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Executive Summary

This document describes the sampling and analysis methods and protocols allowing the analysis of organic compounds (polycyclic aromatic hydrocarbons –PAHs-, polychlorinated biphenyls –PCBs- and polybrominated diphenyl ethers –PBDEs-) and inorganic (cadmium, mercury) compounds according with the selection of pollutants to analyze during the Thresholds project (see Milestone 4.2.1). These compounds are widely spread in the environment and their common properties such as toxicity and persistence makes them relevant candidates to be monitored in the environment, as reported the EU Water Framework Directive (60/2000/EU). Although much has been published on specific analytical methods on these compounds, we compile in this document the specific methods that are being developed and used within the project Thresholds, which have the capability to determine the above mentioned compounds at ultratrace concentrations, needed to determine the potential levels that can cause any effect towards the marine environment. In addition, the methods used are specifically aimed at identifying these compounds in complex matrices, such as marine waters, air, sediment and organisms. Sampling protocols, extraction, and analytical methods are described for each family. Much emphasis is given to quality control analysis, with the aim to provide, throughout the project, high accurate and precise results. In addition, method validation is performed for some compounds.

1. INTRODUCTION

1.1. ORGANIC COMPOUNDS: PCBs, PAHs and PBDEs

Environmental contamination by polychlorinated biphenyls (PCBs) was recognised more than 30 years ago when Soren Jensen detected PCBs in pike from Sweden. Since then, numerous studies have detected PCBs in various compartments of the environment and the occurrence of PCBs as the Arctic is evidence for the long-range atmospheric transport of these contaminants. Although the production of PCBs was stopped, PCBs are still detected in many environmental compartments (Breivik, 2002). As a result, PCBs are being determined in many environmental matrices, such as air, particulate matter, sediments, soils, etc.

Polycyclic aromatic hydrocarbons (PAHs) are one of the most important classes of ubiquitous priority pollutants whose carcinogenic and mutagenic properties and endocrine disrupting effects have been reported on several environmental matrices (Fuoco, 2005). Both natural (such as incomplete high temperature combustions) and anthropogenic sources, such as those from traffic (Lim, 1999) or energy generation emissions (Mastral, 2000) account for their diffusion in the environment, through atmospheric transport, deposition and dispersion (Martinez, 20004). Furthermore, their semi-volatility and high environmental half-lives result in global planetary distribution (Fernández, 2003).

Finally, polybromodiphenylethers (PBDEs) have been widely used in the last decade in many industrial applications as flame retardants (Raham, 2001). These compounds, used as additives in high impact polystyrene, textile coatings, wire and cable insulation, electrical connectors, etc. are not chemically bonded to the plastic structure and are possibly more readily released to the environment. PBDEs have become ubiquitous environmental pollutants as a result of their usage, disposal of PBDE containing products, distribution pattern and, ruled by their physico-chemical properties, increasing levels have been reported in North America (Renner, 2000; Renner 2002b; Erickson, 2002) as well as in Europe (Darnerud, 2002) and Asia (Sakai, 2002). PBDEs behave as persistent organic pollutants and were suggested to be global environmental pollutants due to the high persistence, high bioaccumulation rates and atmospheric long transport potential (Jansson, 1987). In addition, these compounds are suspected to cause endocrine dysfunction by interfering with the thyroid hormone metabolism (Meerts, 2000).

The analysis of PCBs, PAHs and PBDEs in environmental matrices still pose some problems, due to the fact that (i) these compounds are found in complex matrices, e.g. biota, milk, sediment containing high amounts of lipids or organic matter; (ii) trace level determination is required at the low $\text{ng}\cdot\text{g}^{-1}$ dry

weight for sediment samples and $\text{pg}\cdot\text{g}^{-1}$ lipid in the case of biological samples and (iii) unequivocal identification is not so evident since many methods rely only on retention time information. Several methods are described in the literature and involve gas chromatography (GC) with electron capture detector (ECD) which has been used to analyze PCBs and PBDEs but more recently mass spectrometric detection (MS) is the preferred option since it provides structural information. GC-MS is generally performed with quadrupole mass analyzers (Eljarrat, 2002), magnetic sectors (Ikonou, 2002) or ion trap (IT) MS (Manchester, 2001) using either negative chemical ionization (NCI) or electronic impact (EI) as ionization techniques. EI is advantageous since it produces molecular ions and sequential losses of bromine ions and this provides congener specific spectra allowing the use of isotopic dilution method for identification and quantification. However, the sensitivity of EI is lower than with NCI. NCI using either methane, ammonia or isobutane provides lowest LODs among all techniques. Accurate identification and quantification in MS can only be achieved provided an efficient extraction and clean-up is performed to eliminate interferences and coelution of matrix-containing halogenated compounds. The most widely used methods are Soxhlet extraction (Hyötyläinen, 2002; Lacorte, 2003) or liquid-solid extraction (Sellström, 1995) where PBDEs are generally extracted from the matrix using dichloromethane and hexane followed by a clean-up step. Recently pressurized liquid extraction has been used for the determination of PAHs (Martinez, 2004) and brominated flame retardants (Lacorte, 2006). Reported advantages are that the technique is fast and does not need of further extract clean-up.

