



**INTERNATIONAL ATOMIC ENERGY AGENCY
DEPARTMENT OF NUCLEAR SCIENCES AND APPLICATIONS**



***Improving Analytical Capabilities and Quality of
Measurement Results for Mercury Monitoring in the
Marine Environment***

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***“Expert Consultations Meeting on Mercury Monitoring on Soil and Biota”, 13 and 14 of May
2019, Monaco***

The only Environment Laboratory in UN System



1961 - Laboratory established in the Oceanographic Museum

1988 - Temporary facilities in the Louis II Football Stadium

1998 - Permanent facilities on the Port of Monaco



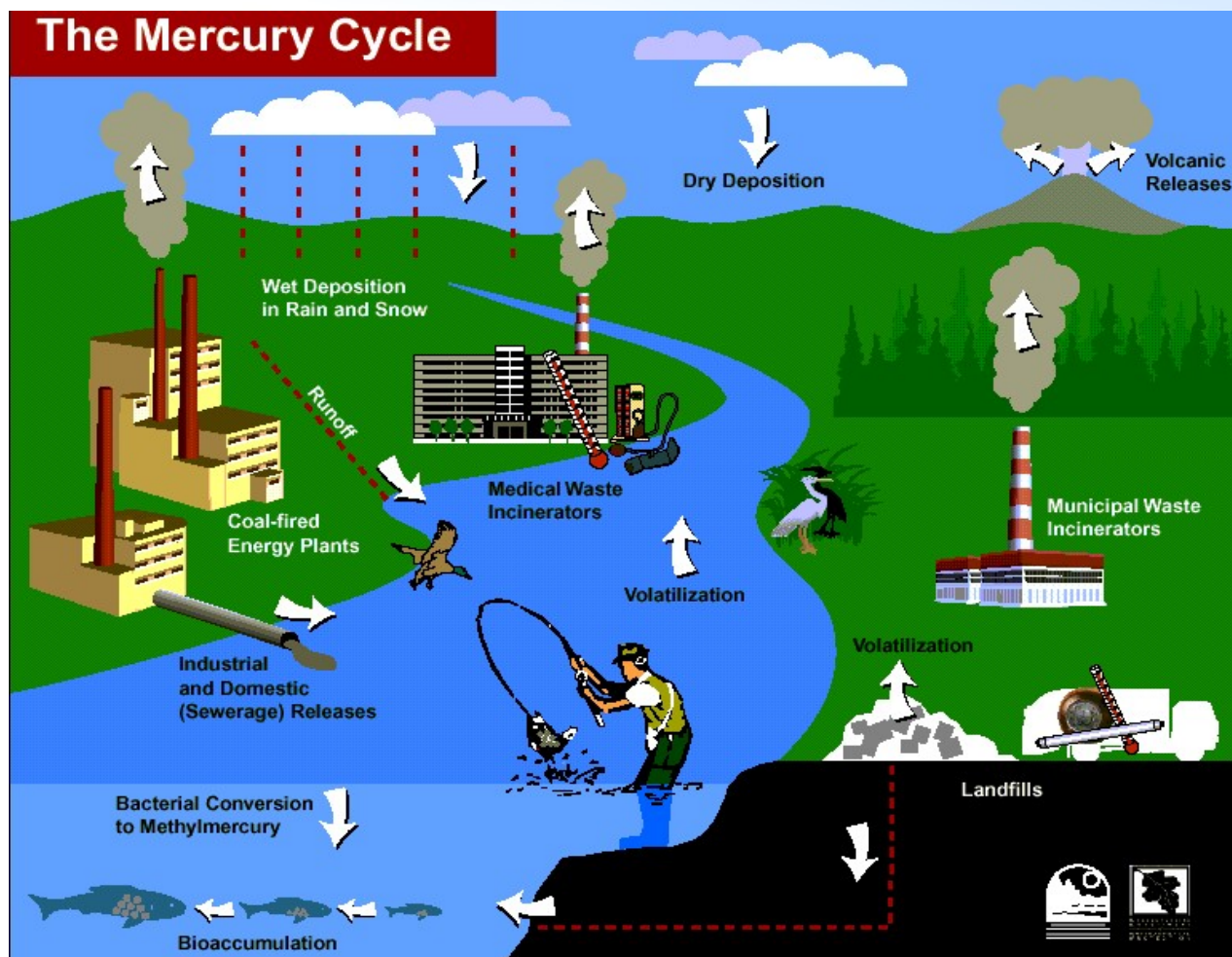
IAEA Environment Laboratories



The primary goal of the International Atomic Energy Agency's Environment Laboratories (IAEA EL) in Monaco is to help Member States to understand, monitor and protect the marine environment.

Among its multiple tasks, IAEA - EL in Monaco acts as the analytical support centre for IAEA Member States laboratories and is **the pillar of the Quality assurance program** for the determination of nuclear and non nuclear pollutants including mercury and methylmercury in the marine environment.

The Mercury cycle



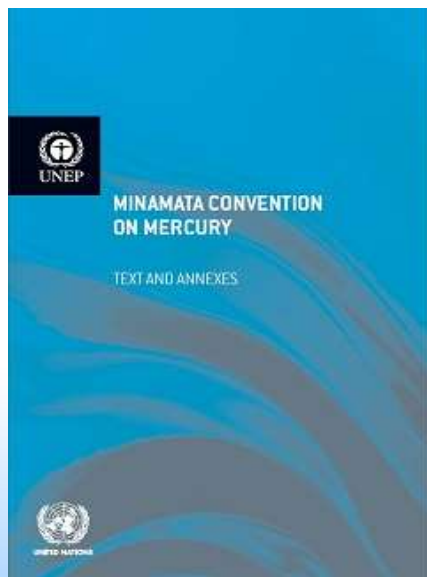
Atmospheric transport

Deposition to land and ocean

Revolatilization

Why mercury is a subject of concern?

- Hg is a global pollutant with high toxicity and possible bioaccumulation.
- Mercury concentrations in the oceans and in marine biota have risen due to anthropogenic emissions.
- Global climate change may complicate the response of global ecosystems to mercury emission reductions.



UNEP report "Mercury as a global pollutant" 2013

Member Nations now needs to establish/strengthen monitoring efforts in order to assess mercury contamination in the environment

Quality assurance of the monitoring data, is essential for their effective use for environmental assessments and decision making

Why is harmonised knowledge on mercury monitoring so important ?



- Comparability of monitoring data
- Accurate environmental assessments
- Effectiveness of mercury pollution control



NAEL activities improving MSs capabilities for mercury monitoring



Build the technical capacity of the national and regional laboratories for assessing mercury pollution by:

- ✓ Organisation of global ILC and targeted proficiency tests
- ✓ Production of matrix certified reference materials
- ✓ Developing fit-for-purpose recommended analytical procedures
- ✓ Reference measurements
- ✓ Training of laboratory practitioners from laboratories of IAEA Member States.

ILC for Trace Elements including Mercury and MeHg



Number of participating laboratories	Number of Member States	Sample Type	OPEN to	Year
81	48	Marine Sediment	All member states	2018
49	32	Fish tissue	All member states (Non EU)	2017
30	19	Marine biota-Oyster	Participants with demonstrated capabilities	2016
108	52	Marine biota-Clams	All member states	2013
23	15	Marine Sediment (No MeHg)	Participants with demonstrated capabilities	2012
72	38	Marine Sediment (No MeHg)	All member states	2011
35	19	Marine Sediment	Participants with demonstrated capabilities	2010
143	59	Marine biota-scallop	All member states	2010

Global ILC Statistics

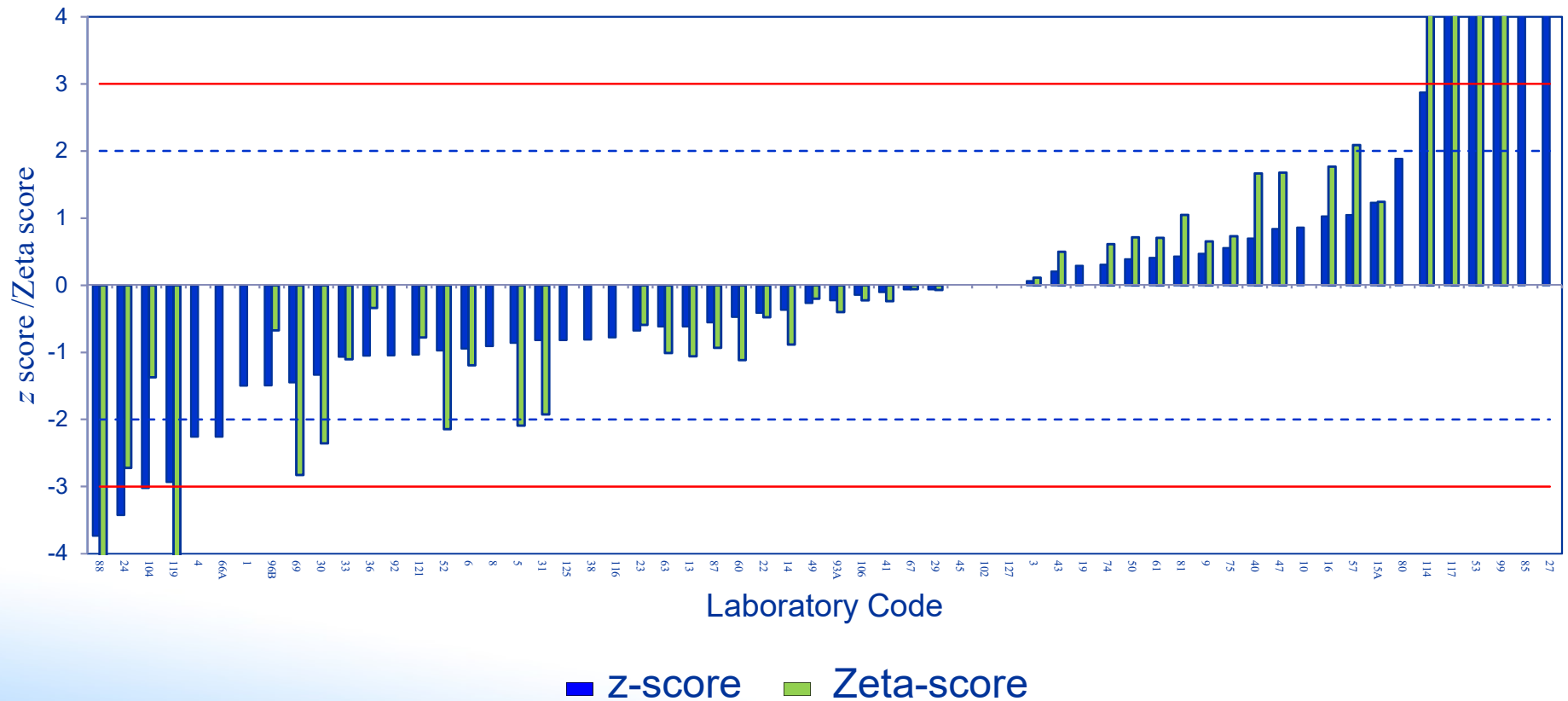
ILC	Participants	Report HgT	Report MeHg	Performed Speciation*
ILC-SEDIMENT-2018	81 laboratories from 48 countries	49 (60%)	4 (5%)	8%
ILC-BIOTA-2017 (Fish)	49 laboratories from 32 countries	35 (71%)	6 (12%)	17%
ILC-BIOTA-2013 (Clam)	108 laboratories from 52 countries	60 (55%)	17 (15%)	28%
ILC-SEDIMENT-2011	72 laboratories from 38 countries	37 (51%)	NA	NA

