrologically traceable Hg isotopic analysis in seawater and atmospheric precipitation. Also the methodology for species-specific Hg isotope ratio measurements in fish tissues has been successfully applied to identify possible sources and transformation of Hg in robust sample archives such as fish tissues from environmental specimen bank of Federal Environmental Agency of Germany.

2 Project context, rationale and objectives

Mercury in its many chemical forms is highly toxic to human, animal and plant health. Its ability to accumulate in terrestrial and aquatic bio systems makes it a particularly insidious threat to environmental sustainability. Its long lifetimes and ability to be transported in air over long distances mean that it is ubiquitous to all environmental compartments and is a pollutant of global concern. The increase in the presence of mercury in the environment has been due to human activity over the last one hundred years, and whilst legislation is in place to limit human releases, the assessment of the ongoing effect of mercury on humans and the environment remains of high importance and is critically dependent on accurate measurements to assess concentrations and trends. This challenge is complicated by the various chemical forms of mercury and its presence in a number of different matrices.

As a result of its highly toxic nature the use of mercury is being phased out for many applications and limited to a mass fraction of mercury in products of less than 1000 mg/kg in any current usage or new application. Mercury is classified as a priority hazardous substance (PHS) due to its persistent, bio accumulative and toxic properties. For priority hazardous substances, European Member States are legally obliged to progressively reduce discharges, emissions and losses to zero within the next 20 years.

According to the UNEP 2013 document "Global Mercury Assessment", mercury emissions to air are thought to have peaked in the 1970s, declined over the following two decades, and have been relatively stable between 1990 and 2005. Conversely it was shown that mercury emissions from fossil fuel combustion, metal and cement production actually increased between 2005 and 2010. Overall, indications are that mercury emissions from industrial sectors have increased again since 2005. Without improved pollution controls or other actions to reduce mercury emissions, they are likely to be substantially higher in 2050 than they are today.

To address the concerns about mercury, the Governing Council of UNEP decided to develop a legally binding instrument on mercury. The Minamata Convention is a global treaty to protect human health and the environment from the adverse effects of mercury. It was agreed at the fifth session of the Intergovernmental Negotiating Committee on mercury in Geneva, Switzerland in January 2013 and adopted later that year on 10 October 2013 at a Diplomatic Conference held in Kumamoto, Japan.

The Convention draws attention to a global and ubiquitous metal that, while naturally occurring, has broad uses in everyday objects and is released to the atmosphere, soil and water from a variety of sources. Controlling the anthropogenic releases of mercury throughout its lifecycle has been a key factor in shaping the obligations under the Convention.

Major highlights of the Minamata Convention include a ban on new mercury mines, the phase-out of existing ones, the phase out and phase down of mercury use in a number of products and processes, control measures on emissions to air and on releases to land and water, and the regulation of the informal sector of artisanal and small-scale gold mining. The Convention also addresses interim storage of mercury and its disposal once it becomes waste, sites contaminated by mercury as well as health issues.

With respect to the atmospheric compartment, at the current time in Europe, it is not possible to defensibly and traceably assess mercury at the concentration levels relevant to the European directives concerned with the measurement of emissions and concentrations in air (European Directive 2004/107/EC, Art. 3; Directive 2010/75/EU) because of a lack of underpinning traceability and validated methodologies for low concentrations and for different mercury species.

Written standards EN15852 and EN15853 (for mercury measurement in air) and EN13211 and EN14884 (for mercury measurement in emissions) still need a metrological backbone to allow the concentration values produced to be confidently compared over time in order to assess trends between countries and locations and to compare relative performance. The same applies to US EPA method 30A "Determination of total vapour phase mercury emissions from stationary sources (instrumental analyser procedure)" and US EPA method 30B "Determination of total vapour phase mercury emissions from coal-fired combustion sources using carbon sorbent traps".

These needs have been addressed by objective 1, with the development and implementation of a new primary measurement standard to link the mercury (Hg(0)) traceability chain to gravimetry instead of the currently used mercury vapour concentration equations.

Mercury provides a serious analytical challenge. It is reactive, difficult to store and handle, and extremely difficult to measure accurately as it easily volatilises or adsorbs onto surrounding media (e.g. surfaces of sample containers) prior to analysis. These effects impinge on measurements of mercury in ambient air and also in emissions from stationary sources.

Sampling methods and sample preparation can effect mercury concentration and speciation over time. Many mercury analysis techniques give inaccurate results due to the volatility of several mercury species, the instability of the samples collected owing to species interconversion or adhesion to surfaces, and analytical interference from organic species present with similar physiochemical properties e.g. natural gas condensates. The proper measurement of speciated mercury in air or natural gas remains a challenge. These problems are exacerbated in harsh environments where these effects are amplified. These effects mean that measurement results may in many cases seem to meet regulatory levels, where in reality this is not the case.

The state of the art in this area still does not provide traceable results with uncertainties low enough to draw conclusions about the effectiveness of abatement measurements or concentration trends over time. Uncertainties for air measurements and measurements from stationary sources are currently as high as 50%.

These needs have been addressed by objective 2, developing underpinning metrology to solve the remaining measurement challenges and biases for continuous and semi-continuous Hg(0) and Hg(II) measurement in (harsh) matrices such as stationary source emissions.

Mercury is widely used in compact fluorescent lamps (CFLs) and the demand for them is increasing in the quest for energy efficiency. According to the EU Directive 2002/95/EC on the restriction of hazardous substances in electrical and electronic equipment (RoHS Directive), mercury content in CFLs cannot exceed 5 mg per lamp. However, the number of CFLs is rapidly increasing, presenting a challenge in use and disposal of mercury (UNEP, 2013, "Mercury, Time to Act"), whereas broken lamps (during use) present relatively long periods of mercury overexposure inside rooms. Fully traceable data on likely concentrations and validated measurement methods to address these problems are currently lacking.

These needs have been addressed by objective 3, investigating mercury concentrations in indoor air from the use of mercury containing compact fluorescent lamps, using traceable methods.

The UNEP 2013 document "Global Mercury Assessment" also includes an assessment of releases of mercury to the aquatic environment and its subsequent pathways and fate.

Mercury released into the environment is eventually deposited on the earth or in the ocean, or in other surface waters. Biological activity transforms inorganic Hg into MeHg which accumulates throughout the food chain and, thus, it is also taken up by humans. Exposure to MeHg is especially dangerous for unborn children as it results in neurological disorders. Furthermore, there is evidence that the exposure of humans to MeHg can influence the cardiovascular system and indications can be found in the literature that it has effects on the immune and reproductive system as well as on vision. The World Health Organisation (WHO) recommends a maximum weekly intake of MeHg of 1.6 µg/kg body weight while the United States Environmental Protection Agency uses a reference dose of 0.1 µg/kg body weight as an exposure without recognised adverse effects.

In Europe, the Water Framework Directive (WFD, 2000/60/EC) is a far-reaching framework Directive for water management and protection in the European Union. It obliges Member States to set up integrated policy plans for river basins and coastal areas, covering both qualitative and quantitative aspects of water management. The goal of the WFD was to achieve a good chemical and ecological status for all European waters by 2015 in the European Union. Within the WFD, a list of prioritised substances including Environmental Quality Standards (EQS) has been identified in a daughter Directive (2013/39/EC).

However, in 2017, this goal is far to be reached. All Member States are required to monitor the environmental concentrations of these substances and to report to the European Commission whether national waters meet the EQS, or not. According to the WFD, the national water bodies of a Member State are considered not to have achieved an acceptable chemical status if one (or more) substance exceeds the respective EQS on a representative spatial scale.

With new legislation coming into force, the EQS for mercury will be measured in prey tissue to account for food chain magnification. Mercury levels from across Europe show that this toxic compound exceeds the respective EQS on an area-wide basis, i.e. those concentrations that are currently considered to be safe for the environment.

Although the WFD refers to “Hg and its compounds” there is no regulation for MeHg, only for total Hg. In water, Hg concentrations are usually quite low (0.03-80 ng/L) except in polluted areas. The fraction of MeHg varies greatly depending on bioactivity, sulphur content and salt content in the different water bodies. The WFD lists Hg and its compounds as priority substances with a maximum allowable concentration in water of 0.07 µg/L and 20 µg/kg wet weight in biota for total Hg content. For quantification of MeHg at these low concentrations, no reference measurement procedure exists.

The state of the art currently does not allow different mercury species to be reproducibly and accurately differentiated and measured.

The needs have been addressed by objective 4, developing measurement procedures to provide full traceability and robust uncertainty statements for dissolved elemental mercury (Hg(0)), inorganic mercury (Hg(II)) and methyl-mercury (MeHg) in water and inorganic mercury (Hg(II)) and methyl-mercury (MeHg) in biota.

Mercury pollution has traditionally been monitored by measuring the concentration of mercury and mercury species in environmental samples. However, a growing number of studies are attempting to obtain a deeper insight into the complicated biochemistry of this element. For this purpose, the potential of isotopic analysis to identify sources, pathways and sinks has recently been investigated. Mercury shows natural variation in its isotopic composition as a result of isotope fractionation. The relative small mass dependent isotope fractionation of this element requires highly precise and accurate isotope ratio measurements (e.g. an expanded uncertainty <0.5‰ for δ 202Hg) to be performed in order to detect small natural differences in the environment. Hence mass discrimination correction for Hg still represents a challenge and a metrological infrastructure based on well characterised and validated methods to achieve comparable results between laboratories is urgently needed.

These needs have been addressed by objective 5, obtaining a deeper insight into the causes of mass discrimination and by assessing the validity of various correction approaches, representing a significant advance over and above the state of the art.

The requirements for environmental quality monitoring must be supported by the archiving of environmental specimens so that the presence of mercury in the environment over longer time periods can be properly understood. Once banked, these specimens can be used retrospectively for quality assurance, application of improved analytical techniques, and spatial and trend monitoring of newly selected compounds. As yet, environmental specimen banks (ESBs) are not systematically used by monitoring programmes such as the Water Framework Directive because storing water samples consumes too many resources. A number of European ESBs systematically archive limnic and coastal fish specimens.

Consistent and interdisciplinary approaches that make better use of high quality ESB samples can overcome the reluctant use of monitoring data in chemicals risk assessment. The application of cutting edge analytical methods to ESB samples is a win-win situation for the value of ESB samples and our understanding of the quality of the environment.

These needs have been addressed by objectives 4, 5 and 6, investigating fish samples from these banks to compare different sampling, processing and storage conditions.

3 Research results

Objective 1: To develop a calibration infrastructure enabling the traceable assessment of mercury in air to support European legislation for gaseous emissions and air concentrations and as part of the global mercury observing system.

Research and outputs in this objective focussed on the development and implementation of a new primary measurement standard to link the mercury (Hg(0)) traceability chain to gravimetry instead of the currently used mercury vapour concentration equations. To ensure robustness and comparability of this innovative new primary gravimetric mercury vapour standard it was also crucial to compare the new standard with currently used calibration techniques to gain the confidence needed for full implementation. Key to this is the demonstration of coherence of the calibrator when compared against measurements of mercury in the particulate phase – since the ratio of particulate and vapour phase mercury concentrations is a key parameter in understanding source apportionment, atmospheric processes, and long-range transport. This

coherence was assessed by a comparison against two types of ICP-MS measurement of mercury in particulate.

Development and implementation of a new primary measurement standard

As part of the EMRP 2010 PartEmission project (EMRP ENV02) a proof of principle assessment of the development of a traceability chain for mercury vapour measurement based upon gravimetry had been made. Within MeTra this approach was further elaborated to develop a gravimetric primary standard for the range 5 ng Hg/m³ - 60 µg Hg/m³, covering key requirements for ambient air monitoring (1-2 ng Hg/m³), health-based exposure standards (50 ng Hg/m³), concentrations relevant to stationary source emissions (upwards of 1 µg Hg/m³), the minimum alveolar concentration value (20 µg Hg/m³), and afford a comparison with the currently used mercury vapour equation based calibration concentrations (0,2 – 60 µg Hg/m³).

Although the PartEmission project had proven that a stable diffusion could be generated by using diffusion cells and a temperature controlled housing, the aim of realising metrological traceability based upon gravimetry wasn't realised because the used (glass) diffusion cells increased in weight, due to oxidation and coating issues, instead of losing (mercury) mass gradually in time.

Within the MeTra project therefore considerable improvements had to be made. The first improvement was the use of metal diffusion cells based upon steel. Very special was the drilling of a hole, by using water at very high pressure, in the steel chimneys/tubes. After realising this important step the different parts of the steel diffusion cells were integrated, and the resulting new diffusion cell was cleaned and filled with 2 mL of pure mercury.



Figure 1: Examples of metal diffusion cells based upon steel In order to cover the range 5 ng Hg/m³ - 60 µg Hg/m³, several types of diffusion cell were made:

- Type 1: range ca. 10 - 100 ng Hg/m³ at 20 °C and 105 kPa
- Type 2: range ca. 12.5 - 125 ng Hg/m³ at 20 °C and 105 kPa
- Type 3: range ca. 90 - 900 ng Hg/m³ at 20 °C and 105 kPa
- Type 4: range ca. 0.7 - 7 µg Hg/m³ at 20 °C and 105 kPa
- Type 5: range ca. 5.5 - 55 µg Hg/m³ at 20 °C and 105 kPa

Adjusting the temperature and/or the flow would enable a coverage of the full range of 5 ng Hg/m³ - 60 µg Hg/m³.