1.2. MERCURY AND METHYLMERCURY

Mercury is one of the most toxic metallic elements to reach the marine environment from natural and anthropogenic sources. Knowledge of its distribution and its transfer in different marine reservoirs, as well as the monitoring of contamination levels, can only be achieved by obtaining reliable data. Here we will describe the tested sampling and analysis techniques providing the dosing of gaseous mercury including elemental mercury (Hg^0) and dimethylmercury (DMHg), of "reactive mercury" (HgR or "easily reducible mercury" which is essentially made up of inorganic forms of mercury), of monomethylmercury (MMHg) and of total mercury (HgT) dissolved in marine waters.

Using the experience gleaned from the development of analytical techniques for the determination of trace metals in natural waters, a systematic study of mercury contamination sources and their inspection have been carried out (e.g., Bloom and Crecelius, 1983; Fitzgerald *et al.*, 1983; Gill and Fitzgerald, 1985). All

these studies point to the prerequisites for obtaining reliable results for natural concentrations in seawater, which include:

- Sampling and conserving water “cleanly” in appropriate recipients. The use of specially cleaned Teflon material for sampling and the storage of samples is necessary.
- Choosing analysis protocols compatible with the dosage of contaminants in trace form, i.e. by selecting those that require a minimum of handling and the lowest quantities of chemical reagents.
- Working in a "clean" environment including the use of a "clean room" or at least a laminar air flow hood.
- Using an analytical detection method that is sufficiently sensitive to measure picograms (pg) of mercury.

The combination of ultra-clean sampling, storage, and processing techniques for samples with a very sensitive detection method with atomic fluorescence spectrometry (AFS), has inspired the firm conviction that in most cases, natural concentrations of total dissolved mercury in most non-contaminated marine waters is approximately 0.1 and 2 ng L⁻¹. In the absence of certified reference material, this conviction results from the convergence between the correct precision of the measurements and the ability to interpret the results.

The principle of mercury dosage used in these protocols is based on the volatility of elemental mercury (Hg⁰) at ambient temperature. Mercury vapour is quantified by AFS, where the cloud of mercury vapour is subjected to agitation by a luminous beam with a wavelength of 254 nm and the fluorescence emitted is measured at the same wavelength. The determination of dissolved gaseous mercury (HgGD) does not require prior treatment of the sample (the sample is degassed under argon), whereas that of HgR requires prior reduction of Hg^{II} to Hg⁰ by SnCl₂ and that of HgT a dissociation of organomercurials with BrCl before reduction (Bloom and Crecelius, 1983). The dosage method of methylated forms (MMHg and DMHg) uses cryogenic chromatography (Bloom, 1989). In the specific case of MMHg it requires the best technique combining hydriding and cryogenic chromatography (Tseng et al., 1998). Whatever the chemical species measured detection is carried out by AFS. AFS is the keystone of the French standard AFNOR T90-113-2 and of the method EPA 1631 used in the United States of America to measure total mercury in water.

1.3. CADMIUM

Cadmium is a trace (or ultratrace) element in the matrices referred to therein (dissolved phase and suspended particulate matter). Which means that the first requirement to ensure good quality results is

a complete and thorough knowledge of contaminations that can affect samples. The personnel in charge of sampling and analysis must therefore be trained and aware of the problems that may arise.

Bottles, sampling (pumps, pipes) and filtration devices are thoroughly cleaned with analytical grade acids prior to sampling.

Sample processing is carried out in a dust-controlled environment. Samples are handled with polyethylene or nitrile gloves only. When transported, samples are bagged in two polyethylene bags closed with plastic ties.

2. ORGANIC COMPOUNDS

Forty PCBs, 16 EPA priority PAHs and 40 PBDEs have been analyzed in water, sediment, and air (table 2.1).

Table 2.1 Organic compounds for which analytical methods have been developed.

PCBs		PAHs	PBDEs	
PCB 17	PCB 149	Naphthalene	BDE #1	BDE #47
PCB 18	PCB 151	Acenaphthylene	BDE #2	BDE #66
PCB 28	PCB 153	Acenaphthene	BDE #3	BDE #77
PCB 31	PCB 158	Fluorene	BDE #10	BDE #100
PCB 33	PCB 169	Phenanthrene	BDE #7	BDE #119
PCB 44	PCB 170	Anthracene	BDE #11	BDE #99
PCB 49	PCB 171	Fluoranthene	BDE #8	BDE #116
PCB 52	PCB 177	Pyrene	BDE #12+13	BDE #85
PCB 70	PCB 180	Benzo(a)anthracene	BDE #15	BDE #126+155
PCB 74	PCB 183	chrysene	BDE #30	BDE #105
PCB 82	PCB 187	Benzo(b)fluoranthene	BDE #32	BDE #154
PCB 87	PCB 191	Benzo(k)fluoranthene	BDE #17	BDE #153
PCB 95	PCB 194	Benzo(a)pyrene	BDE #25	BDE #140
PCB 99	PCB 195	Indeno(1,2,3-c,d)pyrene	BDE #28+33	BDE #138
PCB 101	PCB 199/201	Dibenzo(a,h)anthracene	BDE #35	BDE #166
PCB 105	PCB 205	Benzo(ghi)perylene	BDE #37	BDE #183
PCB 110	PCB 206		BDE #75	BDE #181
PCB 128	PCB 208		BDE #71	BDE #190
PCB 132	PCB 209		BDE #49	
PCB 138				