* % of participant reporting Hg that report MeHg as well

Global ILC IAEA 461(Clam): Total Hg



	Satisfactory	Questionable	Unsatisfactory
z-score	80% (45%)	7%	13%
Zeta-score	74% (43%)	13%	13%



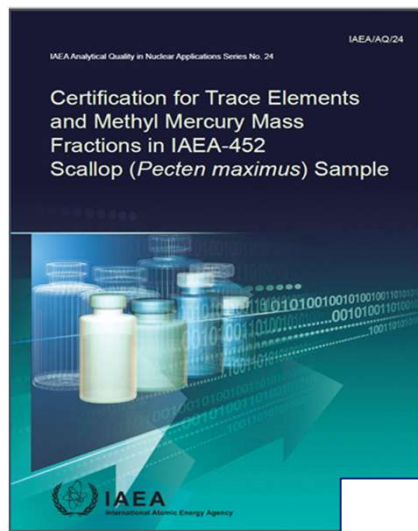
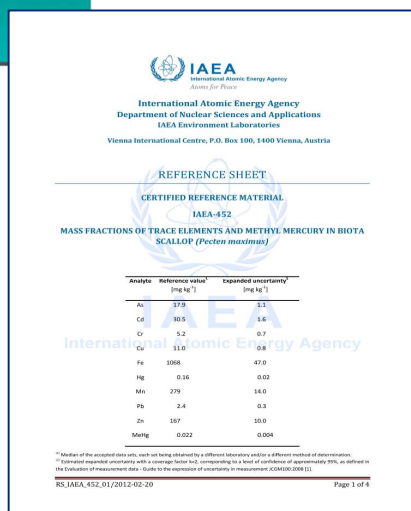
Global ILC IAEA 461(Clam) : MeHg



	Satisfactory	Questionable	Unsatisfactory
z-score	82.4% (12%)	5.9%	11.8%
Zeta-score	84.6% (13%)	7.7%	7.7%



Production of CRM

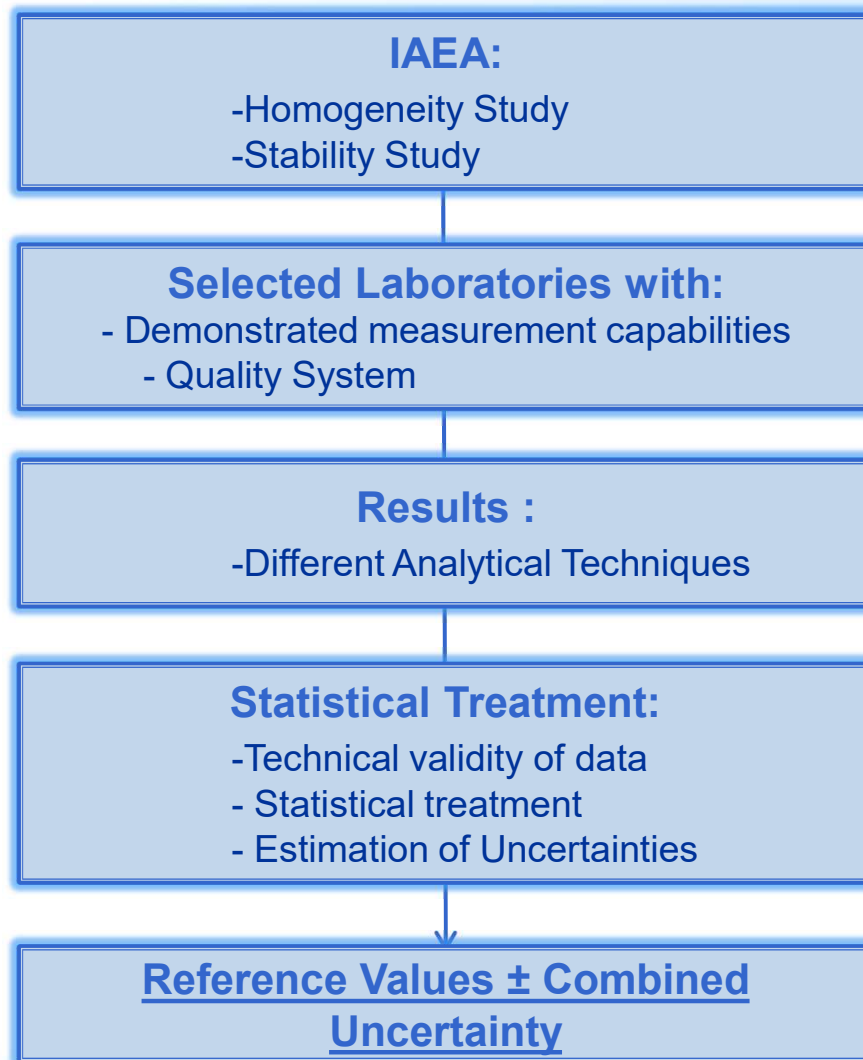
International Atomic Energy Agency
Department of Nuclear Sciences and Applications
IAEA Environment Laboratories
Vienna International Centre, P.O. Box 100, 1400 Vienna, Austria

REFERENCE SHEET
CERTIFIED REFERENCE MATERIAL
IAEA-452
MASS FRACTIONS OF TRACE ELEMENTS AND METHYL MERCURY IN BIOTA SCALLOP (*Pecten maximus*)

Analyte	Reference value* (mg kg ⁻¹)	Expanded uncertainty* (mg kg ⁻¹)
As	37.9	5.1
Cr	36.5	4.6
Co	5.2	0.7
Cd	11.6	0.4
Fe	1068	47.0
Hg	0.16	0.02
Mn	279	14.0
Pb	2.4	0.3
Zn	167	10.0
Methyl	0.022	0.004

* Number of the accepted data sets, each set being obtained by a different laboratory and/or a different method of determination.
* Expanded expanded uncertainty with coverage factor k=2, corresponding to a level of confidence of approximately 95%, as defined in the Evaluation of measurement data - Guide to the expression of uncertainty in measurement (GUM:2008:11).

RS.IAEA.452.01/2012-02-20 Page 1 of 4

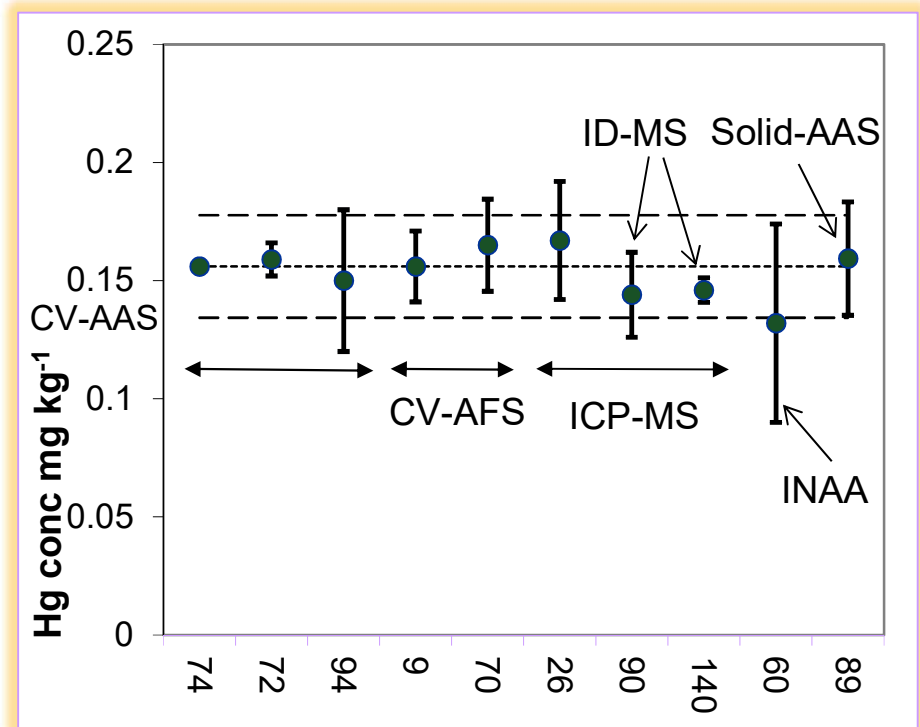
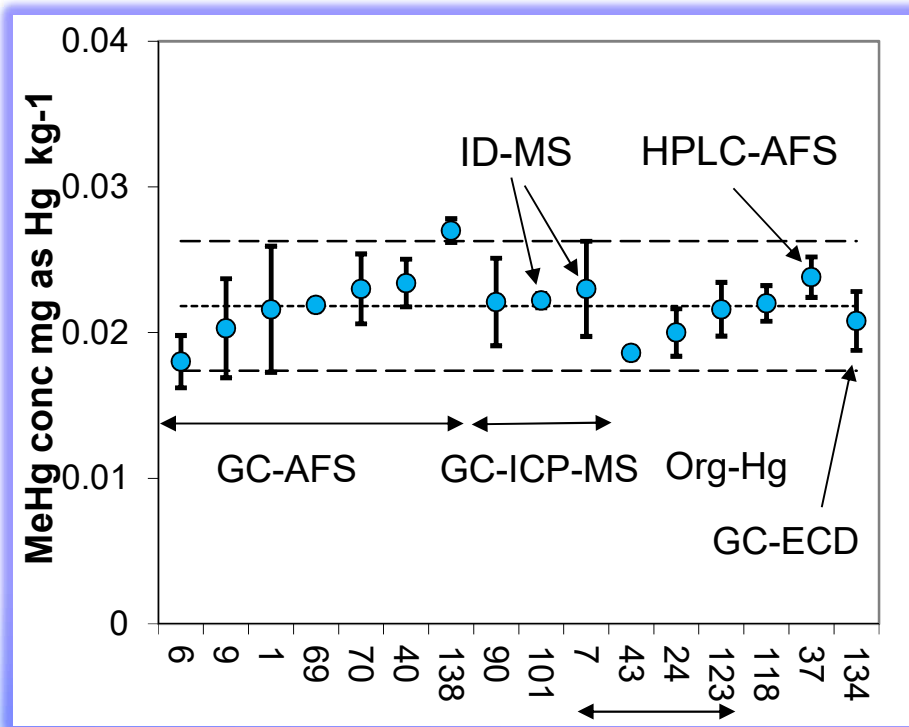