Furthermore, a set of non-passivated mercury generators was built, including dilution and mixing systems consisting of cyclones and thermally cooled piping to generate a homogeneous flow.

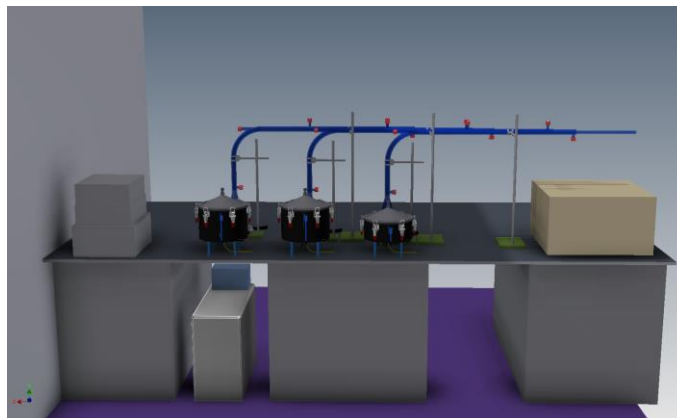


Figure 2: Dilution and mixing systems consisting of cyclones and thermally cooled piping to generate a homogeneous flow

Each of the diffusion cells was weighed after adjusting the internal pressure to 1020,0 mbar. Bringing the internal pressure to 1020,0 was one of the very demanding steps in the weighing process and the most crucial one in the whole weighing process. The reason is that each diffusion cell has (at least) 4 mL of headspace. Each mbar difference of the 4 mL headspace leads to a different mass of (at least) 5 micrograms in the weighing process. We achieved the goal of setting the internal pressure at 1020,0 mbar by first adapting and using a commercial glove box and, based upon the success of using this optimised glove box, by developing a dedicated pressure chamber.



Figure 3: A commercial glove box used to weight the diffusion cells after adjusting the internal pressure

Of course also each mass weighing had to be performed at 1020,0 mbar and the very same temperature during each of the weighing cycles. This goal was realised by using an advanced mass comparator in a pressure and temperature controlled environment, in which up to 3 diffusion cells could be weighed in an automated weighing process.

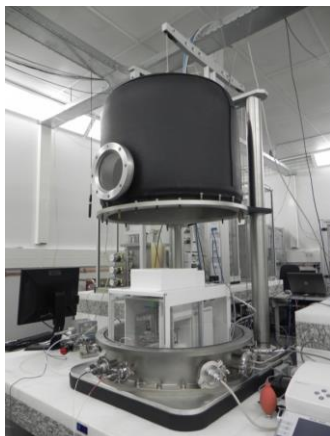


Figure 4: A pressure chamber



Figure 5: New diffusion cells

Using the new diffusion cells, including dilution and mixing steps, once again a stable mercury diffusion was obtained.

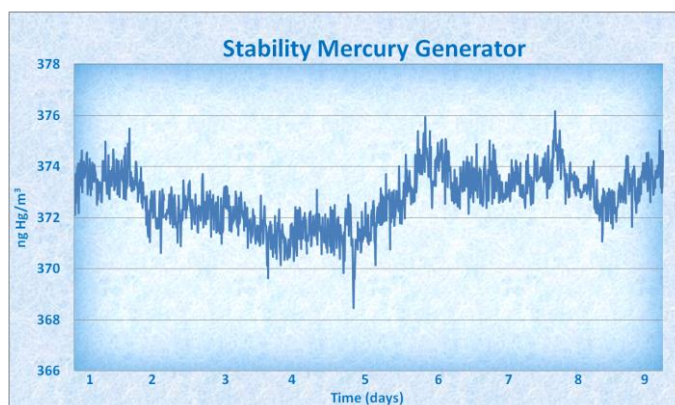


Figure 6: An example of the stability of the mercury diffusion

Furthermore, and most importantly, during each weighing cycle a consistent decrease in mass was measured, ensuring that the goal of realising a novel gravimetric traceability for mercury vapour was achieved.

Comparison of the new gravimetric standard with currently used calibration techniques

A comparison between VSL and NPL showed that the primary mercury generator produced a stable homogeneous mercury flow that could be used to sample gold traps by developing, validating and using an automated loading device.

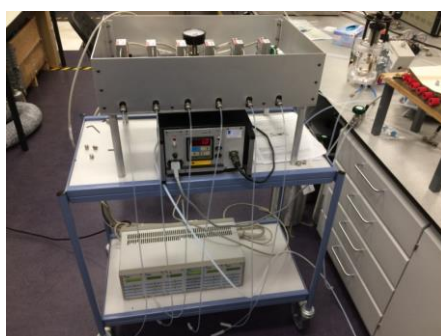


Figure 7: The automated loading device

Results obtained by NPL and VSL, by analysing mercury loaded gold traps by the mercury generator were overlapping as can be seen from the figure 8.

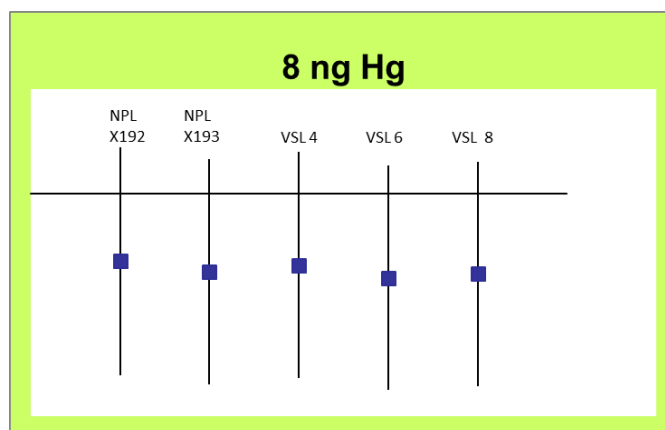


Figure 8. Results obtained by NPL and VSL, by analysing mercury loaded gold traps by the mercury generator.

Another comparison between IJS and VSL showed that at higher loadings breakthrough of elemental mercury might happen. This must be further investigated. If found to be a real effect this can probably be avoided by choosing a more modest flow during loading.

The last comparison was a direct comparison, carried out at VSL, between the new primary measurement standard and the Dumarey equation. Using different temperatures (16 and 18 degrees Centigrade) of the Tekran bell-jar a consistent difference was found of 11,5%. The consistency of the measurement results was a great success and proved that both the primary mercury generator and the set-up, used for this comparison, are working as hoped for. Nevertheless it has to be stated that the $(11,5 \pm 5) \%$ difference found is an indicative difference at the moment as future research has to be performed to optimise this comparison, i.e. lowering the measurement uncertainty by especially an improved characterisation of syringe volumes (now syringes were used with an uncertainty of plus/minus 0,5 %) and more stringent temperature control & measurement.

Comparison against two types of ICP-MS measurement of mercury in particulate

After consulting IJS, LNE and VSL, NPL provided a set of blank and loaded filters to LGC for LA-ICP-MS method development. Using the samples provided by NPL, LGC have developed a strategy to calibrate particle-loaded filters against blank filters doped with acidified Hg solutions at increasing concentrations to generate a response curve. Recoveries relative to concentrations determined by digestion and solution ICP-MS of 98.7% were achieved, and the uncertainty associated with the LA-ICP-MS strategy was estimated to be $\pm 53.8\%$ ($k=2$) compared to 20% for the solution ICP-MS strategy.

IJS developed a technique for temperature desorption of mercury from particles measured by mass spectrometry. Small amounts (between 1-10 mg) of solid samples were put into a quartz tube and heated from room temperature to about 650 °C at a heating rate of 10 °C/min. Sensitivity of the method is sufficient to follow Hg desorption curves at ambient Hg concentrations on particles.

Objective 2: To develop a metrological in-line measurement method and calibration infrastructure enabling the traceable assessment of mercury thresholds specified in European legislation and as part of the global mercury observing system for continuous and semi-continuous Hg(0) and Hg(II) measurement in (harsh) matrices like stationary source emissions or liquid media, including the use of sensor technology.

Research and outputs in this objective have been collated according to the main activity types, relating to the cleaning of gold traps, detection limits of air cleaned traps, novel digestion methods for harsh environments, speciation analysis, improved GMOS methods, novel passive sampling method.

Good practice for cleaning of gold traps

Research was done in order to determine the most efficient method for the cleaning of gold traps, used for the collection and measurement of vapour-phase mercury in ambient air for analysis in the PS Analytical Sir Galahad instrument. The gold traps are usually cleaned at NPL after analysis by thermal desorption in the Sir Galahad for several cycles. Each cycle reaches $> 500^{\circ}\text{C}$ for 60 seconds which removes any excess mercury amalgamated with the gold-coated silica. Argon is used as a carrier gas. This method of cleaning, however, is limited to one trap at a time and extends the time of analysis. In fact in usual operation the cleaning is done as part of the analysis for the trap. That is to say that if the tube undergoes thermal desorption 4 or more times in order to remove all the mercury available for analysis then it is also assumed to be clean after this. The current project gave the opportunity to test the effect of cleaning these tubes in air using the new PS Analytical Trap Cleaner.

The PS Analytical Trap Cleaner is a stand-alone piece of equipment with four trap holders, meaning that several traps can be cleaned at once. The recommended heating time is 60 seconds. It heats the traps to $> 500^{\circ}\text{C}$ and uses compressed air as a carrier gas. Adjustable settings include the number of tubes to be cleaned at one time (between 1 and 4) and the number of heating cycles carried out (between 1 and 9). There is a choice of two modes; A and B. Mode A heats and cools each trap individually, whereas mode B heats each trap individually before cooling them all at once. The use of the trap cleaner results in reduced chance of contamination of the Sir Galahad caused by used/dirty traps, and removes multiple cleaning steps using the Sir Galahad. It is thought that the trap cleaner removes unwanted contaminants from the traps, such as hydrocarbons from the atmosphere, which affect the mercury measurement results.

Various tests were performed to decide the most efficient method for cleaning, the results of which can be seen in the Figure 2.1. Ten traps were used and each test was repeated three times per trap to find an average peak height. One cleaning cycle was found to be sufficient when the traps were spiked with 5 ng mercury on the Gain 10 method. Each test involved six desorptions on the Sir Galahad, the first of which removed the majority of the mercury present. Average peak heights of the final 5 desorptions were taken as the 'baseline'. This baseline was found to lower by 37% after one cleaning cycle carried out before spiking. To see if this effect was permanent, or if cleaning needed to be repeated before each spike, the traps were spiked again and run for 6 desorptions without any cleaning. The lowering of the baseline by 37% remained. A further test was carried out in which the tubes were spiked and then cleaned in the trap cleaner, before being run through the Sir Galahad, which confirmed that the trap cleaner can also remove large amounts of mercury from the traps. No link was found between age/usage and performance of the traps. In conclusion, the recommended cleaning procedure for these gold traps was:

- Desorb the traps a minimum of four times during analysis to ensure a maximum of the collected mercury is removed.
- Following analysis and before subsequent use traps should be cleaned before collecting a sample for 1 cycle in air in the trap cleaner.
- Recommended settings for the trap cleaner are 20 seconds, 60 seconds and 360 seconds are advised for flush time, heat time and cooling time respectively. Mode A is the quickest choice for a single trap to be cleaned, but two or more traps could be cleaned in less time when using mode B.
- Prior to use, and analysis after use, it is recommended that traps are kept in a refrigerator in the dark at below 8°C .

- Previous work (Brown et al, *Environ. Sci. Technol.* 2011, **45**, 7812–7818) has suggested that memory effects associated with traps can become more pronounced over time. Therefore, it is also suggested that if stored for more than 1 month, traps are re-cleaned once in air, prior to subsequently use.

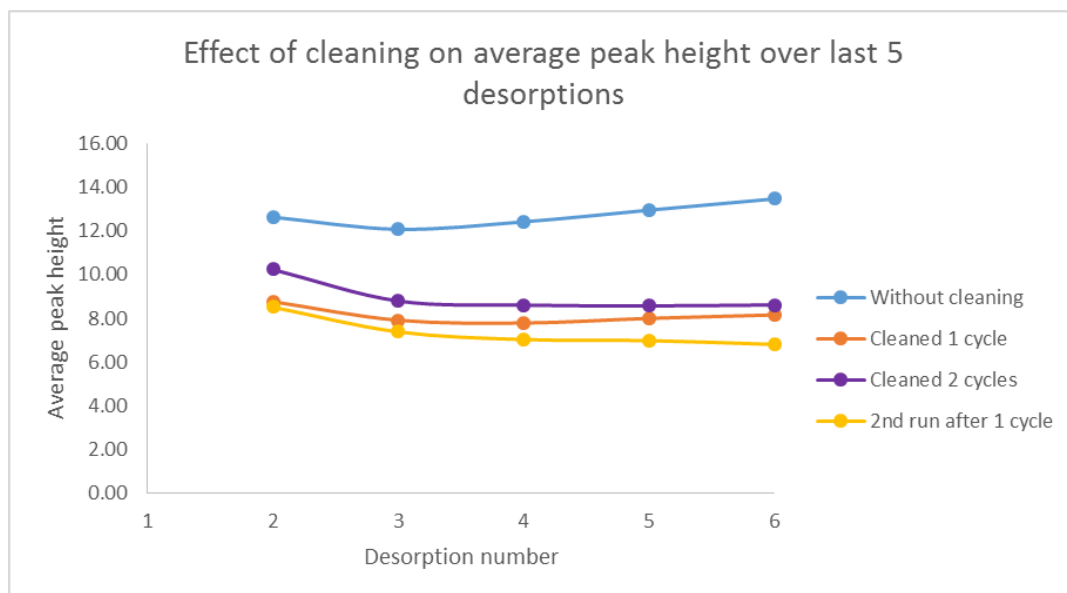


Figure 9 – a graph showing the average peak heights for the 10 traps over the last 5 desorptions on the Sir Galahad. Each trap was spiked with 5 ng mercury. Results are shown with and without use of the trap cleaner.