2.1. EXTRACTION PROCEDURES

2.1.1. *Extraction and purification of air samples*

All PUFs (gas phase) and QM-A filters (particle phase) were spiked in laboratory with perdeuterated PAHs surrogate standards (anthracene-*d*10 and perylene-*d*12, Cambridge Isotopes, Cambridge, UK) before extraction. PUFs and QM-A were Soxhlets extracted for 24 h in hexane:acetone (1:1, v/v)

(Merck, Darmstadt, Germany) and dichloromethane:methanol (2:1, v/v) (Merck, Darmstadt, Germany), respectively. The extracts were concentrated by rotary evaporation to 1 mL and for QM-A solvent-exchanged to hexane. All samples were then cleaned-up on a 3% water deactivated alumina column (1,5 g) with a top layer of anhydrous sodium sulfate to remove any interfering compounds and to fractionate the samples on the basis of polarity. Each column was eluted with a first fraction of 5 mL of hexane, subsequently was eluted with 12 mL dichloromethane:hexane (2:1, v/v) containing PAHs. The first and second fraction containing PCBs and PAHs were concentrated in a rotary evaporator and solvent-exchanged to isooctane under a gentle stream of nitrogen. Neutral aluminum oxide 90 active (63-200 μm) and analytical grade anhydrous sodium sulfate, supplied by Merck (Darmstadt, Germany), were treated at 400 °C for 12 h before use.

2.1.2. *Extraction and purification of water and particulate phase*

The method was optimised using distilled and surface waters. To avoid adsorption of PAHs upon glassware, 10% methanol (v/v) was added to 200-1000 mL of water and the solution was mixed thoroughly. This solution was spiked with target analytes at a concentration of 2 $\mu\text{g/L}$. Samples were filtered through 0.7 μm preweight glass fiber filters. Filters and waters were extracted and analyzed separately. The surrogate standard was added at this stage at a concentration of 0.5 $\mu\text{g/L}$. For the preconcentration step, LLE was used as depicted in figure 2.1.

Filters were spiked with the appropriate surrogates and extracted three times by sonication with 10 ml dichloromethane-methanol (2:1) (Suprasolv®, Merck) for 15 min. As described elsewhere (Maldonado et al., 1999) the recovered extracts were combined, dried with anhydrous sodium sulfate (Merck), concentrated on a rotary evaporator to 0.5 mL, and solvent-exchanged to isooctane (Merck). Cleanup of the dried extracts was carried out by column chromatography (3 g of neutral alumina (Merck), deactivating with 3% (w/w) Milli-Q water), and eluting the compounds of interest with 12 mL of hexane (Merck).