Production of CRM



Certification of mercury and methyl mercury in marine biota IAEA 452 Scallop matrix

Lines represents reference value and associated expanded uncertainty (k=2), error bar represents expanded uncertainty as reported by laboratories, participating in the certification



CRMs for Trace Elements including Mercury and MeHg



IAEA – Code	Sample Type	Analyte Groups	Year	Availability
IAEA 475	Marine Sediment	Trace elements & MeHg	2019	On-going
IAEA-476	Fish Homogenate	Trace elements & MeHg	2017	Yes
IAEA-436-A	Tuna fish	Trace elements & MeHg	2016	Yes
IAEA-470	Marine biota-Oyster	Trace elements & MeHg	2015	Yes
IAEA-461	Marine biota-Clams	Trace elements & MeHg	2013	Yes
IAEA-458	Marine Sediment	Trace elements	2013	Yes
IAEA-457	Marine Sediment	Trace elements	2012	Yes
IAEA-456	Marine Sediment	Trace elements & MeHg	2012	Yes
IAEA-452	Marine biota-scallop	Trace elements & MeHg	2010	Yes

<http://nucleus.iaea.org/rpst/>

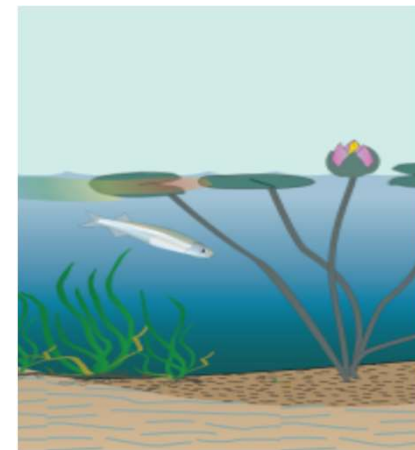
Occurrence in the Environment



Elemental
(Hg^0)

Monovalent Hg (I)
(Hg_2^{2+})

Divalent Hg (II)
(Hg^{2+} , MeHg^+ , EtHg^+ , Me_2Hg)



In the case of mercury



1. Data produced are oceanographically inconsistent
2. “Different methods measuring different fractions of mercury”
3. “Complexity of sample matrix and the definition of the measurant
4. Errors in the quantification of the analytical blank”
5. Lack of matrix matching CRM
6. Sound and realistic combined uncertainty estimation associated with the declared measurement results is nearly always lacking, which makes the comparison of different sets of results difficult

→ ACCURACY OF MEASUREMENT DATA: IT MATTERS!

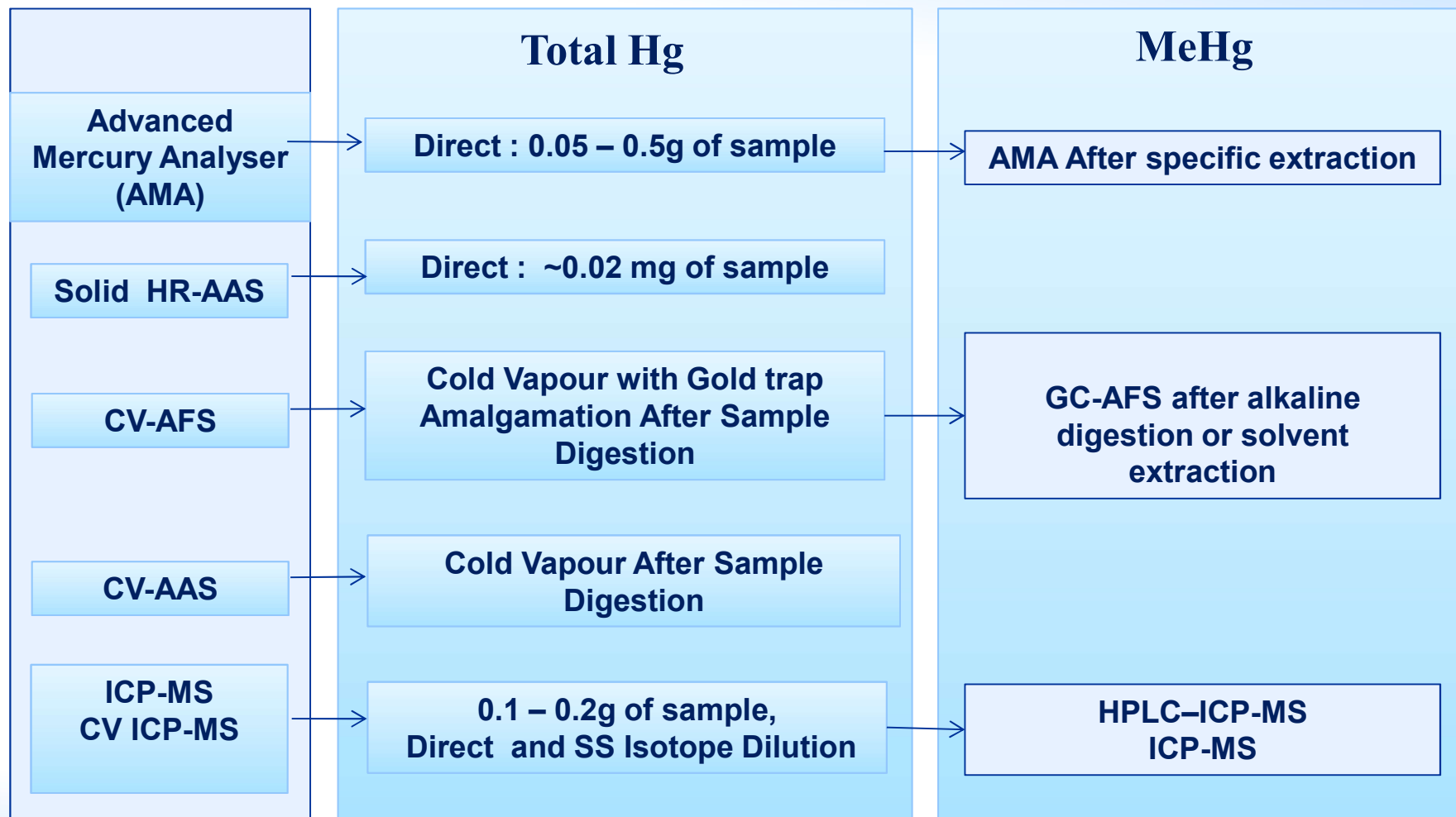
Development, validation and distribution of recommended analytical procedures



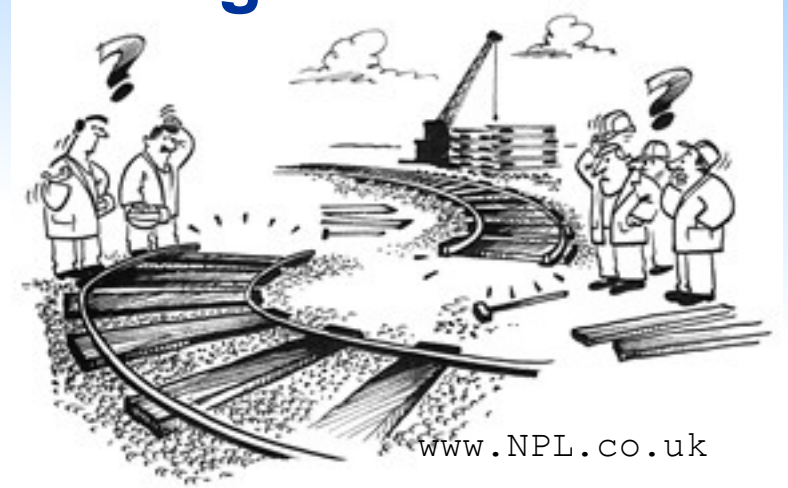
The use of reference and recommended analytical procedures is important for:

- Comparability of data for environmental assessments
- Improving quality assurance of monitoring data, produced in different MSs laboratories
- The effective use of the generated database and decision making