Detection limits using 'air-cleaned' traps

Traps that had been cleaned using the procedure described above were spiked with varying amounts of mercury, ranging from 0.1 – 50 ng. Each trap was then run through the Sir Galahad for 6 desorptions and the peak heights were recorded. A chart was plotted showing % of peak n (n ranging from 2 – 6) relative to peak 1 vs. mass of Hg from peak 1 (See the figure 2.2).

The intercept of these relationships with the y-axis at 100% give a good indication as to the detection limit of the method. This is in very good agreement with what has been previously observed – approximately 70 – 90 pg on a tube – and is perhaps marginally lower (excluding peak 6) than was observed before the cleaning procedure.

Furthermore the plot shows that when a large mass of mercury (>5 ng) is present in the trap, peak height decreases from desorption 2 to desorption 6. However, when only a small mass of mercury (<5 ng) is present, the peak height from desorption 2 is the smallest, and this increases through to desorption 6. This shows that larger masses of Hg require more heating cycles on the Sir Galahad to remove all mercury and reach the lowest peak height. The reason for peak height increasing after the second desorption is unclear, but may be caused by contamination from the instrument.

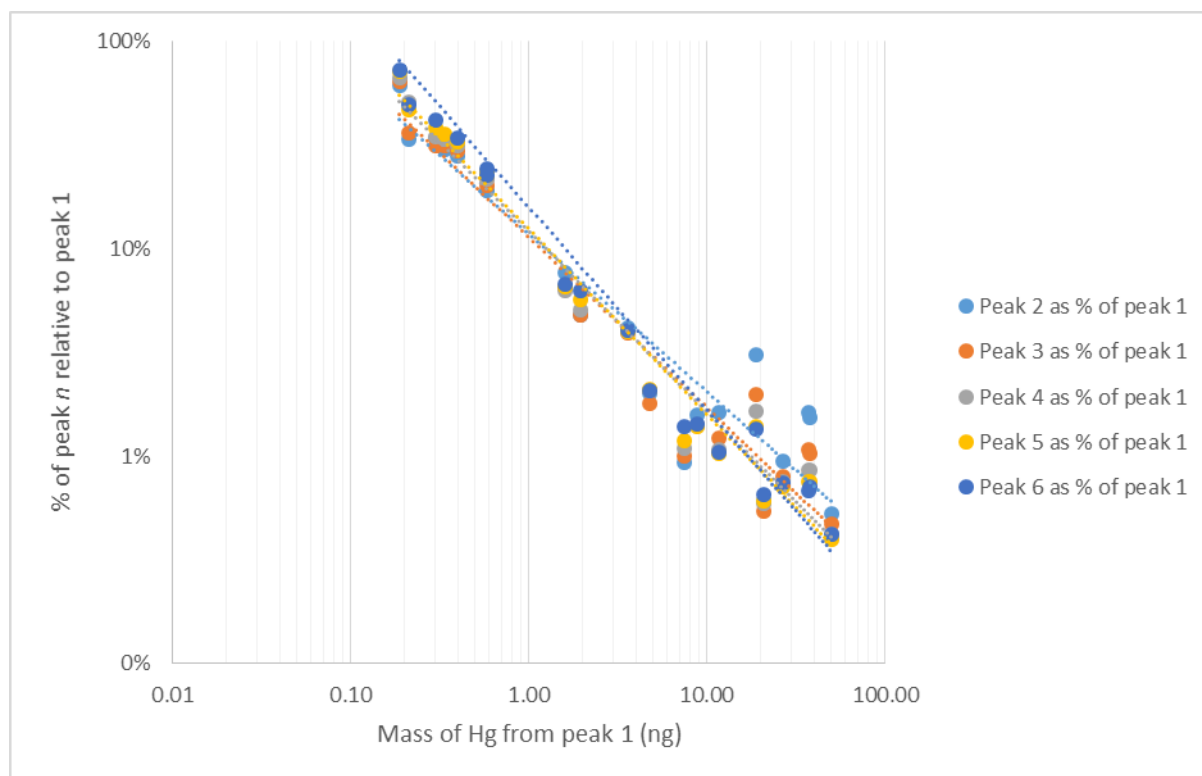


Figure 10 – a graph showing the average peak heights relative to peak 1 for desorption cycles 2 to 6 as a function of the original mass of mercury spiked on the trap.

Novel digestion methods for harsh environments and in-line measurement methods

This activity has concentrated almost entirely on work to revise the EN13211 standard “Air quality — Stationary source emissions — Manual method of determination of the concentration of total mercury” to ensure it does provide underpinning traceability via validated methods with robust uncertainty statements for the determination of total mercury in stationary source impingers. This has been done as part of CEN TC264 WG8 ‘Mercury’.

As well as a number of changes to the main text to update the language and metrology content and reflect more modern practices, the main addition has been to include a new informative annex within the standard, based on work by NPL ([S. L. Goddard, R. J. C. Brown, Sensors, 2014, 14, 21676-21692](#)) which allows a new, HF-free digestion method to be used for the measurement. This will be a great advantage for future users in terms of improving the accuracy of their measurements and also removing many of the health and safety problems which accompany the use of HF.

The new NPL Annex on an alternative digestion method is now integrated in the current prEN 13211:2017. This text is not expected to change significantly prior to formal vote, except for editorial changes.

Speciation analysis

This activity focussed on the development of methods for continuous and semi-continuous Hg(0) and Hg(II) measurement in (harsh) matrices like stationary source emissions or liquid media. The method developed for this analysis works on the principle that bubbling argon gas through a sample prior to adding reductant will liberate any elemental mercury, but will not liberate oxidised mercury. Adding of reductant followed by further bubbling of gas will liberate the remaining oxidised mercury.

This type of analysis may be simply achieved by performing an analysis run with deionised water as the 'reductant' (rather than stannous chloride) to measure elemental mercury, and then swapping the instrument tubing back to use stannous chloride solution as the reductant as usual for a subsequent analytical run to measure the oxidised fraction. Care should be taken in such an analysis to ensure that the sample tubing is well flushed through with the new reductant to be used so no traces of the previous reductant are left – especially when water is replacing stannous chloride.

This method has been proven in principal using two samples: nominally 10 ng/ml Hg in acidified potassium dichromate (where all the Hg is expected to be present as oxidised mercury) and nominally 10 ng/ml in 0.5 % HCl solution with reductant added (where all the Hg is expected to be present as elemental mercury). The samples were analysed first with water being used as the 'dummy' reductant and subsequently using the normal procedure described in the rest of this document using stannous chloride as the reductant. The results are shown in the table below.

Sample type	Average recovery of the nominal mass concentration of 10 ng/ml	
	1 st analysis: water as 'reductant'	2 nd analysis: SnCl ₂ as reductant
Acidified potassium dichromate	0 %	100 %
0.5 % HCl solution with reductant pre-added	90 %	73 %

The results demonstrate the proof of principle of the technique. They show especially clearly that no oxidised mercury is liberated from the acidified potassium dichromate prior to its reduction and subsequent liberation, after which step it is all successfully liberated as elemental mercury. In the case of the 0.5 % HCl solution with reductant pre-added it is clear that the elemental mercury can be successfully liberated directly by just gas bubbling without additional use of a reductant. However, the recoveries of less than unity show that the elemental mercury continues to escape from the sampling even without the additional assistance of gas bubbling. Hence 10 % of the mercury was lost prior to the first analysis and another 17 % loss prior to the second analysis where further reductant was added. Such a loss of elemental mercury from these solutions would also be expected from real samples and this is why most impinger collection methods use highly oxidising solutions to capture the mercury. Nevertheless the method demonstrated should be useful for the speciation analysis of trace level samples of natural origin (such as river water and seawater), but the degradation observed in the case of the HCl test solution highlights the need for appropriate sample storage prior to analysis (for instance in the freezer).

Novel passive sampling method

One of the fundamental goals achieved was the development of a low-cost prototype passive sensor based on nanostructured sensing materials with the advantage of robustness, traceability, sensitivity, reaching ultra-trace levels, and not requiring power supply and gas carrier, as well as highly qualified technical expertise.

The prototype Hg passive sampler has been designed to give information about the average Hg pollution levels over time periods from a few hours to weeks/months providing comparable performance to active samplers in terms of sensitivity and reproducibility. The sampling technique was based on the property of molecular diffusion of Hg gas molecules through a diffusive surface into the region of stagnant air of the sampler and sorbing to the collection surface (passive membrane). The sampler prototype consists in a cylindrical borosilicate glass vial with teflon screw cap at one end. In particular, there are two screw caps: one "opened" used during the sampling time and one "closed" employed before and after the sampling period. To avoid turbulent diffusion inside the vessel during exposition, the open end cap contains a thin-film with an anti-convection porosity which consists of a fine micro-porous nylon screen placed at the inlet end. At the bottom of the vial the passive membrane is placed, consisting of a quartz coated filter (thin layer of the nanostructured material) which during the analytical step is thermally analyzed and regenerated for further

measurements after the desorbing phase. The passive membrane is one of the key components of the passive sampler. The figure 2.3 shows the analytical set-up developed.

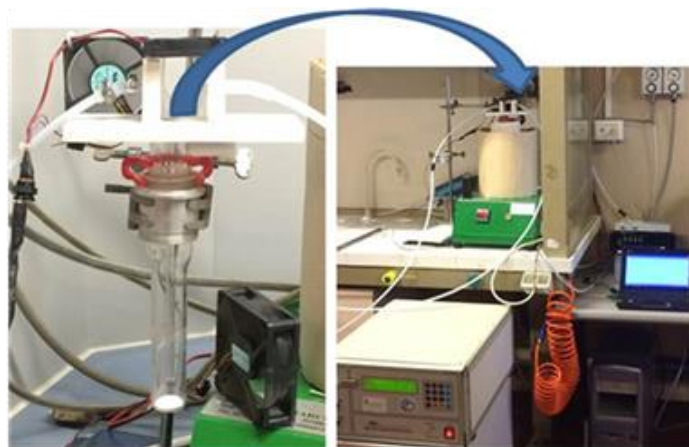


Figure 11. Analytical set-up for desorbing and analyzing the PASs samplers. Mercury collected on the passive membrane is thermally desorbed as GEM (Hg^0) which then passes through and is detected by the automated Tekran 2537A/B analyzer (Tekran Instrument Corp., Ontario, Canada)

The novel passive samplers developed for measuring the most abundant Hg form in ambient air (TGM/GEM) have been employed within the GMOS network across 5 selected GMOS monitoring sites at different latitude, altitude and thus in different conditions (i.e., meteorological etc.) for comparison exercises and testing them with conventional instruments/methods employed in the global network (Tekran and Lumex analysers). The GMOS Sites selected: **1.** Monte Curcio Station, Italy; **2.** EMMA station, Bariloche, Argentina; **3.** Cape Point Station, South Africa; **4.** M.te Ailao, China; and **5.** Listvyanka Station, Russia.

The sampling campaigns have been scheduled over one month and a half during two seasons. 250 Passive samplers were prepared and shipped. The first sampling campaign started in February 2017 and ended during March whereas the second campaign started on 31st of May and ended on 12nd of July. All samples collected during both sampling campaigns have been analyzed at the REG1(CNR) laboratories. The results at two GMOS sites showed a very good comparability and reproducibility between the PASs and Tekran Hg concentrations (figures 12, 13 and 14, 15).

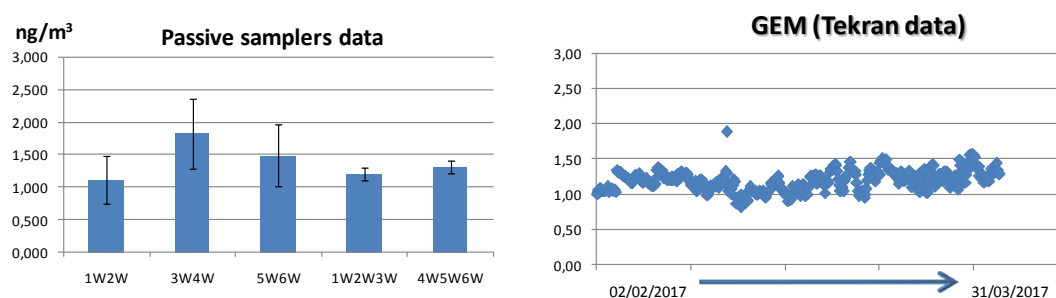


Figure 12 on the right and figure 13 on the left. Hg PASs and Tekran data collected at M.te Curcio, Italy during the first sampling campaign.