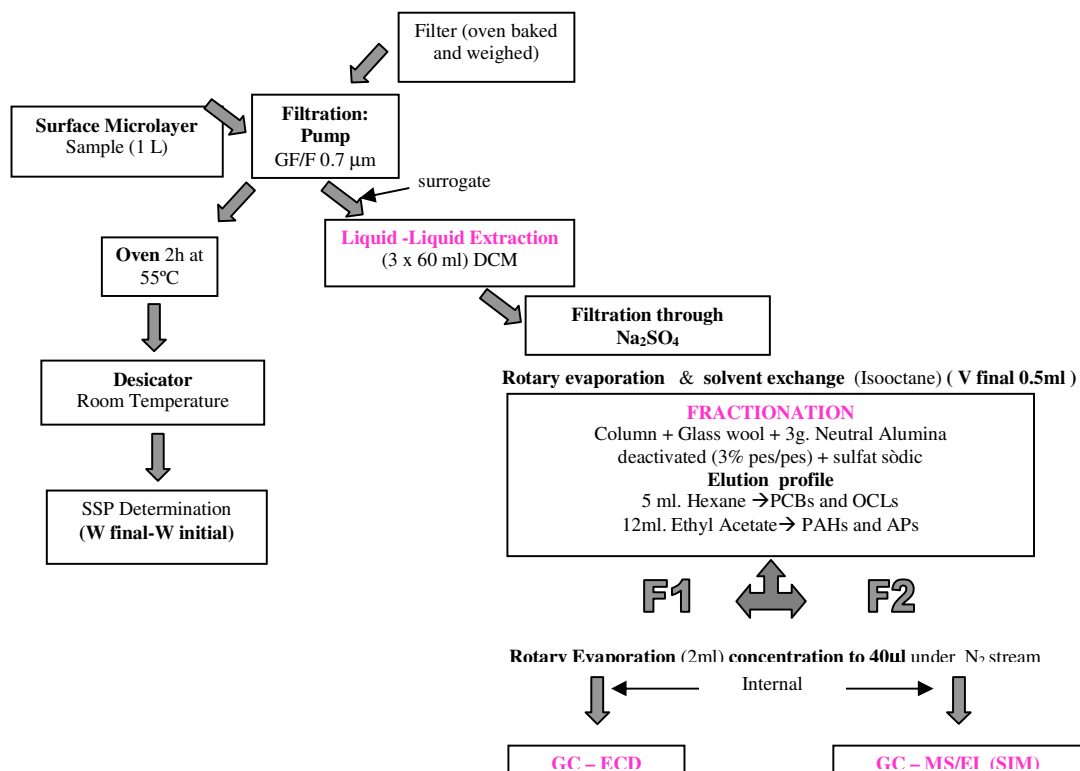


Figure 2.1. Protocol used to extract water microlayer and its particulate phase

2.1.3. Extraction and purification of organisms

Zooplankton or mussels were freeze dried for 48 hours at 10^{-2} mbar vacuum then homogenized in a mortar. One g was spiked with surrogate standard (depending on the compound to be analyzed, see below) and extracted by sonication (10 minutes) using 30 mL hexane/dichloromethane (1:1 v/v). The extract was centrifuged for 5 minutes at 2500 rpm. This was repeated twice more. Extracts were evaporated in a rotary evaporator to 0.5 mL and were purified using 5 g neutral alumina solid phase extraction cartridges (Waters, USA), which were pre-conditioned with 20 mL hexane-dichloromethane (2:1 v/v) and 20 mL hexane-dichloromethane (10:1 v/v) and elution was carried out with 40 mL hexane-dichloromethane (10:1 v/v) and 40 mL hexane-dichloromethane (2:1 v/v). Using this combination of solvents, all target compounds eluted. The two fractions were combined, rotary evaporated and reconstituted in 250 µL hexane.

2.2. ANALYTICAL METHODS

2.2.1. GC-ECD analysis of PCBs

Cleaned extracts were analyzed by GC-ECD using a HP5890 series II chromatograph equipped with a HP6890 automatic injector. The samples were injected in the splitless mode (solvent delay 48 s), using helium 4.6 (1.3 mL min⁻¹) and nitrogen (50 mL min⁻¹) as carrier and make-up gases, respectively. Chromatographic separation was achieved by using a 50 m x 0.25 mm ID x 0.25 µm CPSil8CB column (Chrompack, Netherlands) with a temperature program of 70 °C to 150 °C at 15 °C min⁻¹ and then to 300 °C at 3 °C min⁻¹ holding it for 8 min. The presence of OCL compounds was confirmed by means of a Trace GC-MS (Fisons, Manchester, UK) in the electron impact mode operating at 70 eV of electron energy. The transfer line and ion source were held at 280 and 230 °C, respectively.

The PCB concentrations were calculated from the calibration curves for 41 major congeners (IUPAC # 17, 18, 28, 31, 33, 44, 49, 52, 70, 74, 82, 87, 95, 99, 101, 105, 110, 118, 128, 132, 138, 149, 151, 153, 158, 169, 170, 171, 177, 180, 183, 187, 191, 194, 195, 199/201, 205, 206, 208, 209) based on 37 peaks (CQME-01 Accustandard, New Haven, CT, USA). The OCL quantification was based on standard dilutions prepared from the pure products: α-HCH (97.5%), γ-HCH (99.4%), HCB (99.9%) 4,4'-DDE (99.0%) and 4,4'-DDD (10 mg mL⁻¹, isooctane). All of them were provided from Dr. Ehrenstorfer GmbH (Augsburg, Germany, www.analytical-standards.com) except 4,4'-DDT (99.7%) which was purchased at the Institute of Industrial Chemistry (Warsaw, Poland). Recoveries were calculated from the surrogates and quantification was performed by the internal standard method using 2,4,6-trichlorobiphenyl and 2,2',3,4,4',5,6,6'-octachlorobiphenyl, PCBs IUPAC # 30 and # 204, respectively.

2.2.2. GC-EI-MS analysis of PAH

Samples were analysed by GC (Carlo Erba GC 8000) coupled to a quadrupole mass spectrometer (MS, FISIONS MD 800). The system was operated electron impact mode (EI 70 eV). The separation was achieved with a 30m x 0.25mm I.D. DB-5 column (J&W Scientific, Folsom, CA, USA) coated with 5% diphenyl-polydimethylsiloxane (film thickness 0.25 µm). The oven temperature was programmed from 60°C (holding time 1 min.) to 175°C at 6°C/min (holding time 4min) to 235°C at 3°C/min and finally to 300°C at 8°C/min, keeping the final temperature for 5 min. Injection was performed in the splitless mode, keeping the split valve closed for 48 s. Helium was the carrier gas (50 cm/s). Injector, transfer line and ion source temperatures were 280°C, 250°C and 200°C, respectively.

Peak detection and integration were carried out using Masslab software. For increased sensitivity and specificity, quantification was performed in time scheduled Selected Ion Monitoring (SIM) using three

ions for each compound. Internal standard quantification was performed using the labelled compound present in each elution window. The ion mass program used for quantification is detailed in the table 2.2.

Table 2.2. GC-EI-MS mass windows, retention time, molecular weight and main ions used for the analysis of PAHs. Surrogate standards are indicated.