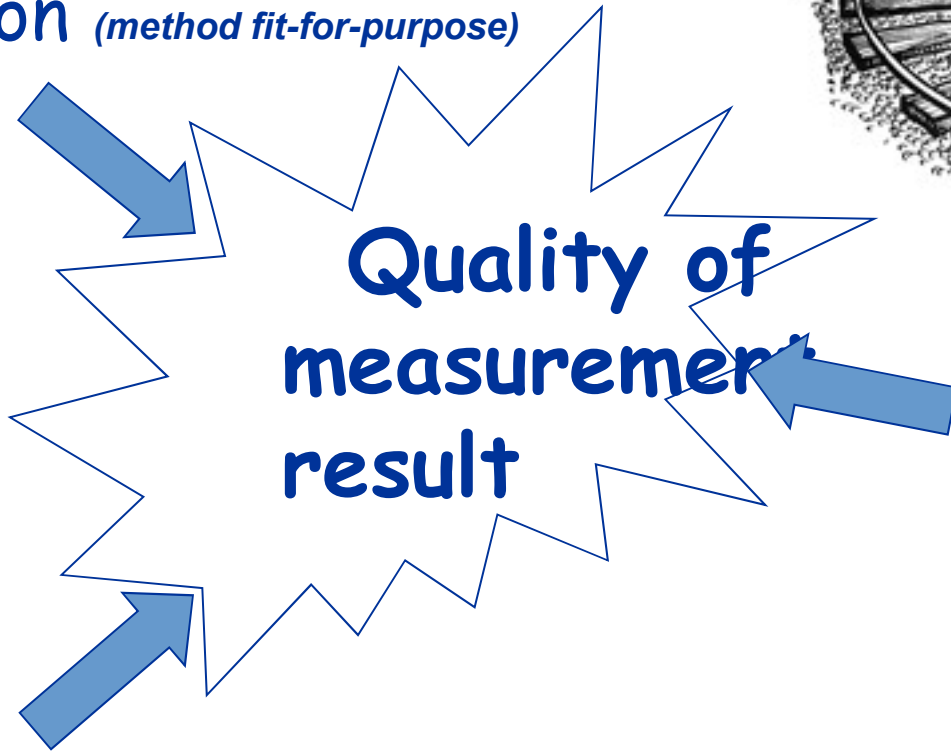
Reference and Recommended Analytical Procedures for Hg and MeHg



Comparability of monitoring data



Validation *(method fit-for-purpose)*



**Quality of
measurement
result**

Traceability
*(my result is comparable
to common reference)*

Uncertainty *(how well do I know the result)*

ISO/IEC 17025



7.2.2 Validation of method

Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled

❖ 7.2.2.1

Note 2: The techniques used for the determination of the performance of a method should be one of, or a combination of, the following:

- ❖ Calibration using reference standards
- ❖ Comparison of results achieved with other methods
- ❖ Systematic assessment of the factors influencing the results
- ❖ Assessment of the uncertainty of the results based on scientific understanding of the theoretical principles of the method and practical experience.

Validation of the analytical procedure



Performance parameters

- Selectivity
- Linearity, measuring interval
- LOD, LOQ
- Repeatability, Reproducibility
- Trueness
 - Recovery
- Ruggedness (robustness)
- Uncertainty
- Traceability

Comparison with primary method of measurements

Estimation of measurement uncertainty

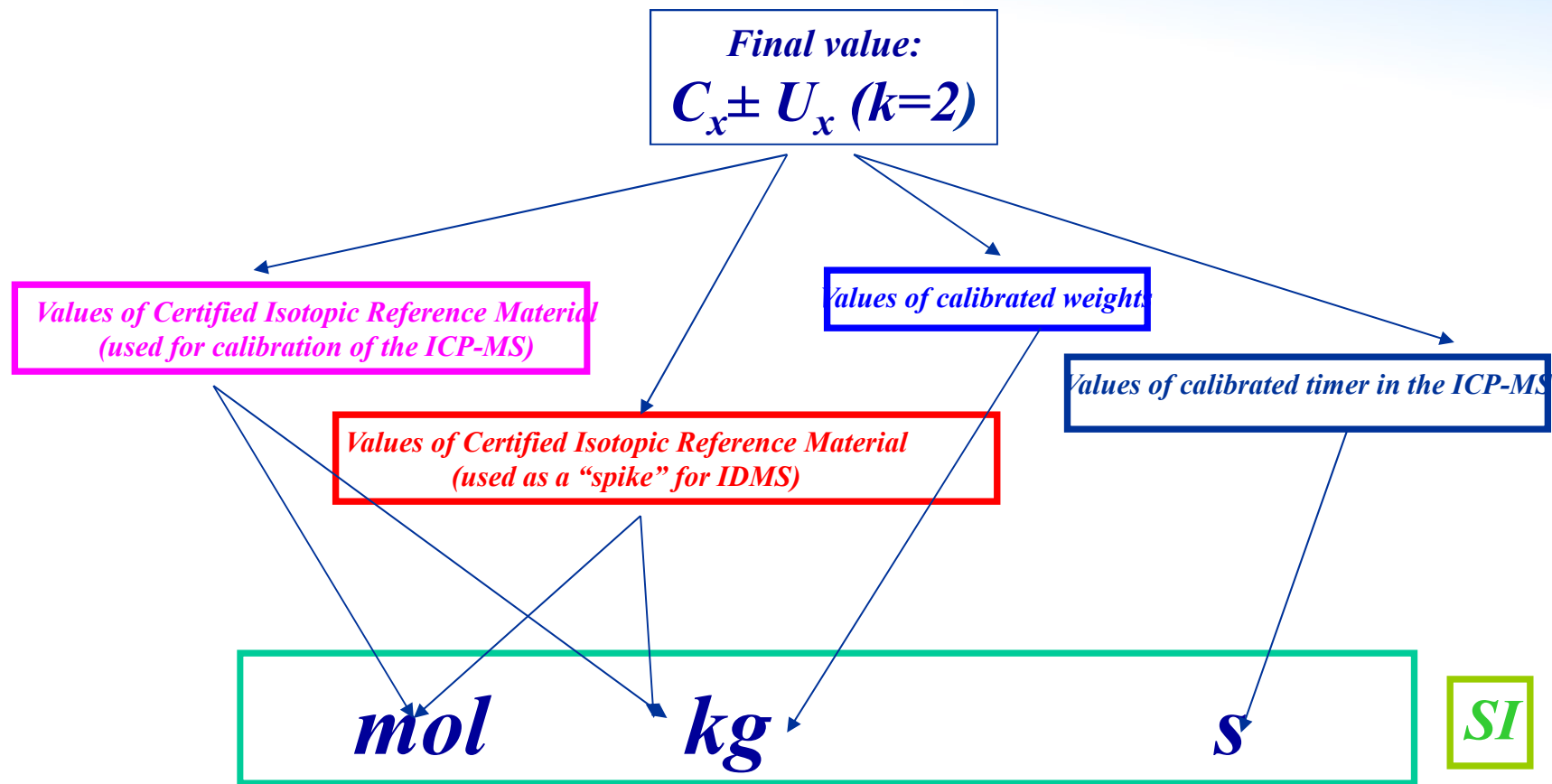


- ❖ Error propagation using partial differentiation
- ❖ More practical to use numerical approach
 - Kragten's method (Analyst, 1994, 119, 2161)
- ❖ Guidelines provided by ISO Guide to the Expression of Uncertainty in Measurement
- ❖ Dedicated software used
 - GUM Workbench[®] (Metrodata GmbH, Germany)
- ❖ Uncertainties reported with coverage factor $k = 2$

2%-15%

Specific treatment for the uncertainties on (semi) additive corrections applied to individual isotope signal intensities (instrumental background, isobaric interferences, dead time effects)

Key steps in the attainment of traceability



Mathematical modeling of the entire measurement system demonstrating the traceability to the mole, the kilogram and the second

Reference methods

Reference methods

The analytical procedure to establish reference values for Hg amount contents in marine samples is based on **ID ICP-MS** applied as a **primary method of measurement**.

$$C_X = C_Y \cdot \frac{m_Y}{m_X} \cdot \frac{R_Y - K_{R_B} \cdot R_B}{K_{R_B} \cdot R_B - K_{(R_1)_X} \cdot R_X} \cdot \frac{\sum (K_{(R_i)_X} \cdot (R_i)_X)}{\sum (R_i)_Y}$$

Correction for mass-discrimination effects

$$K = R_{IRM-true} / R_{IRM-Obs}$$

Blank corrections

$$C_{X_bl} = C_X - n_{bl} / m_x$$

Correction for dead-time effects

$$I_{-dt} = \frac{I_{raw}}{(1 - I_{raw} \cdot \tau)}$$

Moisture correction

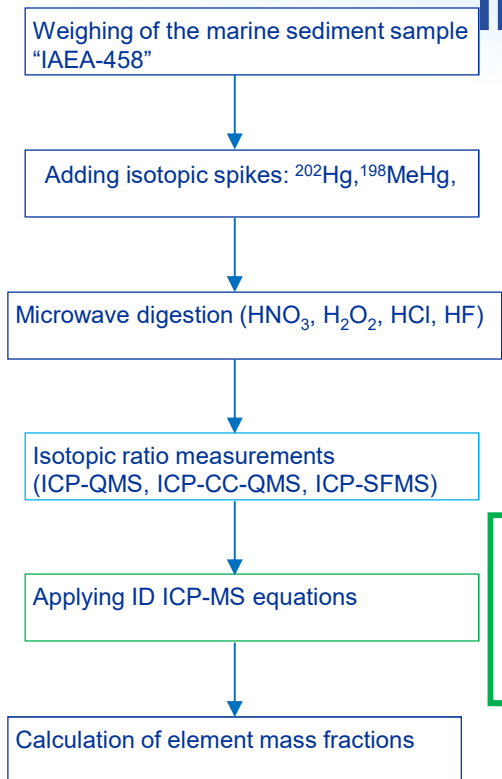
$$C_{X_mc} = C_X \cdot \frac{1}{(1 - W_{corr})}$$

$$R = \frac{I_A}{I_B}$$

Correction for instrumental background

$$I_{-bkg} = I_{raw} - I_{bkg}$$

Direct IDMS and SS ID ICP-MS for Hg and MeHg in marine biota and sediments



Correction for mass-discrimination effects

$$K_b = \left(\frac{R_{IUPAC(ICRM)}}{R_i \cdot \delta_{DT} \cdot \delta_{BG} \cdot \delta_{INT}} + \frac{R_{IUPAC(ICRM)}}{R_{i+1} \cdot \delta_{DT} \cdot \delta_{BG} \cdot \delta_{INT}} \right) / 2$$