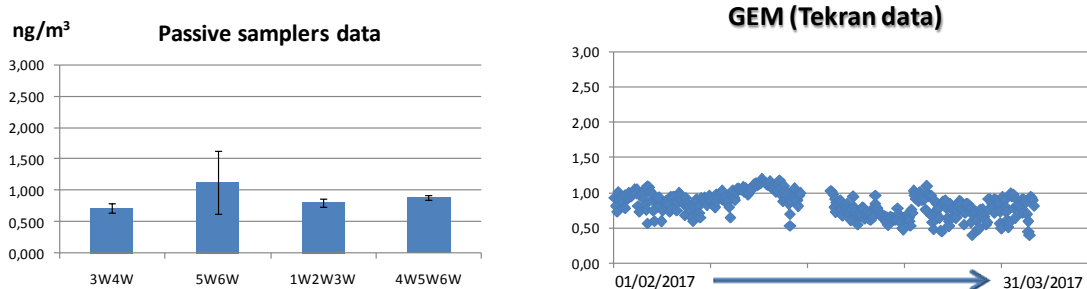


Figure 14 on the right and figure 15 on the left. Hg PASs and Tekran data collected at Bariloche, Argentina, during the first sampling campaign.

Objective 3: To develop a metrological infrastructure for emerging requirements in mercury science such as the evaluation of mercury concentrations in indoor air from the use of mercury containing compact fluorescent lamps.

Fluorescent lamps are available in many shapes and sizes. Many compact fluorescent lamps (CFLs) integrate the auxiliary electronics into the base of the lamp, allowing them to fit into a regular light bulb socket. There are two major types of compact fluorescent lamps on the EU market, screw- (integral design) in and plug-in (modular design). The sample set selected for the measurement of the emission rate consists of the most commonly used types of CFLs on the European market. Mercury in CFLs is present in different matrices depending on age of the bulb and hours of operation. New lamps contain most mercury in vapour or elemental phase, while with increasing age more and more mercury is absorbed by phosphorous powder and glass matrix.

Part of the mercury introduced into a CFL lamp is used to produce visible light while the other part interacts with the phosphor powder, the glass matrix and metal electrodes. Consequently, when the lamp becomes waste, all parts that have been in contact with Hg are contaminated by this metal. The determination of the mercury distribution inside a waste CFL includes the study of mercury vapour that has not reacted, mercury that has reacted with the phosphor powder, and the mercury reacted with metal electrodes. The variation between samples indicates that the total amount of mercury in the lamps not only varies depending on the characteristics and manufacturer but also on the year and place of manufacture and on the operating hours. However, in all cases it is evident that most of the mercury introduced into a CFL is in the phosphor powder when the lamp reaches the end of its lifetime.

The measurement infrastructure to provide traceable measurements of different mercury forms in CFLs is absent in Europe and globally. The inventory of candidate compact fluorescent lamps included the selection of CFLs based on mercury content, quantity on the market, and power consumption.

Mercury was released from a broken CFL bulb inside a closed chamber and trapped (preconcentrated) under a constant flow of Hg free gas (i.e. nitrogen, argon, air) by several adsorbers including gold traps and activated carbon traps. Trapping in oxidising solutions such as $\text{HNO}_3/\text{H}_2\text{O}_2$, KMnO_4 and $\text{K}_2\text{Cr}_2\text{O}_7$ was also tested.

The most frequently used method for pre-concentration of Hg is based on amalgamation of elemental Hg using gold (Au) traps, which is followed by thermal desorption of Hg at 600 °C and detection by CV AAS or CV AFS. The method is suitable for lower masses of mercury (up to 1000 ng). The precision of measurements is less than 15 %. For higher Hg levels (in the range of ng to µg) traps with activated carbon are widely used. Mercury adsorbed on carbon from these traps is further analysed by pyrolysis and CV AAS detection.

The main goal of this work was to develop an easy, on-line, sensitive and traceable method for the determination of released Hg from a broken CFL bulb. A procedure involving a gas tight plexiglass box with bulb crushing system, heated gold amalgamation trap coupled to a CV AAS mercury analyzer with Zeeman background correction was developed. The limit of detection depends on the gold trap capacity for trapping

Hg. Commercial silica coated Au traps can hold up to 1 µg of Hg that is also the upper detection limit of this method.

Calibration of the CV AAS detection was performed using a diluted NIST 3133 Hg standard solution where the mass fraction of mercury is certified. Certified reference materials were used to check performance of analytical methods used. The certified reference standard (SRM 3133) was also used to perform the calibration with Hg(II) reduction. Series of spiked solutions were prepared in MilliQ water after appropriate dilution. SnCl₂ was used as the reducing agent. The amounts of Hg reduced ranged from 1 ng to 100 µg. As the certified value of mercury in NIST 3133 is 9.954 mg/g ± 0.053 mg/g after opening of the ampoule the entire contents were transferred immediately to another container and working standard solutions were prepared. Working standard solutions were in the range of 1 mg/kg to 10 mg/kg, from which more dilute standards were prepared. Diluted HNO₃ was added by mass to bring the solution to the desired dilution. Dilutions were prepared gravimetrically and thus need no correction for temperature. Working standard solutions were prepared daily. To assure good quality of analytical procedures several certified reference materials were analyzed together with samples either analyzed by pyrolysis Lumex or the CV AAS Sansei technique. Measurement procedures for standard reference materials were the same as for samples. ERM-CC141 - Loam Soil (total Hg 73 ± 6 ng/g), RM 277R – estuarine sediment (total Hg 128 ± 18 ng/g) and SRM 1632 – coal (total Hg 90 ± 10 ng/g) were used. Measured values showed good agreement to certified ones.

It was found that the mercury release rate varies proportionally with temperature, which was expected because of the greater volatility of mercury at higher temperatures. One-third of the mercury released occurs during the first 8 hours after breakage. The pattern of emission, with an initial rapid release declining to a slowly decreasing rate, suggests that at least some of the mercury in the bulbs is in a form that can quickly vaporize. The slowly decreasing rate that appears to result after the first 8 hours or so may reflect the gradual release of forms of mercury less subject to volatilization, such as mercury adsorbed to surfaces within the bulb.

New lamps release more mercury vapour, whereas in older or spent (used) lamps the mercury has partitioned to lamp components. Based on different power, shape and availability on EU market several different bulbs from different producers were used to measure Hg evaporation, and amount of Hg on glass and metal parts of CFLs that are in contact with Hg within the bulb. On average, more than 66 % of Hg present in the lamp is bound to glass parts. About 18 % of Hg is bound to metal electrodes, and about 16 % of total amount of CFL Hg is evaporated to the atmosphere after bulb breakage.

The data on mercury releases indicate that breakage of CFLs will result in releases that are near levels of health concern. Real life conditions will vary from the experimental design used to develop these results, such as room size, room temperature, age of lamps, use of lamps, and type of floor covering. However, this variability will unlikely result in higher hazard quotients, because the scenarios used in this study the maximized exposures and comparisons of agency risk values were often calculated with the average maximum hourly exposures, rather than the average exposures.

Objective 4: To develop primary measurement procedures for mercury speciation in water and biota in order to improve mercury monitoring through the aquatic ecosystems and support European legislation. This will include the evaluation of transformation artefacts associated with sample collection and preparation, in order to minimise species conversion post-sampling.

Quantification of Hg species in water

Mercury (Hg) is either directly released into water bodies or introduced via deposition from air. Although the Hg concentration in water is usually low (in the ng/L up to µg/L range), it is accumulated in the food chain and can reach dangerous concentrations in predator fish such as shark or tuna. Hg is usually introduced into water in inorganic form, but it is transformed to the even more toxic methyl mercury (MeHg) by microorganisms and accumulated throughout the food chain. In biota Hg species usually bind to proteins due to their sulphur content and can also cross the blood-brain barrier. Therefore, it is of paramount importance to quantify the various Hg species in water and biota reliably. Within the project MeTra measurement procedures were developed to quantify Hg species both in water and fish as an example of biological matrix. For water, the challenge is mainly the low concentrations of various species. Methods existing in literature based on Isotope Dilution Mass Spectrometry coupled to Gas Chromatography and Inductively Coupled Plasma Mass Spectrometry (ID-GC-ICPMS) were further developed for the quantification of mercury species Hg(II) and MeHg at sub-nanogram levels in freshwater and seawater. Species were extracted from water, derivatised and extracted into an organic solvent such as hexane. After derivatisation and extraction, the

sample volume was reduced to about 100-200 μL using a gentle nitrogen stream resulting in an enrichment of 1000 to 5000 depending on the volume of the original water sample. The whole procedure is shown in Figure 4.1. As all those steps during sample preparation, separation and detection can alter the species composition, the species of interest are added to the samples enriched in one Hg isotope per Hg species (in this project Me^{199}Hg and $^{201}\text{Hg}^{2+}$, hereinafter called spike) before any sample preparation. The quantification is then achieved using double isotope dilution ICP-MS. For this, samples containing a known amount of MeHg and Hg^{2+} are prepared (hereinafter called reference) and treated the same way as the water samples taken in the field. The isotope ratios $^{199}\text{Hg}/^{202}\text{Hg}$ (for MeHg) and $^{201}\text{Hg}/^{202}\text{Hg}$ (for Hg^{2+}) are then determined in both the sample and the reference. The Hg species concentrations in the sample can then be calculated using the equation for double isotope dilution:

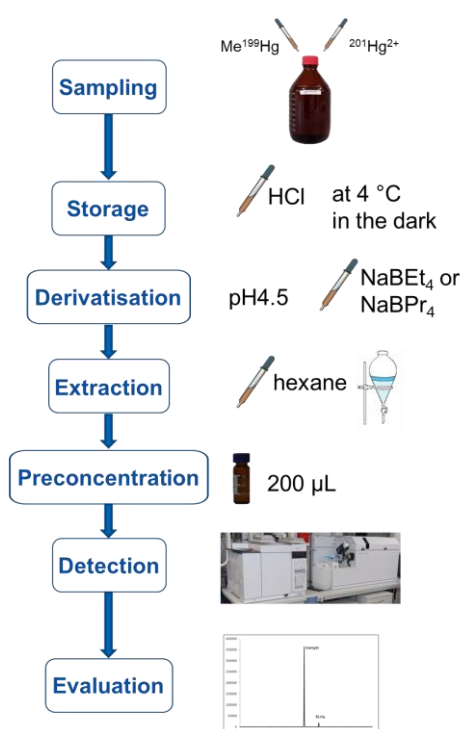


Figure 16: Scheme for derivatisation, extraction, pre-concentration, separation and detection of Hg species in water.

To handle with very low levels of concentration, the principal challenge is to minimize the blank impact, especially for Hg(II) measurements. Hence, the following critical parameters were optimized: sampling volume, pre-concentration factor, container material and cleaning procedures. The choice of bottles was important: amber bottles showed lower contamination levels. Use of 200 ml of sample is possible at sub-nanogram levels, but pre-concentration factor of 1000 is preferable. Higher blank level for Hg(II) resulted in higher bias with reference value and higher uncertainty with respect to MeHg. The methods were tested on Seine river water, seawater collected on the French north coast and Oker river water in Germany: samples were quantified for total Hg and spiked with MeHg. Results were in great agreement with the gravimetric and expected values.

In the frame of the project, a collaboration with IAEA (International Atomic Energy Agency) was established on speciation measurements in seawater. IAEA has large experience in the treatment of seawater samples and in the preparation of reference materials. Comparative measurements between IAEA and LNE have been performed in 2016 on model water, containing synthetic seawater, HA and small amount of fish CRM with endogenous mercury.

Besides the GC-ICP-MS method, a method for the continuous measurement of dissolved elemental Hg (DEM) and Hg(II) in aqueous samples was developed within the project.

Also during storage, there is a risk of species interconversion or loss. Therefore, the influence of different storage temperatures (4°C and room temperature), storage times and storage in the dark or light as well as of different stabilisation agents on the species composition were tested. It can be concluded that the best

storage conditions for Hg species in freshwater is at 4 °C in the dark using hydrochloric acid (HCl) as stabilisation agent. The samples proved to be stable for at least 35 days. Bromine chloride (BrCl), which is often used as stabilisation agent in total Hg determination, cannot be recommended for species analysis as it seems to interfere with the derivatisation.

Besides the concentration of the various Hg species in water, it is also interesting to know to which kind of particle these species are bound in natural waters. One reason is that water samples are usually filtrated before determination of total Hg in water and some Hg is possibly lost during filtration. Different approaches were used within the project. One used field flow fractionation (FFF) to separate the particles according to size (Figure 4.2) and characterise the particles using light scattering detection and UV/Vis spectrometry.

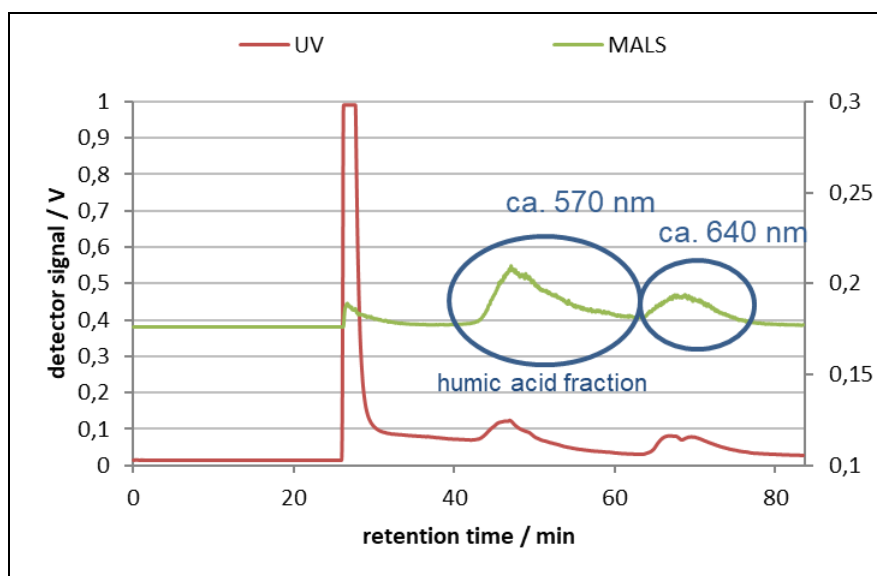


Figure 17: Separation of particles in a river water sample using field flow fractionation (FFF).