Time window (min)	Rt (min)	compound	Compound	MW	Ions	m/z window
6.00-14.50	10.57		Naphthalene	128	128,127,51	51,127,128,136
			<i>Naphthalene-d8</i>	136	136	
14.50-20.50	16.39		Acenaftilene	152	152,76,151	76,82,151,152,154,164,165,166
	17.01		<i>Acenaftene-d10</i>	164	164	
	17.12		Acenaphthene	154	154,152,76	
	19.07		Fluorene	166	166,165,82	
20.50-34.00	23.17		<i>Phenanthrene-d10</i>	188	188	89,101,152,178,188,200,202
	23.30		Phenanthrene	178	178,152,89	
	23.56		Anthracene	178	178,152,89	
	31.64		Fluoranthrene	202	202,200,101	
	33.16		Pyrene	202	202,200,101	
34.00-48.00	42.53		Benzo(a)anthracene	228	228,226,114	114,226,228,240
	42.63		<i>Crysene-d12</i>	240	240	
	42.81		Crysene	228	228,226,114	
48.00-65.00	51.25		Benzo(b)fluoranthene	252	252,250,126	126,138,139,250,252,264,274,27
	51.49		Benzo(k)fluoranthene	252	252,126	
	53.64		Benzo(a)pyrene	252	252,250,126	
	54.06		<i>Perylene-d12</i>	264	264	
	59.06		Indeno(1,2,3-c,d)pyrene	276	276,138	
	59.27		Dibenzo(a,h)anthracene	278	278,276,139	
	59.87		Benzo(g,h,i)perylene	276	276,274,138	

2.2.3. GC-NCI-MS analysis of PBDE

Gas chromatography-mass spectrometric analysis using negative chemical ionization was used for the analysis of PBDEs. The PBDE Solution EO-4980 was from Cambridge Isotope Laboratories, Inc. (MA, USA) and contained 3 monoBDEs (BDE # 1, 2 and 3, following IUPAC nomenclature), 7 diBDEs (BDE # 7, 8, 10, 11, 12, 13 and 15), 8 triBDEs (BDE # 17, 25, 28, 30, 32, 33, 35 and 37), 6 tetraBDEs (BDE # 47, 49, 66, 71, 75 and 77), 7 pentaBDEs (BDE # 85, 99, 100, 105, 116, 119 and 126), 6 hexaBDEs (BDE # 138, 140, 153, 154, 155 and 166) and 3 heptaBDEs (BDE # 181, 183 and 190). Decachlorobiphenyl (Cromlab. S.L, Barcelona, Spain) was used as surrogate standard. The EO-4980 standard was used to construct a calibration curve over the range from 5 pg/μL to 200 pg/μL. This calibration curve allowed quantification of congener concentrations over the range 10-400 pg/g-

dw. EO-4980 was also employed to spike sediments for recovery studies following the standard addition method.

Solvents used were from Merck (Darmstadt, Germany) and 2 g alumina cartridges were purchased from Waters (Milford, MA, USA).

GC-NCI-MS was performed on an Agilent 6890 gas chromatograph connected to an Agilent 5973 Network (Agilent, Waldbronn, Germany) mass spectrometer. A HP-5ms (30m x 0.25 mm i.d., 0.25 μ m film thickness) containing 5% phenyl methyl siloxane (model HP 19091S-433) capillary column was used with helium as the carrier gas at 10 psi. The temperature program was from 110°C (held for 1 min) to 180°C (held for 1 min) at 8°C/min, then from 180°C to 240°C (held for 5 min) at 2°C/min, and then from 240°C to 280°C (held for 6 min) at 2°C/min. 2 μ L of sample was injected using the splitless injection mode over an interval of 1 min. The GC-NCI-MS operating conditions were as follows: the ion source temperature was 250°C, methane was used as the chemical ionization moderating gas at an ion source pressure of $2.7 \cdot 10^{-4}$ torr, and acquisition was performed in selected ion monitoring as described elsewhere (Eljarrat *et al.*, 2002; Lacorte *et al.*, 2003). A quadrupole mass analyser with unit resolution was used. With GC-NCI-MS, the target compounds were easily distinguishable and retention time and spectral information provided confirmation of such results.

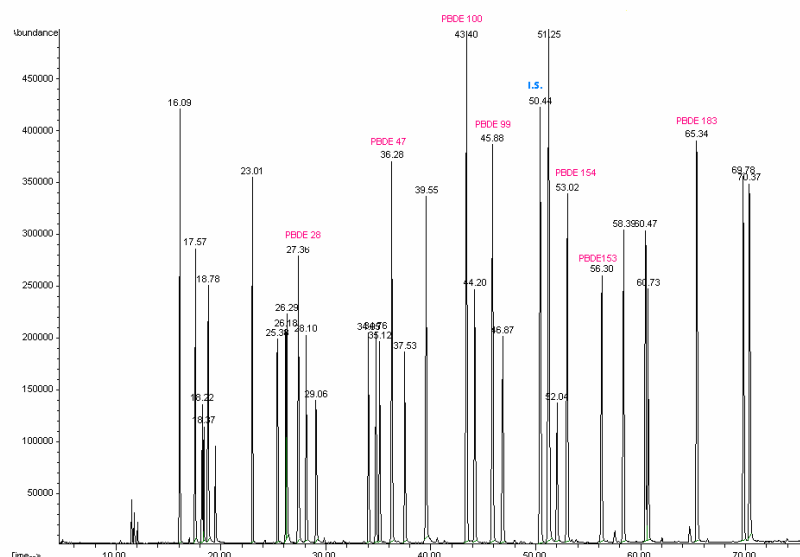


Figure 2.2. GC-NICI-MS chromatogram of PBDE standard mixture solution at 70 pg/ml.