Correction for dead-time effects

$$I_{DT_corr} = \frac{I_{raw}}{1 - I_{raw} \cdot \tau}$$

Correction for instrumental background

$$I_{INT_corr} = I_{raw} - I_{INT}$$

$$R_b = I_{a1_corr} / I_{a2_corr}$$

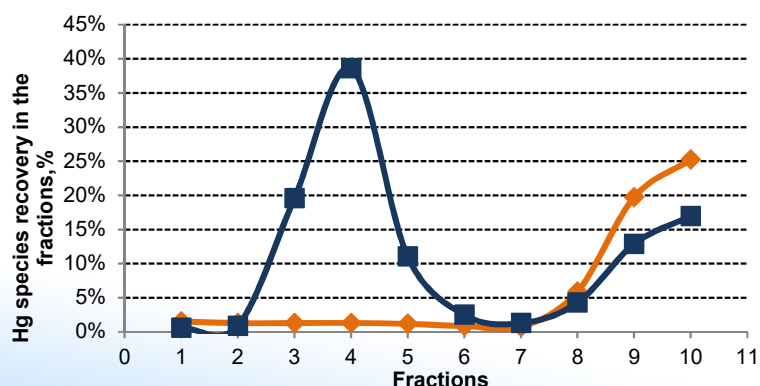
Blank corrections

$$C_{x_Bl_corr} = C_x - n_{Bl} / m_x$$

Moisture correction

$$C_{x_Wcorr} = C_x \cdot \frac{1}{1 - W_{corr}}$$

$$c_x = \left(\frac{m_y}{m_x} \right) \cdot c_y \cdot \left(\frac{R_y - K_b \cdot R_b \cdot \delta_{DT} \cdot \delta_{BG} \cdot \delta_{INT}}{K_b \cdot R_b - R_x \cdot \delta_{DT} \cdot \delta_{BG} \cdot \delta_{INT}} \right) \cdot \left(\frac{\sum_{i=1}^n R_{xi}}{\sum_{i=1}^n R_{yi}} \right)$$



ID-ICP-MS reference measurements

- ✓ Systematic assessment of all actors influencing measurement results
- ✓ Modeling of the analytical procedure and estimation of combined uncertainty
- ✓ Validation of all steps of measurement procedure
- ✓ Use of reference standards (isotopically enriched spikes)
- ✓ Demonstrated traceability of the obtained results

Cold vapor matrix-independent ID ICP- MS for reference measurements of mercury in seawater, sediments and biota



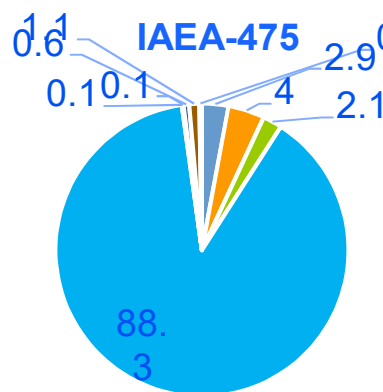
Method development and validation studies for total Hg

$$n(^{200}\text{Hg})/n(^{202}\text{Hg})$$

IAEA-475: sediment

IAEA-470: oyster

Seawater: ~ 2 ng/kg



- s.u. on repeatability within bottle
- s.u. on concentration of diluted ERM AE-640
- s.u. on mass of added spike to sample
- s.u. on repeatability of $^{200}\text{Hg}/^{202}\text{Hg}$ ratio in 4 replicate blends
- s.u. on instrumental background

Materials	Isotope dilution $C \pm U, \mu\text{g}/\text{kg} (k=2)$	U, %	Certified value / PT value	U, %
IAEA-470	20.1 (0.7)	3.5	21.2 (2.1)	9.9
IAEA-475	30.2 (1.3)	4.5	29.9 (1.5)	5.0
BCR-579	$1.96 (0.06) \cdot 10^{-3}$	3.0	$1.9 (0.5) \cdot 10^{-3}$	26
PT Seawater I	$4.83 (0.14) \cdot 10^{-3}$	3.0	$4.84 (0.11) \cdot 10^{-3}$	2.2
PT Seawater II	$9.85 (0.23) \cdot 10^{-3}$	2.4	$9.86 (0.90) \cdot 10^{-3}$	9.1
PT Seawater III	$34.2 (0.5) \cdot 10^{-3}$	1.6	$34.1 (1.8) \cdot 10^{-3}$	5.3

➤ Gas phase sample introduction

✓ separate Hg from matrix

^{200}Hg : $^{184}\text{W}^{16}\text{O}^+$ and ^{202}Hg : $^{186}\text{W}^{16}\text{O}^+$

✓ reduce memory effects

✓ increase sensitivity

Hyphenated techniques for mercury species using direct and species-specific Isotope Dilution ICP-MS



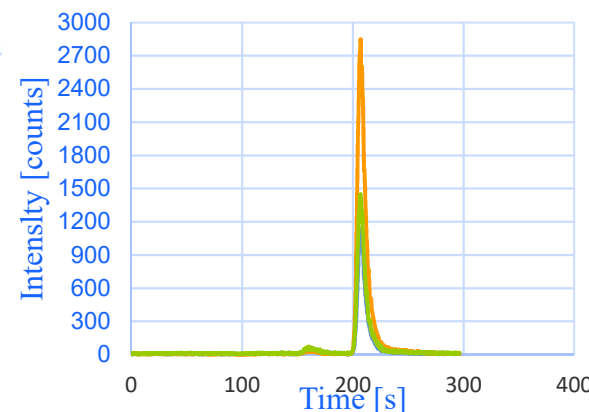
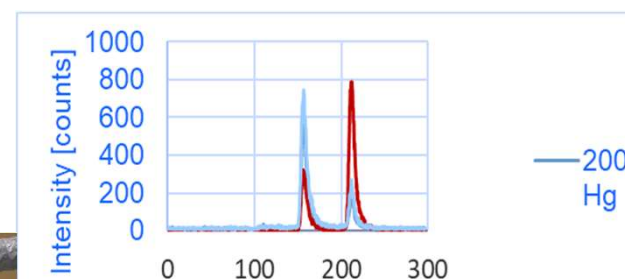
HPLC ICP-MS

$$n(^{200}\text{Hg})/n(^{202}\text{Hg}) \text{ and } n(^{201}\text{Hg})/n(^{202}\text{Hg})$$



Method development and validation studies for total Hg and MeHg

- IAEA-476: fish homogenate
- IAEA-461: Gafrarium tumidum clam
- IAEA-470: oyster



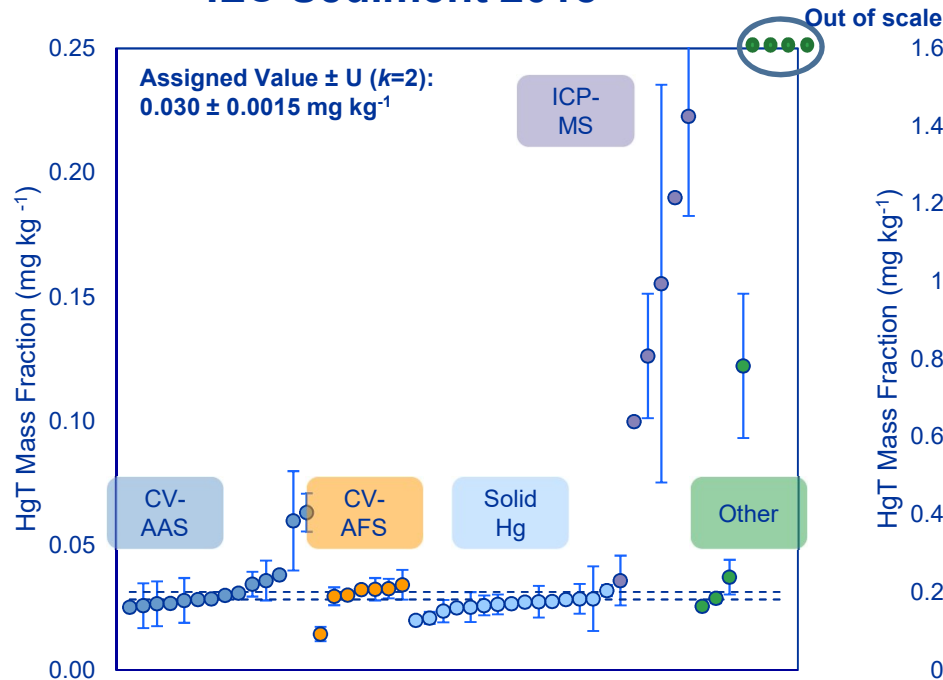
Testing methods

Global Total Hg : Example of Results

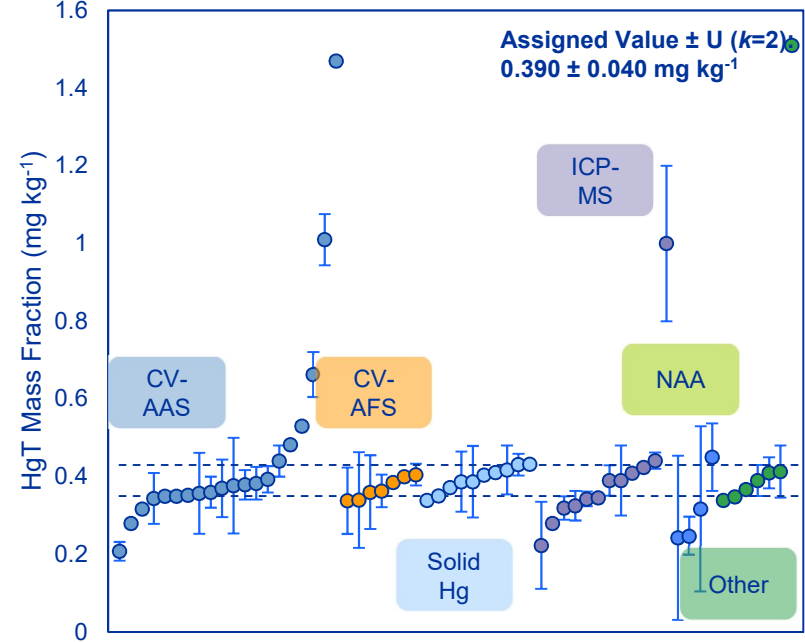


Lines represents reference value and associated expanded uncertainty ($k=2$), error bar represents expanded uncertainty as reported by laboratories, participating in the ILC