The various fractions were then collected, extracted and the Hg species content was determined using GC-ICP-MS. It was found that only a small fraction of inorganic Hg (5-14%) and a slightly higher fraction of MeHg (up to 26%) was dissolved in water. The rest was more or less evenly distributed between the two fraction that can be identified in Figure 4.2. Most likely the Hg species are bound to the organic content or sulphur containing part of the particles.

The other approach used filters with different pore sizes (0.45 µm and 0.7 µm). As also the material of the filter may influence the species composition as well as the total Hg content by absorption, glass fiber and polyvinylidene difluoride (PDVF) material were investigated. No difference between the various filter types could be observed. However, as already indicated by the FFF investigations, the main part of Hg (about 50%) is bound to particles. From these results, it can be concluded that water samples have to be used without filtration for a reliable determination of Hg or Hg species in water. To mimic particle rich water, seawater was collected in Monaco harbour, filtered and the reference material CRM IAEA 458 (marine sediment) was added. For biota containing water the same filtered seawater was spiked with CRM IAEA 452 (scallop). In both cases the fraction of Hg bound to the particulate phase was even higher (60% for biota and even 80% for sediment rich water). The applied method was able to quantify ppt levels of total Hg in highly charged synthetic waters (SPM > 25 mg/L) with very good recoveries > 80%.

In routine laboratories, mainly total Hg is measured. There are several guides and standards available for the measurement of total Hg in water samples. There are different recommendations available for storage times of water samples intended for total Hg measurement. Two most essential standard methods are the ISO 17852 and EPA Method 1631. The first one suggests that samples should preferably be preserved on-site using HCl, potassium bromide (KBr) and potassium bromate (KBrO₃) reagents. ISO 17852 suggests also carrying out total Hg measurement within seven days of collection. According to EPA Method 1631, the samples for total Hg measurement should be either preserved or measured within 48 hours of collection. EPA 1631 suggests preserving samples with BrCl reagent containing HCl, KBr and KBrO₃. After preservation, the samples are stable for up to 90 days from the date of collection. Therefore, the different

storage conditions recommended were applied using enriched ^{196}Hg stable isotopic reagent to test the stability of the Hg concentration in water. Two stability study campaigns for total Hg in natural water were carried out with the samples having different total organic carbon (TOC) concentrations, different storage temperatures, and storage times up to three months.

The first campaign was carried out in 2016 using the water samples from Lake Keravanjärvi (60°37'06.1"N 25°05'40.9"E) and River Vantaa (60°15'26.1"N 24°58'36.5"E). After sampling, the sample water was acidified with HCl and half of the sample batch was spiked with enriched ^{196}Hg isotope (enrichment 52%, natural abundance 0.15%). The total Hg concentration levels were between 4-10 ng/kg. The samples were divided into 112 borosilicate bottles. Cold vapor ICP-MS (CV-ICP-MS) technique (Perkin Elmer Elan DRC II) with external calibration was applied for total Hg measurements of the spiked and non-spiked samples. The measurement method is accredited according to ISO/IEC 17025 standard.

The second stability study campaign was carried out in 2017 with the water of River Kymijoki (60°52'23.9"N 26°36'54.7"E) using exact matching double isotope dilution CV-ICP-MS method for measurement of mass fraction of total Hg. The concentrations of the total Hg were 2-4 ng/kg. TOC concentration and colour of the water were lower than in River Vantaanjoki in the first campaign. The sensitivity of the instrument (ICP-MS instrument Thermo iCAP Q with cold vapour unit from Elemental Scientific) applied in this campaign was better than during the first stability study campaign. Expanded measurement uncertainty was estimated to be 2% at 4.5 ng/kg mass concentration level.

As a conclusion, total Hg was noticed to be stable for at least 78 days, if samples were preserved with HCl (5 ml per 100 ml sample) and samples were refrigerated in the dark. BrCl treatment was not necessary at the time of sampling. It can be added before measurement to remobilise the Hg adsorbed to the walls. 50 ml borosilicate glass bottles with PTFE sealed screwcaps were used throughout the study.

Quantification of Hg species in biota

In biota, the main challenge is the complete extraction of the Hg species from the matrix without changing the species composition.

To develop and validate measurement procedures for Hg species in fish, four different fish materials were prepared within the MeTra project. Two fresh fish samples (pike and roach) and two Environmental Specimen Bank (ESB) samples (bream) were sampled, processed, characterised and distributed within the JRP consortium. The materials consisted of whole fish (roach, bream) and fillet (pike). The sampling and preparation is described in more detail below. The materials vary in total Hg concentration (approx. 7 – 280 µg/kg) and fat content (approx. 0.8 – 5.3 %). Half of the material was gamma irradiated.

Fillets and whole fish samples (pike, roach and bream) were processed under defined conditions such as freeze drying, cryo milling and homogenisation. The resulting materials were characterised regarding the fat content as well as the concentrations of total Hg and MeHg, respectively.

Different methods were implemented by the participants for total Hg and Hg species measurements: acidic, alkaline and enzymatic extraction in combination with ID-GC-ICP-MS as well as alkaline extraction in combination with GC-AFS. Microwave assisted acidic and alkaline extraction methods as well as ethylation and propylation for derivatisation were tested for sample preparation. It was found that alkaline extraction gave the better uncertainty while acidic extraction led to better recovery. Acid extraction has also the advantage to not use toxic reagents such as tetramethylammonium hydroxide (TMAH) in the extraction steps. Heating, filtering, purification steps were avoided as possible in order not to change the original speciation.

For validating the method, different reference materials with different origin, fat content and mercury level were used, such as NIST 1947 (fresh fish CRM), IAEA 407 (mussels) and the processed pike material. Limits of detection and quantification were determined within the validation procedure as well as the accuracy and the precision.

For the separation of Hg species from biological matrices and tissues common acidic or alkaline digestion strategies or solid/liquid extractions were used. Moderate temperatures and pH values are essential to avoid the loss of Hg and possible transformation reaction of Hg species. Besides, enzymes such as trypsin, protease type XIV, and/or bile salt-dependent (bsd) pancreatic lipase have been used to extract various compounds or pollutants from different biological matrices such as fish. The so called enzymatic hydrolysis has the advantage that enzymes act only on specific chemical bonds of proteins or fat and do not modify Hg species. An enzymatic digestion method was evaluated and established. Here two different enzymes were applied to digest the complex fish matrix in terms of lipids and proteins. The results were compared with the common extraction methods described above.

Commercial fresh fish (tuna fillet) was used to characterise possible loss or transformation of Hg species during processing. Two different strategies for processing were elucidated: pureeing – cryo-milling – freeze

drying vs. pureeing – freeze drying – cryo-milling. For each processing level, the Hg content was controlled and the best processing method was used to produce a spiked fresh fish material with isotopic labelled Me¹⁹⁹Hg for the spiking experiments.

a)

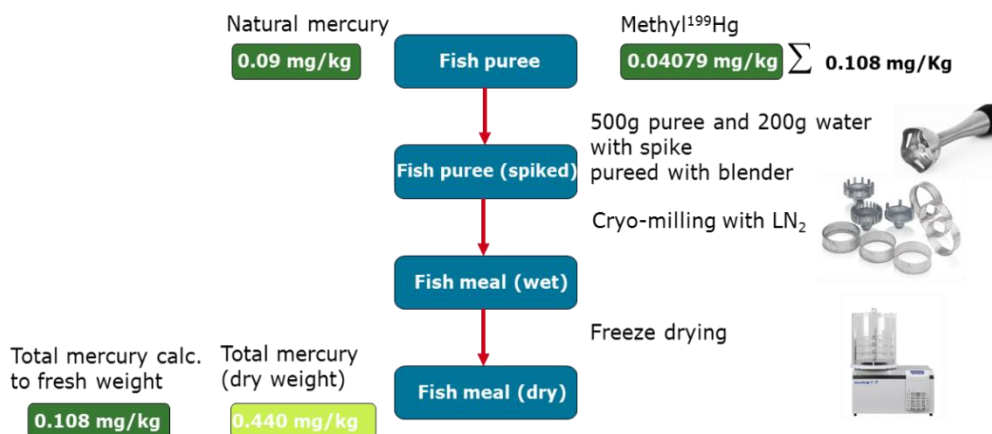


Figure 18: a) Spiking experiments of fresh fish material with Me¹⁹⁹Hg to investigate possible losses or species transformation

b)

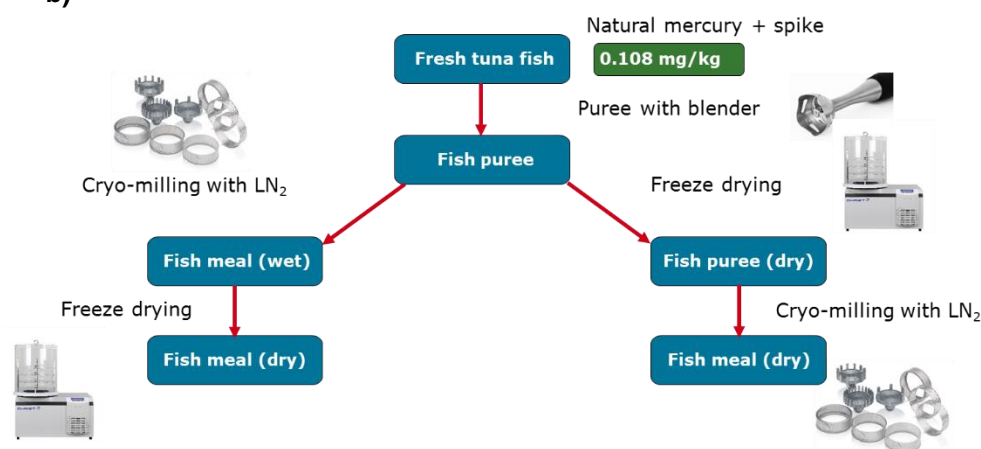


Figure 19: b); b) Different strategies for processing of fresh fish samples.

For an accelerated microbial activity selected fish samples were stored under higher temperature (room temperature and 60°C). Subsamples were taken after distinct time intervals. These samples with a higher microbial and enzymatic activity were characterised regarding to the total Hg and MeHg content in comparison to a reference sample which was stored at -20°C. On the other hand, sample extracts from the matured and stressed materials were elucidated by GC-MS regarding to possible transformation products or metabolites.

For verification of measurement results and validation of measurement procedures matrix reference materials are needed. Therefore, samples were collected and two fresh fish and two Environmental Specimen Bank samples were prepared to check for their suitability for this purpose. The aim was to source material with different concentration levels and different fat contents. The matrices were roach (whole fish material), pike (filet) and bream (two whole fish materials, one with a Hg contamination close to the Hg environmental quality standard (EQS) level). These materials were applied to test the methods developed within this project on different real environmental samples and to compare the results of the different partners. As available reference materials are often delipidated they do not mimic realistic environmental samples.

In March 2015, a fish sampling campaign was performed at Lake Stechlin (Germany) to collect pikes and roaches. 15 kg of pike filet and 15 kg of roaches were collected. During sampling the fish were measured, weighed and scales and operculum were taken for age determination. Whole roaches and pike filets were cut into pieces and immediately stored under liquid nitrogen.



Figure 20: Fish sampling



Figure 21: length determination



The pike and roach samples were freeze dried (water content: 70-78%) and cryomilled to a particle size < 200 µm (for more than 80% of the particles).

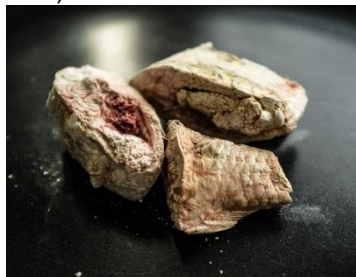


Figure 23: Whole roach cut into pieces **Figure 24: roach after freeze-drying** **Figure 25: pike filet after freeze drying**

From the German ESB bream whole fish material (collected 2013) was used. The two bream materials were already cryomilled (without previous freeze drying) and stored under liquid nitrogen. The freeze drying was performed afterwards by one project partner.








All four materials were homogenised using a drum hoop mixer. Samples were portioned in 4 g aliquots via cross riffing. Half of the three fish materials were gamma-irradiated (^{60}Co , 8 kGy).



Fish material assembled in ~4 g portions; left to right	
Bream_2013_F3	ESB-sample, one fish, whole fish
Bream_2013_HOM	ESB-sample, homogenate of seven fish, whole fish
Pike_2015_HOM	Fresh fish sample, homogenate of filets of 14 pikes
Roach_2015_HOM	Fresh fish sample, homogenate of 48 fish, whole fish

Figure 26: Final four fish samples: Bream_F3, Bram_HOM, Pike_HOM, Roach_HOM
Total Hg and fat content were determined. Figure 27 on the left Following table shows total Hg concentration, residual moisture, average age of the used fish, fat content and how much samples were available to the partners.

Figure 28: Characterisation of various fish samples provided for investigations of Hg species in fish.