3. SPECIATION OF MERCURY DISSOLVED IN MARINE WATERS: DETERMINATION OF TOTAL MERCURY, GASEOUS MERCURY, REACTIVE MERCURY, MONOMERCURY AND DIMETHYLMERCURY

3.1. SAMPLING, FILTRATION AND CONSERVATION OF SAMPLES

Several documents have set out the principles and described working practices concerning trace elements. The pioneering contribution of the Conseil National des Recherches in Canada in the domain is put forward in a summary in which Sturgeon and Berman (1987) describe the state-of-the-art for sampling and storing water for the dosage of trace metals. They appraise the materials that can be used and their cleaning, samplers, sampling methods and their evaluation, filtration, preconcentration, preservation and storage. Clean rooms, or clean laboratories, have been described in various articles (e.g., Boutron, 1990). Sampling and analytical methods are described in several others (e.g., Kramer, 1994; Nolting and Jong, 1994). Quality inspection techniques in this specific area are described *inter alia* by Taylor (1987) and Keith (1991). At this stage, it is important to stress that these last two authors insist specifically on including sampling and prior treatment of sample stages in the quality control process as they have often been neglected in the past. In a more exhaustive manner, Howard and Statham (1993) provide the philosophy that supports the fine-tuning of trace element analysis. The first precautions are quality control of air and materials used and sample protection from the influence of the operator. In what follows we will describe the protocols chosen for their suitability to marine waters.

Sampling is carried out either directly in the Teflon storage bottle (FEP or PFA) for surface water (the operator having taking care to don polyethylene gloves), or with a pneumatic Teflon pump of the type PFD-1 (ASTI) to which is attached Teflon tubes for sub-surface water and water from up to 100 metres in depth. For deep-water we will use bottles of the Go-Flo (General Oceanic) type with an interior Teflon coating. These techniques do not differ from those described by Chiffolleau et al. (2003) for other trace metals and to which we can refer in more detail. The main difference is in the storage material for seawater samples. In the case of the dosage of mercury, we only use a PFA or FEP Teflon bottle that is easier to clean than PTFE due to a surface condition that is less favorable to adsorption.

The dosages of HgGD and DMHg are carried out on unfiltered samples, those of HgR and dissolved HgT after filtration of samples on hydrophilised LCR type Teflon filters with 0.5 µm porosity and with a 45

mm diameter (Millipore). The filtration device and the method are similar to those described for other trace elements (Chiffolleau et al., 2003). Samples are kept out of the light and in the cold immediately after sampling.

The dosing of HgGD, DMHg, and HgR must be carried out within a few hours after sampling at natural pH. Samples for dosages of MMHg are acidified to pH 2 by the addition of HCl (Suprapur, Merck type) and kept in the dark at +4°C until analysis that must take place as quickly as possible. Samples for the dosage of HgT are acidified with HCl (type Suprapur, Merck) to a pH lower than 2 (0.5 %, v/v). Out of the light and in the cold these samples can be kept for several weeks on the condition of hermetically screwing on the lids of the containers in order to avoid gaseous exchanges. This operation is carried out using multigrip pliers made from plastic. The Teflon containers are kept in a double wrapping of polyethylene bags.

3.2. CLEANING OF BOTTLES AND OTHER EQUIPMENT

Any sampling equipment used on the site or in the laboratory is packaged, handled, and stored in a manner to avoid any contamination. Washing in single purpose polyethylene trays must be conducted in a clean environment. Handling requires the systematic usage of polyethylene gloves. Dust proof storage is carried out in a double wrapped polyethylene bag.

The following procedures must be observed for cleaning new materials:

1. Washing with detergent or ethylated alcohol or methanol.
2. Rinsing with tap water and then with de-ionised Milli-Q (Millipore) type water.
3. Submerging in a tray containing 50 %(v/v) ACS type HNO₃ for five days.
4. Rinsing with tap water then de-ionised water.
5. Submerging in a tray containing 10 % (v/v) type ACS type HCl for five days.
6. Rinsing with de-ionised water.
7. If they are flasks, they are filled with de-ionised water and concentrated purified HCl of the Seastar or Suprapur (Merck) type is added to obtain a solution at 0.5 % (v/v).
8. Closing the flasks using multigrip pliers and placing them in two polyethylene bags.

Flasks can be kept as they are until use. The flasks designated to receive the samples for the dosage of HgT must receive an addition of HCl in phase 5, 0.1 % (v/v) of the BrCl solution. The other materials in plastic are dried and kept in a double wrapping of polyethylene bags. Stages 2, 3, and 4 may be disregarded for used material

3.3. REAGENTS

Solutions are prepared and kept in 125 mL Teflon FEP flasks that have been washed according to the technique previously described. Handling is carried out under a laminar airflow hood, class 100 (US standard). To avoid any contamination by ambient air during storage, lids are screwed on with multigrip pliers and the flasks protected by two polyethylene bags. In the case of the BrCl solution, the stages are carried out under a chemical suction hood paying close attention to the very toxic bromine vapours that can form.

3.3.1. *BrCl solution*

1.1 g of KBrO_3 then 1.5 g of KBr are dissolved into 20 mL of de-ionised water. 80 mL of concentrated and purified HCl of the type Seastat type is added. This solution can be stored at $+4^\circ\text{C}$ for several days. KBrO_3 and KBr crystals are previously purified in the oven at 250°C for at least 12 hours.