ILC Sediment 2018



ILC Clams 2013



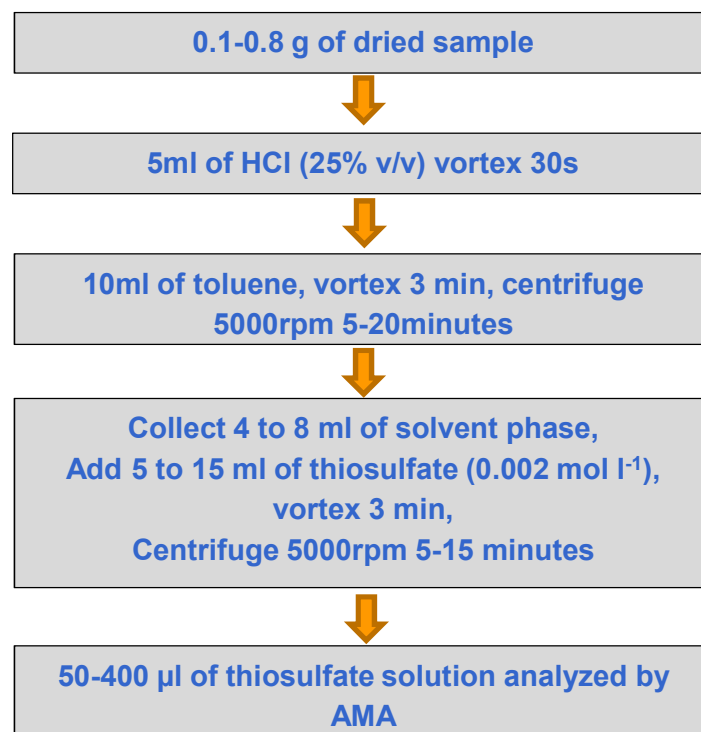
Determination of Hg and MeHg in Marine Biota Samples by using Advanced Mercury Analyser



Advanced Mercury Analyser

Sample preparation procedure

MeHg in Marine Biota



S.Azemard and E.Vassileva, Food Chemistry, 176, 2016, 367-375.

Validation Parameters



Marine biota samples

Parameters	MeHg ⁺
Recovery	92-108%
Repeatability	1.3-3.9%
Reproducibility	1.7-4.5%
Working range	0.002-20 $\mu\text{g kg}^{-1}$
Linearity	0.9932
LOD	0.5 ngkg^{-1}
LOQ	1 ngkg^{-1}
Traceability	SI system via CRM
Uncertainty	17.5% (k=2)
<i>Procedural Blank</i>	<i><0.68 pg</i>

Determination of Hg in marine samples by using CV AFS



- Analysis by Dual-Stage Gold Pre-concentration and Direct Atomic Fluorescence
- Direct measurement without pre-concentration
- Applicable to seawater, marine biota and sediment samples

Validation Parameters CV-AFS sediment and biota samples



Parameter	CV-AFS
Working range	0.1 to 5 ng kg ⁻¹
Detection limit	0.02 -0.05 ngkg ⁻¹
Quantification limit	0.2-0.5 ngkg ⁻¹
Repeatability	2-5%
Reproducibility	4-8%
Recovery	96 - 102%
Uncertainty (k=2)	14% (k=2)

THg in Seawater by CV-AFS



Validation Parameters :

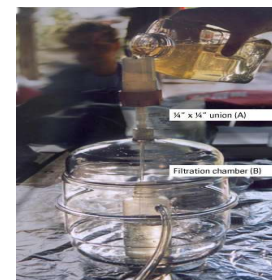
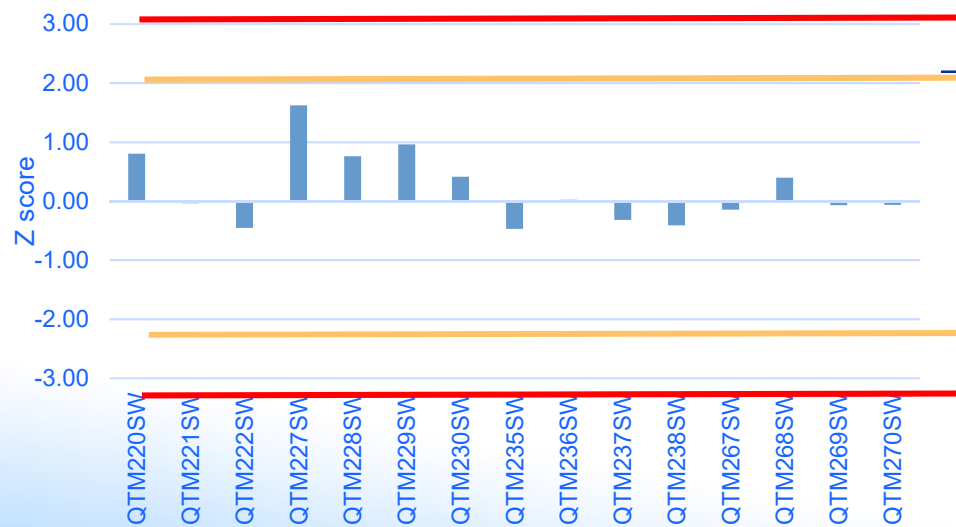
Summary of the Method

- Filtered or Unfiltered sea Water (25ml)
- Digestion 12h with BrCl
- Pre reduction Hydroxylamine ammonium
- Reduction with SnCl₂
- Double Gold trap Amalgamation
- AFS Detection (automated analyzer Brooks Rand or Tekran)

Parameters

Linearity	Demonstrated up to 900pg (36 ng kg ⁻¹)
Working range	0.8 to 900pg (0.03 to 36 ng kg ⁻¹)
LOD	0.009 ng kg ⁻¹
LOQ	0.03 ng kg ⁻¹
Repeatability	7.0 -2.4% (for 0.2 to 5 ng kg ⁻¹)
Intermediate precision	7.3 -2.4% (for 0.2 to 5 ng kg ⁻¹)
Recovery	94 – 103 %
Uncertainty (U)	30% (k=2) with BCR 579 15% (k=2) with Spike for recovery

Results of 3 years external PTs on seawater



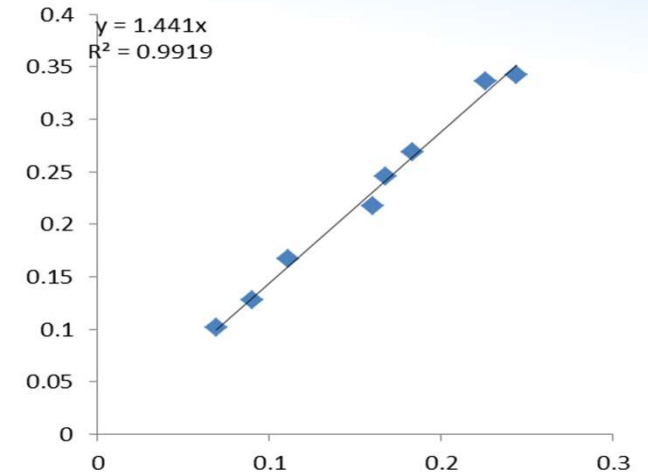
Validation of: $HgT_p = HgT - HgT_D$

Samples are analyzed Filtered (Hg_T_D) and unfiltered (Hg_T)

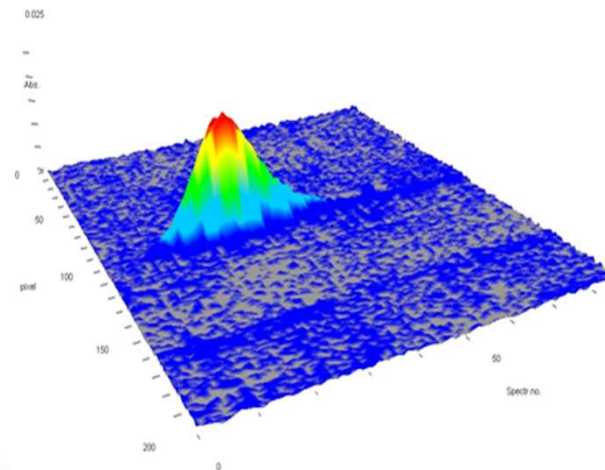
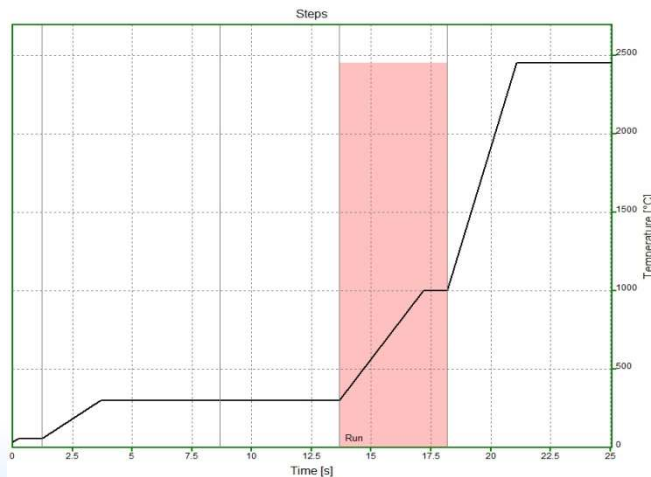
Solid Sampling CS HR AAS for Hg in marine samples: biota and sediments