FISH		Hg wet weight [µg/kg]	Hg dry weight [µg/kg]	Residual moisture [%]	Average age [years]	Fat content [%]	Available amount
Bream_2013_F3		~7*	~25*	7,5	n.d.	5,0	30 x 4 g
Bream_2013_HOM		~16*	~60*	6,1	n.d.	5,3	160 x 4 g
Bream_2013_HOM_γ		~16*	~60*	6,5	n.d.	5,3	85 x 4 g
Roach_2015_HOM		~45*	~200*	5,3	7,3	1,7	190 x 4 g
Roach_2015_HOM_γ		~45*	~200*	5,9	7,3	1,7	185 x 4 g
Pike_2015_HOM		~280*	~1300*	3,4	5,6	0,8	90 x 4 g
Pike_2015_HOM_γ		~280*	~1300*	4,6	5,6	0,8	85 x 4 g

γ: gamma irradiated (⁶⁰Co 8 kGy, Helmholtz-Zentrum Berlin)

* total Hg determination using a direct mercury analyser (DMA)

Samples were stored at -20°C before distributing them to the partners. Short-time stability at room temperature (7 days) was evaluated for shipment at room temperature. Samples were extracted using alkaline microwave extraction and the MeHg content was measured using GC-ICP-MS. Quantification was done with species-specific isotope dilution. Fig. 7 shows Hg concentration as dry weight.

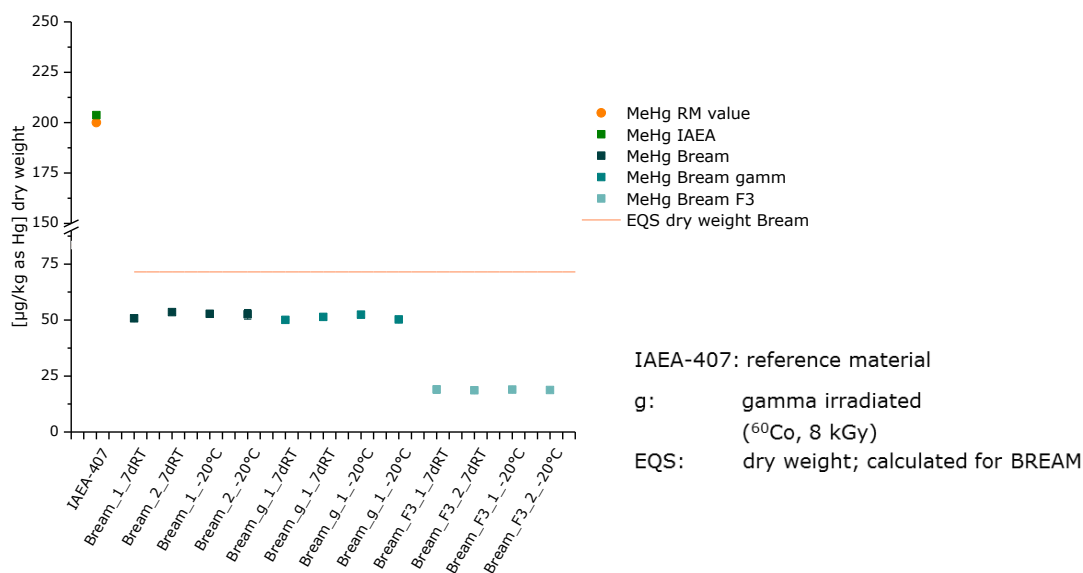


Figure 29: Total Hg content in the various fish materials stored at different temperatures.

The different methods implemented by the partners and described before, were applied to characterize the fish material (Figure 4.8).

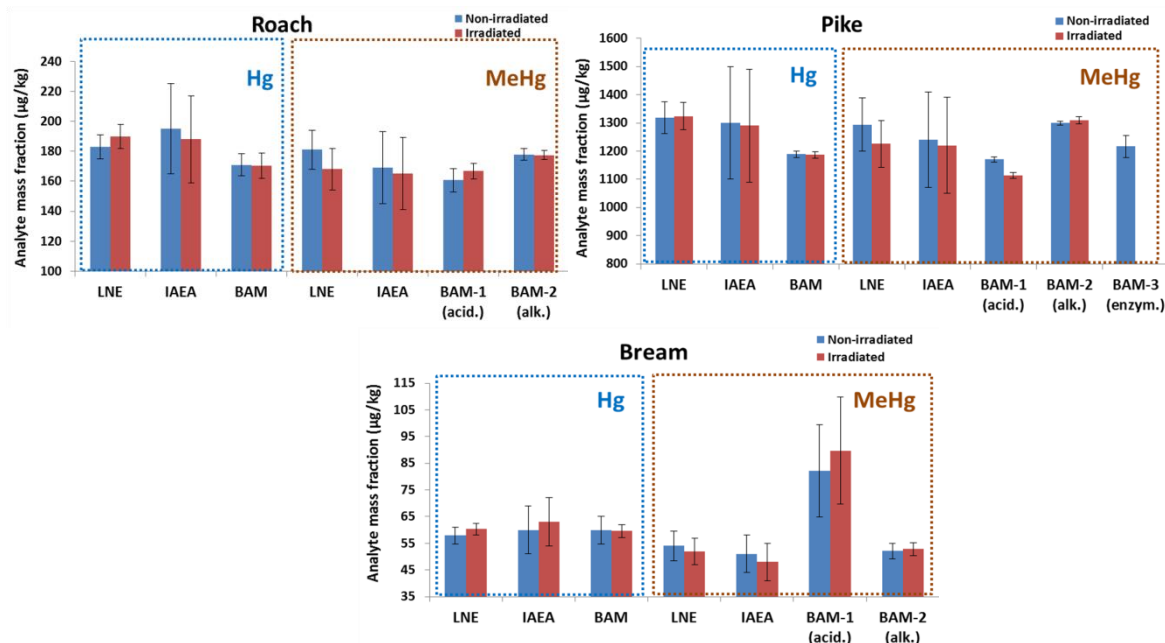


Figure 30: MeHg and tHg measurement in roach, pike and bream fishes

For roach fish, the average value of total Hg was about 170 µg/kg, mostly constituted of MeHg. Results among partners and methods were coherent. Higher uncertainties were associated to the alkaline extraction GC-AFS method that uses external calibration instead of isotope dilution. BAM alkaline extraction method seems to overestimate the MeHg content with respect to total Hg. For pike fish, results are more dispersed in the range between 1150-1300 µg/kg. The enzymatic extraction method was here applied and results were consistent to the BAM reference method, which is the acidic extraction method, and to total Hg measurement. For bream fish, whole fish from Specimen Bank, collected in 2013, the Hg content is lower, about 60 µg/kg. The results are coherent among partners and different methods with the exception of acidic extraction for BAM, which gave surprisingly overestimated values. For each sample the γ -irradiation as sterilization approach was tested on part of the fish to see if there is an impact on speciation: the results show no impact of irradiation.

In conclusion different methods to quantify total Hg and MeHg in fish samples have been developed and compared: these methods have been successfully applied to the characterization of three fish samples collected in collaboration with the German ESB. The robustness of the methods has been proven and complete uncertainty budgets have been established.

Objective 5: To develop and accurately perform bulk and compound specific isotope signature measurement methods for Hg(0) and Hg species

Although separation of different Hg species can be achieved by many analytical techniques, including gas chromatography, liquid chromatography, electrophoresis, dialysis and selective chemical reactions, the determination of natural variations in the isotopic composition of Hg species remains a significant analytical challenge. Yet, these data were shown to be a very useful tracer in geo- and environmental sciences. Natural variations in the isotopic composition of Hg species have been used for interpreting natural mechanisms of interconversion between Hg species and provided valuable insight into distribution of mercury between different biogeochemical compartments.

Key challenges in obtaining accurate and precise Hg isotope ratio data at environmentally relevant concentration levels include measurements at low Hg concentration, significant potential for artificial Hg isotope fractionation of analyte due to its non-quantitative conversion during sample preparation steps, matrix interferences and bias induced by instrumental mass discrimination. In order to achieve the above objective the work undertaken was aimed at the following: (1) development and validation of methodology for the accurate and precise bulk and compound specific Hg isotope ratio measurements in fish tissues by multi-collector ICP-MS; (2) development of strategies to correct for instrumental mass bias of Hg isotopes; (3)

assessment of the influence of sample introduction techniques, sample matrix and instrumental parameters on the instrumental mass bias; (4) evaluation of the potential occurrence of mass-independent isotope effects during sample preparation and analysis.

Evaluation of the performance of different sample introduction systems and instrumental parameters for precise and accurate Hg isotope ratio measurements by MC-ICPMS

The sample introduction systems evaluated included conventional pneumatic solution nebulisation, Apex-Q desolvating inlet system and cold vapor generation MC-ICPMS. First two systems were selected because they have an advantage in that no chemical transformation of mercury occurs during sample introduction. Pneumatic solution nebulisation is the least sensitive method but can be advantageous when SI traceable Hg isotope amount ratios need to be determined due to minimum extent of instrumental bias introduced and low measured uncertainty. The Apex-Q desolvating system was found capable of providing up to 8 time increase in sensitivity for mercury and up to 10 time increase for internal standard, thallium, relative to those achieved by using pneumatic solution nebulisation. By using this technique it is possible to determine Hg isotope ratios in solutions with concentration of mercury as low as 25 ng g⁻¹ and with in-run standard deviation better than 0.2 ‰ per u. The most sensitive sample introduction technique is cold vapour generation, capable of providing signal enhancement with a factor of 50 and higher relative to pneumatic solution nebulisation. Quantitative release of Hg in Cold Vapour Generation (CVG) reaction chamber is an important requirement in the measurements as incomplete conversion of Hg species into purgeable elemental Hg can be accompanied with Hg isotope fractionation. The effect of different measurement parameters and conditions on the magnitude of instrumental mass bias was investigated.

It was shown that normalisation to internal standard (TI, NIST SRM 997) was an efficient strategy in correcting for drift in instrumental mass discrimination and improving repeatability of measured Hg isotope ratios. Different methods of implementing the normalisation to TI were investigated, including the Rayleigh law, exponential law, power law, linear law and the regression model. The exponential model was found to be superior in performance over the other models in terms of accuracy and precision (Figure 4.1). No mass-independent Hg isotope fractionation was observed during sample preparation, storage and measurements of bulk Hg isotope ratios.

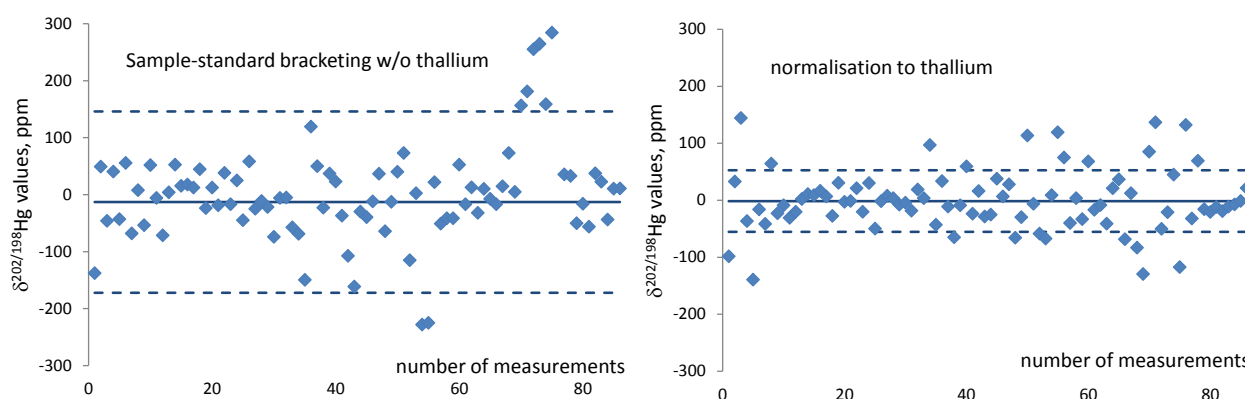


Figure 31 ²⁰²Hg/¹⁹⁸Hg isotope ratio measurements of NIST SRM 3133 standard solution at Hg concentration of 10 ng g⁻¹ by cold vapor generation MC-ICPMS with and without online normalisation to TI internal standard using the exponential model. Solid lines are the arithmetic mean values; dotted lines are standard deviation ($\sigma=1$). It can be seen that normalisation to the internal standard significantly reduces drift in the measured isotope ratios.

Development of an on-line pre-concentration method to determine Hg isotope ratios at ultra-trace concentration levels in liquid samples by cold vapor generation and dual gold amalgamation MC-ICPMS

The cold vapor generation dual gold amalgamation MC-ICPMS coupling allows the isotopic analysis of bulk Hg in samples with Hg concentration in solution down to 5 ng L⁻¹ and sample volume of 90 ml (450 pg of Hg). This represents a decrease in Hg concentration of a factor 200 as compared to the conventional cold vapor generation MC-ICPMS technique and will allow Hg isotope ratio measurements in liquid samples with very low concentration of the element such as seawater and atmospheric precipitation. The developed method is fully automated and allows rapid sample treatment. The short transient signals can be efficiently treated

using data reduction strategy based on linear regression analysis of measured isotope data for analyte (Hg) and internal standard (TI). Figure 5.2 shows the principle of measurements by CVG-DGA/MC-ICP-MS.