3.3.2. *NH_2OH , HCl solution*

30 g of hydroxylamine chlorhydrate are dissolved into 70 mL of de-ionised water. The solution is purged by nitrogen in the mercury for several hours after the addition of 100 μL of the SnCl_2 solution. Storage cannot exceed 2 weeks at $+4^\circ\text{C}$.

3.3.3. *SnCl_2 solution*

20 g of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ is dissolved into 12.5 mL of concentrated and purified Seastar type HCl . The Steam bath is warmed until dissolution without exceeding 60°C . After cooling, it is filled up to 100 mL with Milli-Q water. The solution is purified by bubbling nitrogen for several hours. The solution thus prepared can be kept approximately 15 days at $+4^\circ\text{C}$.

3.3.4. *NaBH_4 solution*

1 g of NaBH_4 is dissolved into 100 mL of Milli-Q water. The solution must be renewed every 4 hours. Before use ultrapure Argon without mercury is bubbled for 10 minutes. The solution is kept in an ice bath sheltered from the light during use.

3.4. INORGANIC MERCURY STANDARD SOLUTION

The dilutions are carried out in gauged Teflon vials PFA (Savillex) of 100 mL in size. The solutions are stored in 125 mL Teflon FEP flasks at $+4^\circ\text{C}$ and sheltered from light.

3.4.1. *Dilution solution*

All solutions of inorganic mercury are prepared using a dilution solution that allows the stabilisation of mercury. This solution, which is made up of the zero of the calibration range, contains 10 mL of

concentrated HNO₃ (Seastar or Suprapur, Merck type) and 2 mL of K₂Cr₂O₇ 10 % (p/v) and a quantity of de-ionised water (Milli-Q, Milliporetype) sufficient for 1 litre.

3.4.2. Stock solution

We dilute a certified solution to 10 g L⁻¹ (type NIST-3133) to a tenth with the dilution solution to obtain a working solution at 1 g L⁻¹. This solution can be kept for one year.

3.4.3. Work solution

Using the main solution, a work solution at 1 ng L⁻¹ is prepared to a hundredth by three successive dilutions. This solution can be kept for three weeks.

3.5. MMHg STANDARD SOLUTION

Dilutions are carried out in gauged 100 mL vials made from Teflon PFA (Savillex). The solutions are stored in 125 mL Teflon FEP flasks at +4°C sheltered from light.

3.5.1. Stock solution

0.1252 g methylmercury chloride are dissolved in isopropanol in a 100 mL gauged Teflon PFA (Savillex) vial. This solution can be kept for three months.

3.5.2. Work solution

The solution at 1 g L⁻¹ is diluted in an aqueous solution of HCl at 0.5 % in order to obtain a solution at 0.2 ng mL⁻¹. This solution can be kept for several weeks. In order to evaluate the variation of the concentration in MMHg of this solution, it is recommended that the total mercury and inorganic mercury concentration (“reactive” mercury) be measured and the difference (MMHg = HgT-HgR) between these two dosages will test the conservation of the solution.

3.6. DETECTORS AND TRAPS

The atomic fluorescence detectors that have been used successfully come from three manufacturers: (1) the Tekran 2500 model, (2) the Merlin PSA analytical model and the (3) Spectra-France MLD-500 model. The other detectors available on the market have not been tested.

The purification of gases and the pre-concentration of mercury are carried out on golden sand traps (Figure 3.1) prior to measurement. The gas-phase mercury, present in the gas, is amalgamated on a thin film of gold that covers the grains of sand in a quartz tube. The sand used is a sand known as “Fontainebleau” sand previously purified by being washed in nitric acid, rinsed with de-ionised water, and calcinated at 600°C. The sand is then covered with a thin layer of gold using a plater or a cathode evaporator of the type used in electronic microscopy (e.g.: Hammer II, Technics).

The trapping of acid vapours is carried out on soda lime cartridges. This trap is conditioned by heating to 120°C. It also allows the trapping of a section of the water vapour present in the vector gas.

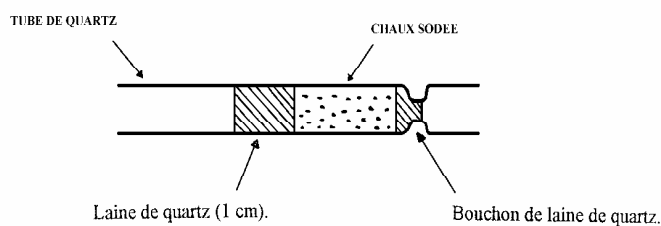


Figure 3.1. Gold trap

3.7. DOSAGE OF GASEOUS, REACTIVE AND TOTAL DISSOLVED MERCURY

3.7.1. Principle

The dissolved gaseous mercury (HgGD) includes elemental mercury (Hg^0) and dissolved methylmercury ($\text{Hg}(\text{CH}_3)_2^0$). The latter molecule is unstable at pH less than 7.5 and under light, however, it can still be traced in significant quantities under oceanic thermocline (Mason and Fitzgerald, 1990; Cossa et al., 1997). 'Reactive' mercury (HgR) essentially includes the mineral chemical species and possible labile organic complexes at the pH of the reduction, i.e., around 2. The total dissolved mercury (HgT) is made up of all of the chemical species that can be oxidised by BrCl , which includes inorganic and organic species, in particular alkylated compounds, including monomethylmercury. All these chemical species must be reduced to volatile elemental mercury (Hg^0), pre-concentrated by amalgamation on the golden sand trap (Fig. 3.1) then analysed by AFS.