Step	Name	Temp. [°C]	Ramp. [°C/s]	Hold [s]	Time [s]	Ar flow
1	Drying	55	100	1	1.2	Max
2	Pyrolysis	300	100	5	7.5	Max
3	Gas Adaption	300	0	5	5.0	Stop
4	Atomize	1000	200	1	4.5	Stop
5	Clean	2450	500	4	6.9	Max



Sample mass 0.111mg



Validation Parameters

Parameters	Total Hg
Repeatability Reproducibility	RSD=3.2 % RSD=11 %
LOD	0.12 ng Hg
LOQ	0.36 ng Hg
Recovery	95-101%
Uncertainty	8.5% (k=2)
Traceability	SI system via CRM

Determination of MeHg in Marine Samples with GC-pyr-AFS



**Automatic purge and trap
iso-thermal packed GC-Pyr-AFS**



**Automatic purge and trap
Capillary GC-Pyr-AFS**

Methods for MeHg determination in seawater, marine biota and sediment samples are based on aqueous ethylation derivatization, purge & trap pre-concentration, GC separation and AFS detection

Assay of Different Extraction Conditions



MAE-HCl (5M)

0.5 g IAEA-452 10 mL of 5 M HCl and 0.25 M NaCl

Teflon vessels were irradiated at 60 °C for 10 min
Centrifugation at 3000 rpm for 20 min

Injection volume 20 µL

94%

MAE-TMAH (25%, w/v)

0.1 g IAEA-452 4 mL of 25% (w/v) TMAH

Teflon vessels were irradiated at 70 °C for 8 min
Centrifugation at 3000 rpm for 20 min

Injection volume 30 µL

20%

MAE-KOH (25%, w/v)

0.15 g IAEA-452 6 mL of 25% (w/v) KOH/MeOH

Teflon vessels were irradiated at 70 °C for 8 min
Centrifugation at 3000 rpm for 20 min

Injection volume 30 µL

49%

Furnace-KOH (25%, w/v)

0.25 g IAEA-452 10 mL of 25% (w/v) KOH/MeOH

Teflon vessels heated in a furnace at 75 °C for 3 h
Dilution to 50 mL

Injection volume 20 µL

97%

Protease digestion

0.2 g IAEA-452 0.02 g of protease XIV
8 mL of 0.1M phosphate buffer pH 7.2/0.05% (w/v) Cys

Teflon vessels heated in a furnace at 37 °C for 4 h while being shaken

Injection volume 20 µL

99%

Validation Parameters



Parameters	MeHg in biota
Recovery	91%
Repeatability	9%
Reproducibility	11%
Working range	1-800 pg
Procedural Blank	<0.45 pg
LOD	0.45 pg
LOQ	0.85 pg
Traceability	SI system via CRM
Uncertainty	14% (k=2)

L. Carrasco and E. Vassileva, Analytica Chimica Acta [853](#), 2015, 167–178.

L. Carrasco and E. Vassileva, Talanta, 122, 2014, 106-114.

MeHg in Seawater by GC-AFS



Tekran



Brooks Rand

Summary of the Method

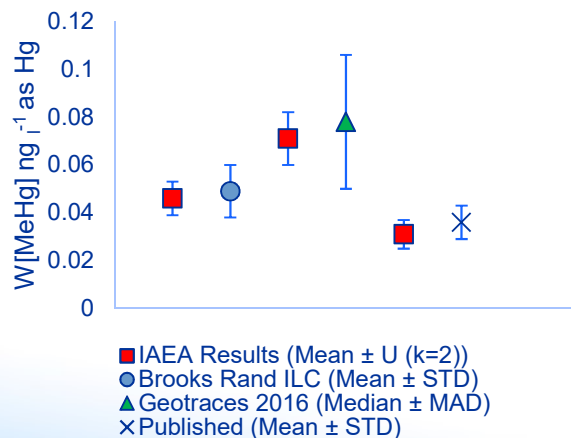
200ml filtered acidified Seawater

Solvent Extraction

Back Extraction Aqueous

Analyse with GC AFS

Validation via External comparisons

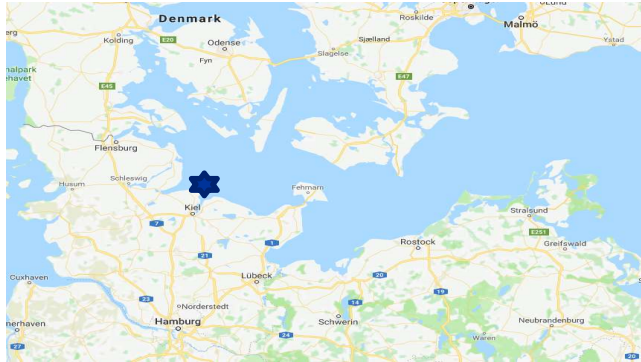


(Manuscript under preparation)

Validation Parameters :

Parameters	
Linearity	Demonstrated up to 7pg (0.12ng/l)
Working range	0.2 to 7pg (0.0035 to 0.12ng/l)
LOD	0.001 ng/l
LOQ	0.004 ng/l
Repeatability	3.3% at 0.05ng/l (4.5% at 0.007ng/l)
Intermediate precision	2.9% at 0.05ng/l
Recovery	94 %
Uncertainty (U)	15-25% (k=2)

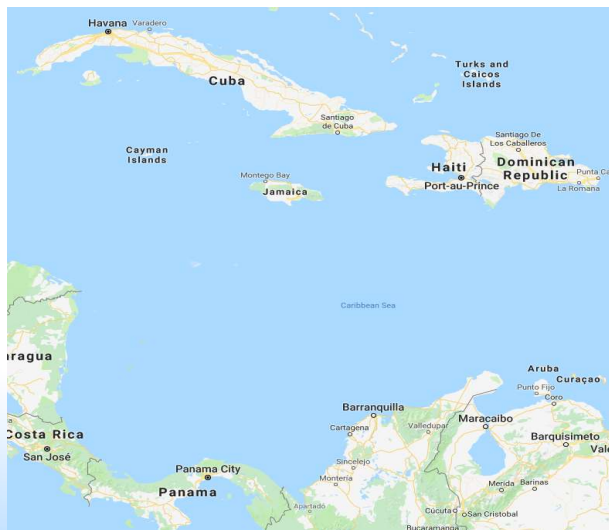
Regional monitoring studies for Hg and MeHg



Baltic Sea: Hg and MeHg determination. Pollution history study

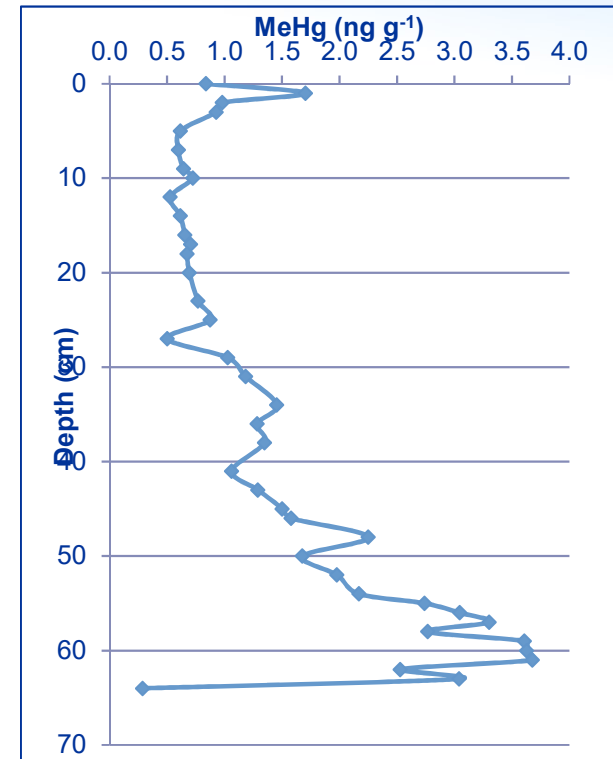
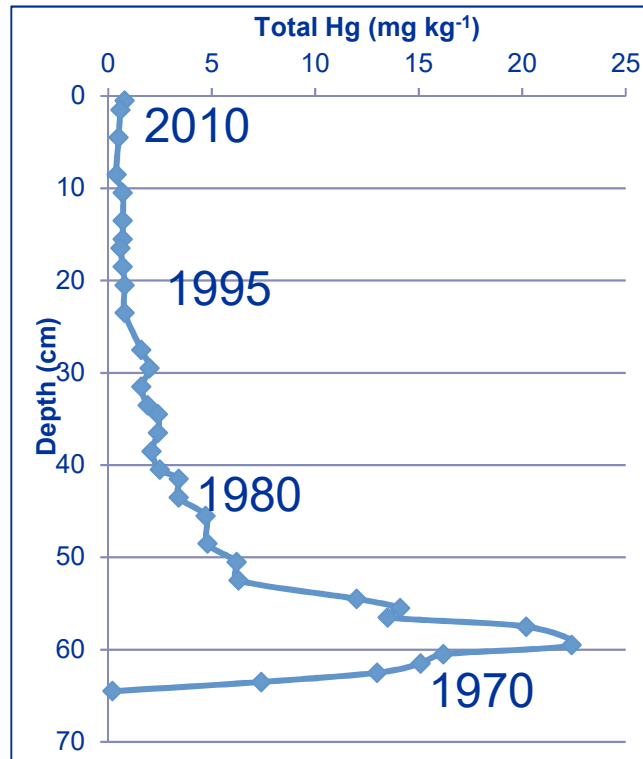


Namibia: Baseline monitoring of total Hg along the Namibian coast.