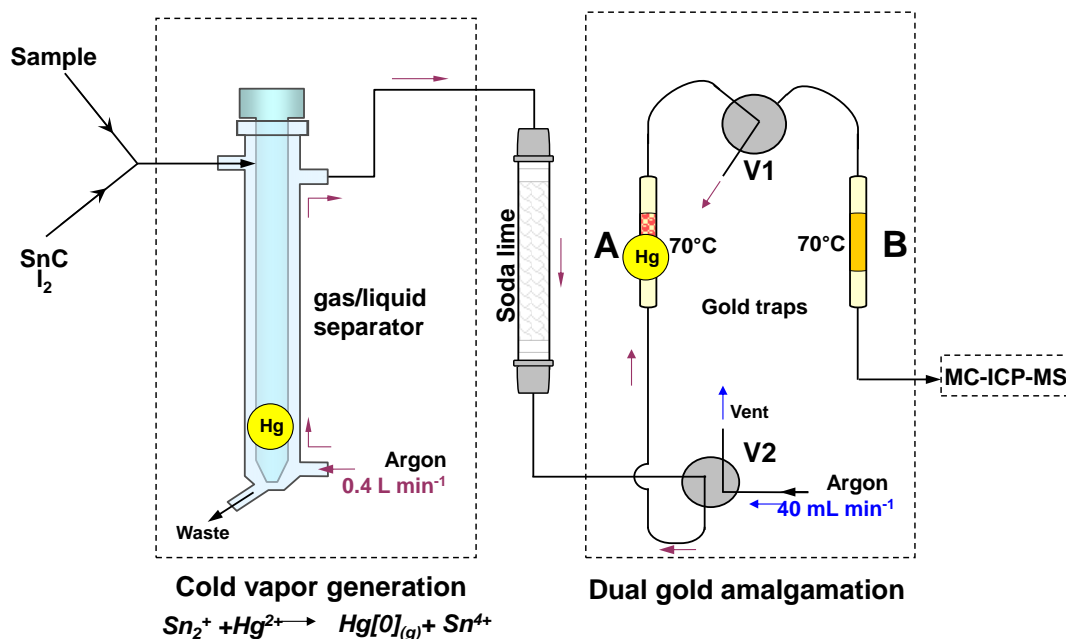


Figure 32. Illustration of the CVG-DGA/MC-ICP-MS coupling. GLS is the Gas Liquid Separator, A is the gold sand filled trap, B is the pure gold trap, and V1 and V2 are two electronic valves.

Development of a new methodology for precise and accurate species-specific Hg isotope ratio measurements in fish tissues by using HPLC and cold vapor generation (CVG) MC-ICPMS

The developed methodology involved improved Hg extraction procedure, HPLC separation and MC-ICPMS measurement protocols for determination of Hg isotope ratios in methylmercury and inorganic Hg. It was shown that off-line measurements of Hg isotope ratios were a robust and reproducible way of obtaining species-specific Hg isotope data. In contrast, on-line coupling of HPLC to CVG MC-ICPMS was subject to additional bias in measurements due to incomplete conversion of methylmercury, required for CVG, and mass-independent isotope fractionation in the case when photochemically assisted dissociation of methylmercury was employed. An efficient approach for minimising the matrix effect from sulphur based HPLC complexing agents through oxidation was developed. An approach for estimating the isotopic composition of inorganic Hg based on mass balance calculations was suggested in the cases when inorganic Hg constitutes only a small fraction of the bulk mercury present in fish tissues.

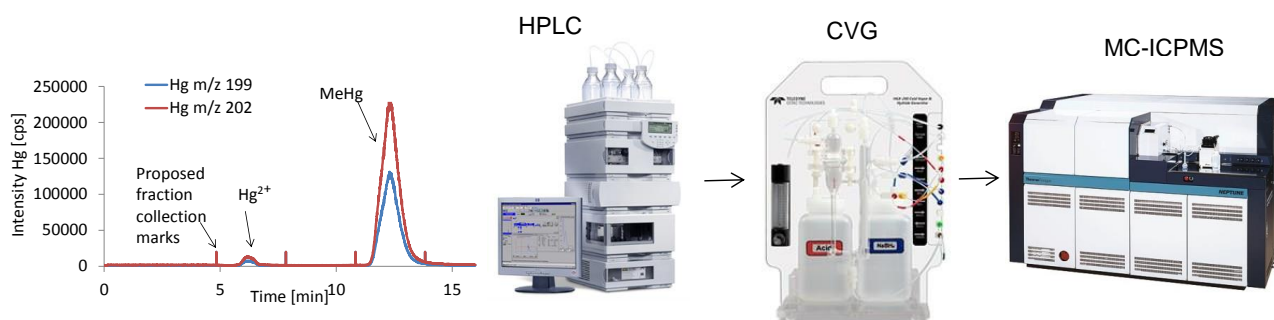


Figure 33. Schematic illustration of Hg isotope ratio measurements by HPLC MC-ICPMS

A significant improvement in developing metrological infrastructure for accurate and precise Hg isotope ratio measurements has been achieved. A thorough investigation of instrumental operating parameters provided a deeper insight into the causes of instrumental mass bias of Hg isotopes. It was shown that data reduction strategy based on normalisation to the internal standard provided the best means of minimising the drift in the magnitude of instrumental mass discrimination over a measurement session.

For the first time, a pre-concentration method was developed to determine Hg isotopic composition at ng L⁻¹ concentration level in liquid samples. This method opens up the unique possibility of metrologically traceable Hg isotopic analysis in seawater and atmospheric precipitation.

Also for the first time, a methodology for species-specific Hg isotope ratio measurements in fish tissues by HPLC-CVG-MC-ICPMS was developed. It addressed technical challenges of the measurements due to non-quantitative dissociation of methylmercury and matrix effect and allows determination of natural variations in the isotopic composition of Hg in methylmercury and inorganic Hg. It has been shown that Hg isotopic data can be useful in identifying sources and transformation of Hg when applied to robust sample archives such as fish tissues from environmental specimen bank of Federal Environmental Agency of Germany.

Objective 6: To develop and accurately perform ratio measurement for light isotopes (C, N, H, O) in organo-Hg species in order to detect contaminant transformations and migration

Determination of the isotope ratios of the elements bound to mercury in organo-mercury compounds has the potential to yield information regarding the source of the organic moieties within the environment, as well as affording more variables with which to distinguish the origin of organo-mercury compounds for pollution.

Measurement of light element isotope ratios in organo-mercury species has been investigated using the carbon isotope ratio in methyl Mercury (MeHg) as an example. Carbon isotope ratios are typically reported as so-called isotope-delta values relative to the Vienna Pee Dee Belemnite (VPDB) measurement standard ($\delta^{13}\text{C}_{\text{VPDB}}$ values). These $\delta^{13}\text{C}_{\text{VPDB}}$ values are measured using isotope ratio mass spectrometry (IRMS) on CO₂ gas which therefore necessitates conversion of the analyte material/compound into CO₂. For the analysis of MeHg isolated from fish tissues, this is achieved via a combustion process following gas chromatographic separation of the MeHg from other extracted analytes (i.e. gas chromatography-combustion-IRMS, GC-C-IRMS). The presence of mercury in the analyte, at potentially high concentration necessitated modification of the usual GC-C-IRMS instrumentation by the addition of a gold trap immediately after the combustion reactor to ensure that gaseous mercury or mercury compounds could not be vented into the laboratory.

Chromatographic separation of MeHg from other organo-Hg compounds was achieved using two distinct methods. Firstly, MeHg and other organo-Hg compounds were derivatized using sodium tetraethylborate to form methylethylmercury (MeEtHg) and other similarly ethylated species. These could then be chromatographically separated using a 100 % dimethyl polysiloxane coated capillary column. Alternatively the organo-mercury compounds could be directly separated without derivatization using a similarly polar GC column, but with a pre-treatment involving the injection of methanolic HBr prior to the organo-mercury compounds. Both methods resulted in baseline separation of MeHg from other organo-Hg compounds, which is a prerequisite for accurate and precise isotope ratio determination.

The ethylation reaction was modified from that applied during Objective 5 in such a way that the process could be carried out in a single vial, precluding the need to transfer the volatile derivatized mercury species. Briefly, the aqueous MeHg analyte solution, the sodium tetraethylborate (in THF) and the hexane extraction solvent were all added to a GC vial, then shaken; meaning that the derivatized MeEtHg would be present in the upper hexane phase following the completion of the reaction. This phase could then be sampled directly using the autosampler syringe of the GC.

Derivatization of MeHg to MeEtHg necessitates the correction of measured isotope ratios for the presence of carbon from the derivatization agent. This is generally achieved via a mass-balance approach where the carbon isotope ratio of the derivatization agent is determined and then used to correct the measured isotope ratio of the derivatized compound. In the case of the derivatization approach employed in this work, the sodium tetraethylborate was obtained as a solution in THF precluding direct determination of the carbon isotope ratio (given that THF also contains carbon). This was overcome by the use of inorganic Hg solutions either as standards, which would be derivatized within the same batch to Et₂Hg. Provided that the derivatization went to completion and that the derivatization agent was present to significant excess, then the carbon isotope ratio of the Et₂Hg should be equal to that of the ethyl groups within the sodium tetraethylborate.

This approach to correcting for derivative carbon was tested using commercially available MeHg and iHg solutions. The iHg was either used as an internal standard (and thereby directly added to the MeHg solution prior to derivatization) or as an external standard and prepared/derivatized/analysed within the same sequence as the MeHg. There was no difference within measurement uncertainty between the values obtained using these two approaches. Conversion efficiency of MeHg separated by GC to CO₂ via the combustion reactor was tested by comparison of peak areas for the obtained CO₂ from MeHg to compounds known to exhibit favourable conversion characteristics.

The effect of the correction for derivative carbon on measurement uncertainty was also investigated. Increasing the number of derivative carbon atoms was found to increase the measurement uncertainty by approximately 0.3 permil for each additional carbon atom. The selection of derivatization approach (e.g. between use of ethylation and propylation) is therefore a balance between increasing the measurement uncertainty from the correction for derivative carbon and increasing the signal amplitude which results in better precision of replicate injections.

Calibration of measured carbon isotope ratios (typically relative to a working standard gas) to the VPDB scale requires the use of two reference materials of known isotope ratio, the first to anchor results to the VPDB scale and the second to account for any scale contraction or expansion effects that may be present. Three commercially available MeHgCl solids/aqueous solutions were characterised by flow injection-chemical oxidation-IRMS (FIA-CO-IRMS) against commercially available secondary reference materials with traceability to the VPDB scale. Two of the MeHg solutions were very distinct in carbon isotope ratio which afforded a suitable isotope ratio calibration range for use as calibration materials during GC-C-IRMS analyses.

Measurement uncertainty considerations related to scale calibration of measured carbon isotope ratios were also investigated. These included the estimation of the increase in measurement uncertainty associated with calibration of measured isotope-delta values by extrapolation of the calibration range afforded by reference materials of known isotope ratio. Each each permil increase in distance from the calibration range was shown to result in an increase in the combined uncertainty of the scale-calibrated $\delta^{13}\text{C}$ value of 0.01 ‰.

The mass of carbon injected required to produce the minimum 1 V signal was determined via the analysis of decreasing amounts of 1000 ppm aqueous MeHg standard solutions. For the approach using ethylation, approximately 30 ng of carbon on column was required, which is the equivalent of 5 µg of carbon from MeHg or 100 µg of MeHg in total for each material being analysed. Further changes to the derivatization approach can be estimated to reduce this amount to 20 µg of MeHg: these changes include reducing the volume of hexane added during derivatization, or the use of a propylation derivatization reaction. However, even with such an improvement in sensitivity, the applicability of this method to environmental samples is limited: For example, the NIST 1947 CRM contains $0.233 \pm 0.010 \text{ mg Kg}^{-1}$ MeHg and each unit is 8g and therefore 11 units would be required to obtain the 20 µg of MeHg. For the GC-C-IRMS approach not using derivatization, the amount of MeHg required during each analysis is three times higher as there is no derivative carbon contributing to the signal making this approach less useful. None of the ESB samples distributed within this project contained sufficient MeHg to allow analysis of carbon isotope ratios of MeHg.

This limitation of sensitivity is inherent to the analysis of compounds such as MeHg where the moiety or element of interest is only a small percentage of the compound by mass. While the carbon isotope ratio of MeHg can be determined using the methods developed during this objective, the amounts of fish material required greatly limit the practical application of the methods. Nonetheless in cases where there is significant mercury pollution in the natural environment, there may be instances where the developed methods can be used to glean additional information regarding MeHg from environmental samples.

4 Actual and potential impact

This JRP put in place an underpinning traceability framework for the measurement of mercury in all environmental compartments which will have global impact on the quality, comparability of measurement results. This will provide SI traceability for all Hg measurements and quantities required to be measured by current European and international legislation. The framework will ensure the comparability of measurement across Europe and the world and between environmental compartments, the ability to accurately assess changes over time, and assurance of the coherence between any different measurement methods used.

The main actions to reach this impact are described below.

Dissemination activities:

Stakeholder Committee

A Stakeholder Committee (SC) has been created within the first month of the project, including 12 members. These include representatives from the industrial sector (energy sector, fluorescent lamp production, cement industry, gas and oil industry), policy making representatives, academic sector, non-European NMIs, and instrument manufacturers. The active participation of the SC members in the project meetings has shown the external interest for the outputs produced by the project. With several of these SC members, active collaboration has been built during the project (see below, paragraph Stakeholder Engagement).

Scientific publications

The project has generated 22 high impact publications in key journals and some others are in preparation. These incorporate the significant scientific outputs of the project. A list is provided in Annex 1.

Conferences

In the framework of the MeTra project, two Special Sessions were organized at the International Conference on mercury as a Global Pollutant (ICMGP): the first one, entitled "traceability for mercury measurements" was held in Jeju Island, Korea, in July 2014, and the second one, entitled "Comparable measurement results for mercury analysis and speciation", was held in Providence, Rhode Island, USA, in July 2017.

In both cases the conference attendees came from about 50 different countries and the total number of participants was around 1000. In particular for the second event, there were 8 oral presentations and 18 posters. The oral session was attended by over 110 participants, which filled the session room completely. Several MeTra partners attended the conferences. The rest of the people were from the academia and industrial sector, particularly the instrument producers. This was a success beyond the expectations and clearly shows how the project outcomes were useful for the external audience.

In addition, more than 30 presentations (oral and posters) have been presented in international conferences.

Workshops and trainings

The first stakeholder workshop was held in conjunction with the kick-off meeting in Paris on the 5th November 2014. About 30 participants attended the workshop. The main objective was to fine tune the needs and ideas of the potential stakeholders at the start of the project to maximise the JRP impact and to ensure that proper relationships between participants' channels of communication were established from the initiation of the JRP. The workshop was structured in three main sessions, according to the JRP structure, i.e. mercury measurements in air, mercury speciation analysis in water and biota and isotopic mercury measurements in biota. For each session, invited speakers and project partners gave technical presentations. The invited speakers, were from USEPA (USA), NIST (USA), JRC-IRMM (EC), the Technical University of Braunschweig (Germany), the University of Bremen (Germany) and the Health and Safety Laboratory (UK) and presented the state of the art on the achievements in mercury measurements. The importance of reliable measurement results in order to understand the biogeochemical cycle of mercury and to contribute to address the needs related to the implementation of the Minamata Convention was stressed.

A second workshop was organised on "Metrology of Hg measurements". This was organised as preconference one day workshop at the 13th ICMGP in Providence, USA. The workshop was composed of two parts: a training course on "Issues and Solutions: Quality Assurance in Laboratory Measurements" and a workshop on "GEOTRACES intercalibration exercises for Hg species in seawater". There were about 30 participants. 3 MeTra partners and collaborators such as NIST, USEPA and IAEA also actively participated in the training.

A training course entitled "Hyphenated techniques to MC ICP/MS", organised by REG2(CNRS) with the support of other project partners, was held from 30th November to 2nd December 2016 in Pau, France. The audience was mainly young researchers. About 20 students attended the training course.

The final stakeholder workshop on “mercury monitoring and regulation” was held in Berlin on the 4th and 5th April 2017. The workshop aimed to combine science, technology, policy and regulation and gathered about 60 participants.

The essential outcomes of the workshop have been:

- Monitoring and support of environmental regulation: need to fill the gap between the regulation requirements and what is possible with regards to monitoring of mercury in the environment. Improved discussion is needed between regulation, research and routine laboratories. National Metrology Institutes (NMIs) play an important role in these discussions providing focussed research and elaborating appropriate answers to researchers and regulators.
- Research field: need to better understand the mercury cycle, e.g. mechanism, transportation and fate in the environment. More data are needed to improve the modelling approaches. Novel methodologies have the potential to distinguish between natural and anthropogenic sources of mercury, to characterize anthropogenic emission scenarios and quantify mercury species in the environmental compartments. For example mercury stable isotopes are an important emerging analytical technique to understand the geochemical cycle of mercury. To be used in regulation, guidelines on how to interpret isotopic signature data need to be developed. A database of reference isotopic values for various environmental and human samples is needed. Projects such as MeTra play an essential role to improve the reliability of the data and provide guidelines.
- Data comparability: Overall, more data are needed on mercury in the environment and in the human population to ensure comparability. Analytical methods with lower uncertainties are needed as well as the development of standards. For example measurements in air are prone to high uncertainties due to handling of equipment and different equations that are applied to calculate the mercury mass fraction. Therefore the MeTra outcomes are fundamental to ensure better comparability and traceability for mercury measurements.

Early impacts

Metrology achievements

The project aimed to address a number of limitations in primary standards to support the implementation of the Minamata convention and of the European Directives in support of air and water quality monitoring. This has been done cooperatively and ensuring that the underpinning SI traceability objectives were achieved.

One of the main metrological achievements of this project is the development and implementation of a new primary measurement standard to ensure the traceability chain for mercury vapour measurement based upon gravimetry. This will ensure the comparability of measurement across Europe and globally, the ability to assess accurately changes over time with small uncertainties, and assure measurement coherence between different environmental compartments and between the different measurement methods used.

Scientific and technical innovation

The project has developed innovative methods and instrumentation to deliver its objectives. As a major example, the knowledge gained in this project has brought to the development of a low-cost prototype passive sensor based on nanostructured sensing materials with proven records for robustness, high sensitivity, ability to detect ultra-trace levels, and not requiring power supply and gas carrier, nor highly qualified technical expertise. The novel passive samplers developed for measuring the most abundant Hg form in ambient air have been employed within the GMOS network across 5 selected GMOS monitoring sites at different latitude, altitude and thus in different conditions. This represents a step forward towards a reliable monitoring of mercury in air.

Stakeholder Engagement

The large involvement of researchers and stakeholders outside of the project Consortium is one of the major impacts created by this project. The main synergies created are described below.

Global Mercury Observation System

The Global Mercury Observation System (GMOS) is a coordinated global observational network which has been established in the framework of several EU initiatives (FP7 GMOS project) and international programmes i.e., UNEP F&T, Group on Earth Observations (GEO), Task 09-02d “Global Monitoring Plan for Atmospheric Mercury”/GEOSS) and conventions (UNECE-LRTAP TF HTAP) aiming to build a global observation system for mercury. GMOS includes more than 40 ground-based monitoring sites distributed in the northern and southern hemispheres with a central QA/QC database to harmonize and enhance integration of both mercury and air quality measurements from different national and regional networks assuring a full comparability of site specific observational datasets. Harmonized Standard Operating Procedures (GMOS-SOPs) as well as common Quality Assurance/Quality Control (QA/QC) protocols have been indeed addressed based on available European Standard methods (EN-15852 and EN-15853) and the Canadian CAMNet/CAPMoN and US-AMNet SOPs to ensure the collection of high quality, intercomparable atmospheric Hg measurements at all GMOS sites in accordance with the measurement practice adopted in well-established regional monitoring networks and based on the most recent literature. A great novelty of the network is the centralized system (GMOS-Data Quality Management, G-DQM) based on the GMOS-SOPs able to acquire atmospheric Hg data in near real-time and, assure and control quality of collected Hg datasets. This system introduced a service approach that facilitates real-time adaptive monitoring procedures, thus being essential in preventing the production of poor-quality data. The SOPs developed within objective 2 have been adopted by GMOS within the global network to use them as quick reference guide when starting up Hg measurements in ambient air.

Environmental specimen banks

Environmental specimen banks (ESBs) systematically store high quality samples from the environment and human populations in support of chemicals management and innovative research for a better environmental quality. ESBs are operated by environment agencies as part of the national long-term environmental research infrastructure or by environmental research institutes. There is a large potential to make better use of ESB samples in research and chemicals regulation. ESB operations are guided by strict protocols for sampling, processing and archiving. The samples are stored at low or ultra-low temperatures in the archives which provides for their long-term biological and chemical integrity. There are around 30 ESBs in the world, of which around 20 are located in Europe. The specimens are used to monitor the quality of the environment and the efficacy of regulatory efforts to control known hazardous substances like metals, PAHs, PBDEs, dioxins and other organochlorines. However, the main objective of ESBs is retrospective analysis of chemicals of emerging concern (CECs). The systematic use of high quality ESB data and samples has the potential to significantly increase our understanding of the fate of regulated and non-regulated contaminants in the environment. ESB data is already being used to some extent to identify persistent organic pollutants (POPs) under the Stockholm Convention and potential persistent and bioaccumulative compounds in REACH legislation. However, consistent and interdisciplinary approaches that make better use of high quality ESB samples can overcome the reluctant use of monitoring data in chemicals risk assessment. The application of cutting edge analytical methods to ESB samples is a win-win situation for the value of ESB samples and our understanding of the quality of the environment.

MeTra project has made use of ESB samples for two different activities. Two fresh fish material (roach and pike) and two ESB fish samples (bream) of Lake Stechlin Germany were used to source material with different concentration levels and different fat contents. These materials were needed to test the applicability of the developed methods for speciation analysis on different real environmental samples and to compare the results of the different partners.

ESB samples of the river Mulde and Saale and Lake Belau (EQS sample) in Germany were distributed to the partners for isotopic measurements. Each sample set of the rivers included samples between 1995 and 2013. The purpose of these samples was to apply the different developed methods for Hg isotope ratio measurements (bulk and species) and compare the results and also to see if a time trend concerning isotopic signatures during the time period of the samples is present.

International Atomic Energy Agency

The IAEA Environment Laboratories (IAEA-MEL) in Monaco is the only marine laboratory within the UN system. Its unique nature means that, besides carrying out an IAEA-focused core programme, IAEA-MEL is

designed to respond to regular requests for technical assistance from other UN agencies and programmes, e.g. UNEP, IOC (UNESCO), FAO, WHO and WMO.

A strong collaboration has been established between the project partners and the IAEA-MEL. This has brought to the implementation of the reference primary procedures developed within the project for the Hg and Hg speciation in biota matrices to the characterisation of an IAEA certified reference material. This material, when available, will help analysis laboratories in their QA/QC procedures.

The IAEA-MEL has also intensively supported the project, contributing with several dissemination activities, such as one training and several conference contributions.

USEPA and NIST

The United States Environmental Protection Agency (USEPA) is the institution in charge of the US Hg regulations and measurement requirements. In terms of metrology, EPA published interim traceability protocols used to establish NIST-traceability of mercury gas standards and EPA and NIST are continuing to collaborate on the topic, to solve the lack of SI traceability for Hg measurements. During the lifetime of the MeTra project, USEPA and NIST have constantly worked together with MeTra partners, in order to achieve an international consensus for Hg standards and to exchange on practical options for providing traceable Hg gas standards.

Impact on standards

A new digestion method developed for mercury in particulate matter based on an *aqua regia* digest. This had two advantages: first it was safer than the currently used HF digestion solution and second it resulted in a lower ionic strength matrix following digestion which meant that routine ICP-MS analysis could be performed with greater repeatability and therefore a lower overall uncertainty. NPL were members of CEN/TC264/WG8, the working group would be revising the EN13211 "reference method for the analysis of mercury in stationary source emissions", and were able to convince the committee of the value, benefits and impact of the new digestion method. As a result an Annex was written describing the use of this new digestion method which has been included in the revised standard. This will allow analysis of mercury in stationary source emissions across Europe to be safer and be performed with a lower uncertainty.

Novel information was acquired about the stability of total Hg in water samples with different Total Organic Carbon (TOC). SYKE is member of the ISO/TC working group 147 (Water Quality). This new information will be taken into account during the revision of the ISO 17852 standard "Water quality - Determination of mercury - Method using atomic fluorescence spectrometry".

Long term impact

An overarching target of the project was to support the implementation and assessment of the Minamata Convention on mercury – the global and legally binding treaty aimed at reducing global mercury emissions – together with EC Directives and Member State objectives to reduce the presence of mercury in the environment. However, the decisions on how internationally recommended references are made and implemented and, further, on the ownership of these decisions is an issue that cannot be solved in three year project. This situation has been extensively discussed in the position paper: "*Who should take responsibility for decisions on internationally recommended datasets? The case of the mass concentration of mercury in air at saturation*"; R.J.C. Brown, P.J. Brewer, H. Ent, P. Fisicaro, M. Horvat, K.H Kim and C.R. Quétel; *Metrologia* 52 (2015) L25–L30.

It is clear that assessment against legislative limit values requires metrological traceability. This is to ensure stability over time so that measurements can be accurately assessed against limit values, and trends be elucidated for a given emissions source, and to ensure coherence, meaning that the same measurement values are obtained regardless of the measurement method. The coherence provided by metrological traceability has another important benefit: it ensures that measurements of emissions and concentrations in air can be compared to the measurements of the exposure of humans or animals in health studies, which are used to specify the requirement.

Such an improvement in scientific understanding prompts a difficulty: who owns and makes the decision about the recommended values for the mass concentration of mercury in air at saturation, and what body would be respected enough to ensure that such a decision was adopted universally?

The point has been reached now where a decision is required as to whether to continue with the current equation describing the mass concentration of mercury in air at saturation, or whether to suggest a change to this relationship based on the new data produced in the frame of the project.

In terms of the practicalities of such discussions and decisions the paper suggests that the best bodies to take this forward in order to ensure universal acceptance for any change would be either IUPAC or CCQM with the formation of an expert committee to review the available data and to provide recommendations.

It is probable that any such change or official pronouncement would take a long time to filter down the academic, industrial and science education communities and to make its way into text books and collections of reference data, but this should not necessarily prevent a change, as it has not for the proposed redefinition of the SI. The most important step is to ensure that key stakeholders, especially legislators and regulators, accept, understand and embrace the change and are prepared to adopt it in relevant environmental and other legislation.

5 Website address and contact details

<http://projects.lne.eu/jrp-metra/>

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For information about mercury measurements in water and biota please contact Claudia Swart (Claudia.Swart@ptb.de)

For information about mercury isotopic measurements in biota please contact Philip Dunn (Philip.Dunn@lqcgroup.com)

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