Caribbean region: Cuba, Haiti, Dominican Republic, Colombia. Surface and core samples collected in areas with specific historical Hg contamination

Results: Total and methyl mercury profiles in Colombian sediment



Mercury was used in the production of chlorine in **Alkalis plant in Colombia** and waste waters were discharged directly into the sea.



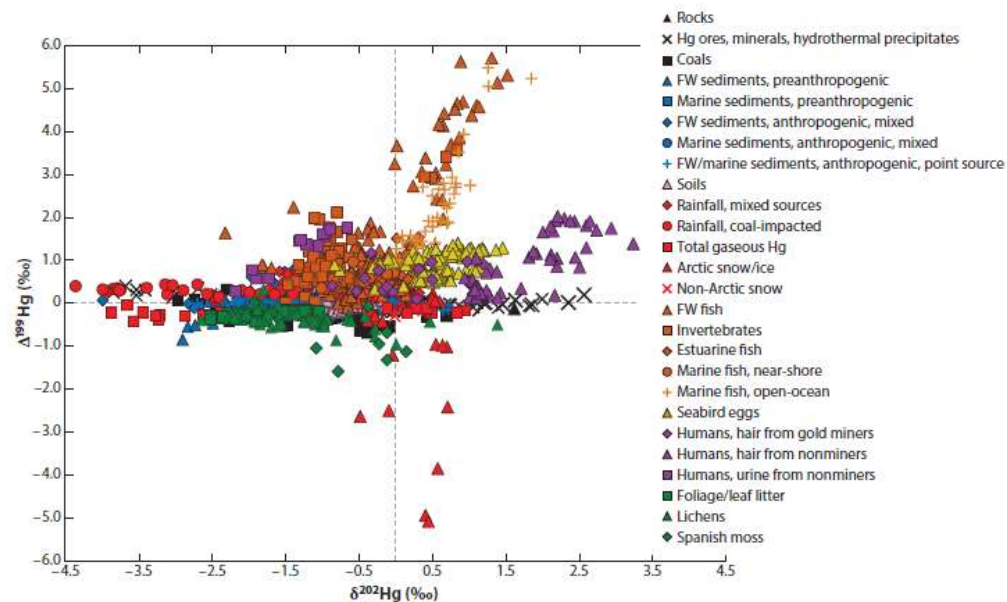
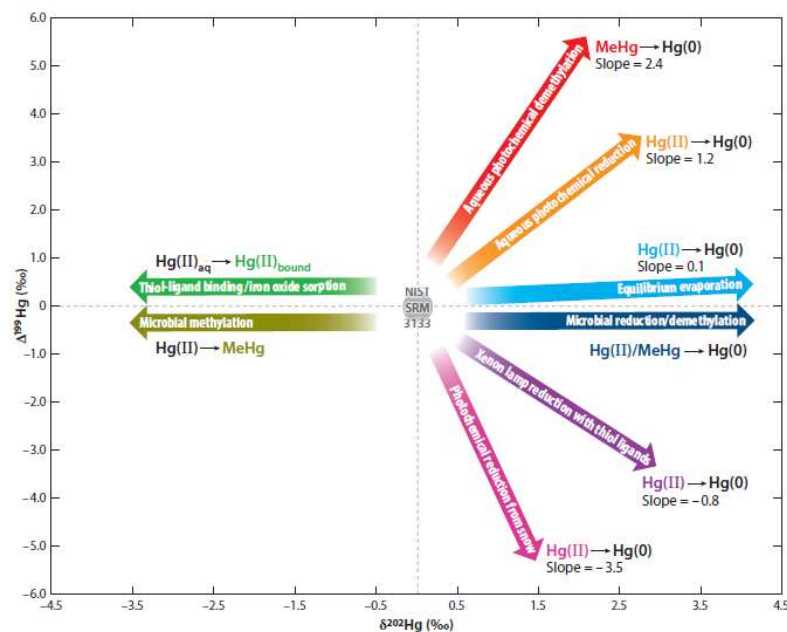
New Developments and Progress in the Environmental IAEA Pollution Studies

International Atomic Energy Agency
Atoms for Peace and Development

A photograph of a rocky coastline with waves crashing against the shore. The water is a deep blue, and the waves are white with foam. The rocks are dark and jagged.

Hg isotopes are used to fingerprint, identify and trace the source of pollution

Mercury Isotope Ratios



Hg isotopes can be used to trace pollution sources, biogeochemical cycling and reactions involving Hg in the environment

Application of Hg isotope ratios for pollution source evaluation



Hg isotopes can undergo two types of fractionation, depending on chemical/physical processes in which they are involved:

- **MDF = Mass Dependent Fractionation**

$$\delta^{xxx}Hg(\text{‰}) = \left\{ \left[\frac{(xxxHg/^{198}Hg)_{sample}}{(xxxHg/^{198}Hg)_{SRM3133}} \right] - 1 \right\} \times 100$$

where ^{xxx}Hg = selected Hg isotope

SRM 3133 = Hg standard

As convention, the equation for $\delta^{202}Hg$, involving the ratio $^{202}Hg/^{198}Hg$, is used to express the MDF

- **MIF = Mass Independent Fractionation**

$$\Delta^{xxx}Hg = \delta^{xxx}Hg - (\delta^{202}Hg \times \beta)$$

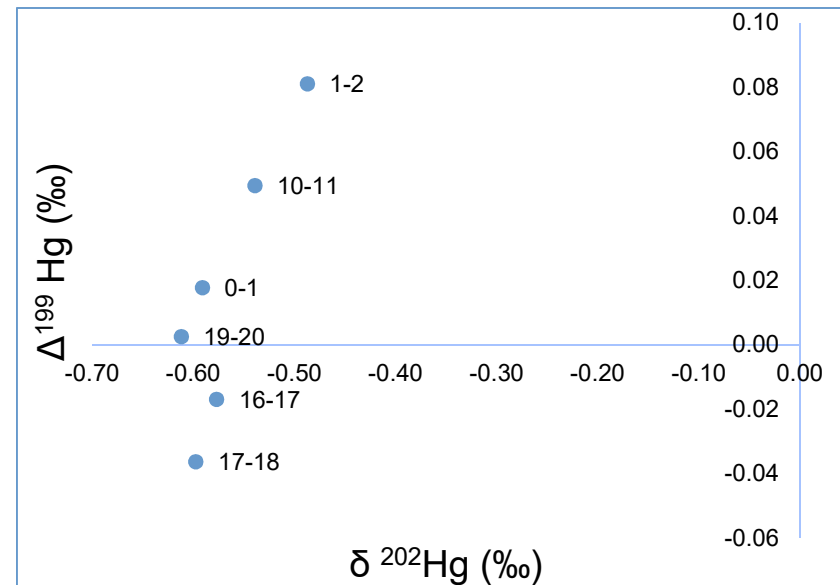
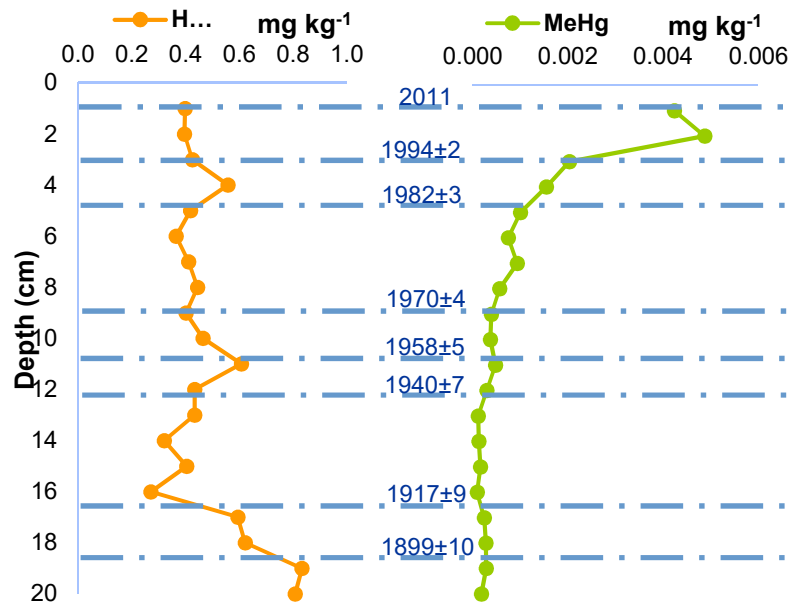
where ^{xxx}Hg = selected Hg isotope

$\beta = 0.252, 0.502$ and 0.752 for $^{199}Hg, ^{200}Hg$ and ^{201}Hg respectively

Hg in Baltic sediment core



Hg and MeHg profiles: pollution history Hg isotope ratios in sediments



The most probable source of Hg is coal combustion. Highest Hg inputs recorded at the beginning of the 20th century.

Support Capacity Building of Member States to Protect the Marine Environment



Promote excellence in analysis - training of scientists via TC projects and Regional Seas programmes

Dissemination of recommended analytical methods for mercury and methylmercury via peer review papers, scientific presentations and IAEA reports

PUI project on Seafood safety



Conclusions



Sound strategies for marine chemical monitoring are based on measurement systems, capable of producing comparable analytical results with demonstrated quality.

The IAEA's EL supports the improvement of the worldwide performance of monitoring laboratories and the availability of reliable analytical data for global mercury monitoring.

Acknowledgment

- Mr. Lius Carrasco
- Mr. P. Mandjukov
- Ms. Agnieszka Krata
- Ms. Irena Wysocka

Thank You