The analytical chain and device are described in Figure 3.3. To measure HgGD, the sample of seawater is first bubbled through purified argon in an FEP Teflon recipient. The gaseous mercury is recovered on a golden sand trap, which is then heated to 550°C to break down the amalgamation and free the mercury vapour in an AFS. Bloom and Crecelius (1983) initially described this method. The measuring of HgR is based on the same principle. However, it is necessary to reduce the Hg^{II} to Hg^0 via SnCl_2 solution before bubbling with argon. For HgT dosing, the transformation is added to easily reducible Hg^{II} for all the organic forms; in particular alkylated compounds. The break in the Hg-C or Hg-S link is carried out by the BrCl action and a hydroxylamine hydrochloride solution is then used to neutralise the excess BrCl , before proceeding with the reduction to SnCl_2 .

3.7.2. Analytical system

The analysis must be carried out under a Class 100 laminar airflow hood fitted with an active carbon filter. The analytical system is described in Figure 3. This includes a line made up of a Teflon tube (PFA) which the argon successively passes through:

- A vector gas in a purification device including a golden sand trap and a Teflon filter (Vacu-Gard L3749, Whatman)
- A flowmeter (the Aalborg or Platon type)
- A four-way solenoid valve (Nresearch type) that enables the inclusion or exclusion of the degassing cell (Teflon PFA Teflon PFA type wash flask) in the argon circuit
- A soda lime trap (Figure 2)
- A golden trap (Figure 1), fitted with a Ni-Cr heating strip (1.5Ω at 24 volts, the frequency being programmable on the control box when it is turned on)
- A three-way solenoid valve (Nresearch type) enabling the orientation of the argon flow either to the AFS detector, or to free air
- An atomic fluorescence detector and a data acquisition system on a PC (or a recorder).

The part of the line between the four-way valve and the golden trap can be kept permanently at 65°C by a heated wire in order to avoid the possible condensation of the mercury vapour on the tubes, the septum or the soda lime trap. The solenoid valves and the heating are controlled by an automatic control box (the CREA-Automatism type).

3.8. ANALYTICAL PROCEDURE

3.8.1. HgGD

As a rule, a sufficient volume (300 to 600 mL) of a non-filtered sample of seawater is introduced into the degassing cell. Upon exiting the cylinder at a pressure of 1.8 bar and after purification through a golden sand trap and a Teflon filter (PTFE), the argon passes through a flowmeter fitted with an adjustment valve, the sample in the degassing cell, then the acid vapour trap (soda lime trap, Fig. 2) and the amalgamation trap (golden sand trap, Fig. 1). The sample is purged of its volatile mercury under an argon stream of $250 \text{ mL}/\text{min}^{-1}$ depending on the expected level of concentration of HgGD. The golden trap is then heated to 550°C for 1 min and the fluorescence signal recorded under an argon stream of 150 mL min^{-1} . Two minutes are allowed between each dosing to enable the cooling of the golden trap. The concentration of elemental mercury (Hg^0) is calculated from the difference between HgGD and DMHg.

3.8.2. *HgR*

In the degassing cell, 0.5 mL of the SnCl₂ solution is added to 50 or 100 mL of the sample. The argon is bubbled with a flow of 250 mL/min⁻¹ for 6 to 12 minutes (the degassing cell for the measurement of HgR and HgT is statistically different from that of HgGD, so that there is no trace of stannous chloride that could reduce the Hg^{II} during the measurement of HgGD). The preconcentration trap is heated to 550°C for 1 min under an argon stream of 150 mL / min⁻¹.

3.8.3. *HgT*

0.2 mL of the BrCl solution is added to 100 mL of seawater filtrate in a 125 mL Teflon flask (PFA). It is necessary to then check that colouration continues during the reaction time of one hour and the quantity of BrCl to be added must be adjusted if colouration disappears. After one hour, it is necessary to add 0.5 mL of the hydroxylamine solution required for the disappearance of yellow colour showing the neutralisation of the excess BrCl. 50 to 100 mL of the sample thus processed is poured into the degassing flask. 0.5 mL of the SnCl₂ solution is then added and the previous procedure is followed.

3.8.4. *Whites*

The white is defined as the quantity of mercury contained in the reagents (as well as a permanent background noise that must be minimised as per the procedure described in section 5.4.4); it must not go beyond 10 pg. The value of the white is obtained after an analytical cycle carried out immediately after the passing of a sample. As a result, the already degassed sample receives 0.5 mL of the SnCl₂ solution, in the case of dosage of HgR, and 0.2 mL of BrCl, 0.5 mL of SnCl₂ and NH₂OH, HCl in the case of dosage of HgT. For HgGD only argon bubbling is suitable.

3.8.5. *Calibration*

This is carried out from the height (or the area) of the peak in comparison to the height (or the area) of a standard peak for approximately 200 pg of mercury. The standard can be either an aqueous solution of 1 ng L⁻¹ used according to the normal procedure or gaseous mercury. In the latter case, we inject 0.01 to 0.02 mL of mercury-saturated air, through the septum (Figure 3.2). Knowing the temperature makes it possible to calculate the quantity of mercury ejected. For example, 0.01 mL of saturated vapour at 20°C contains 132 pg of elemental mercury.

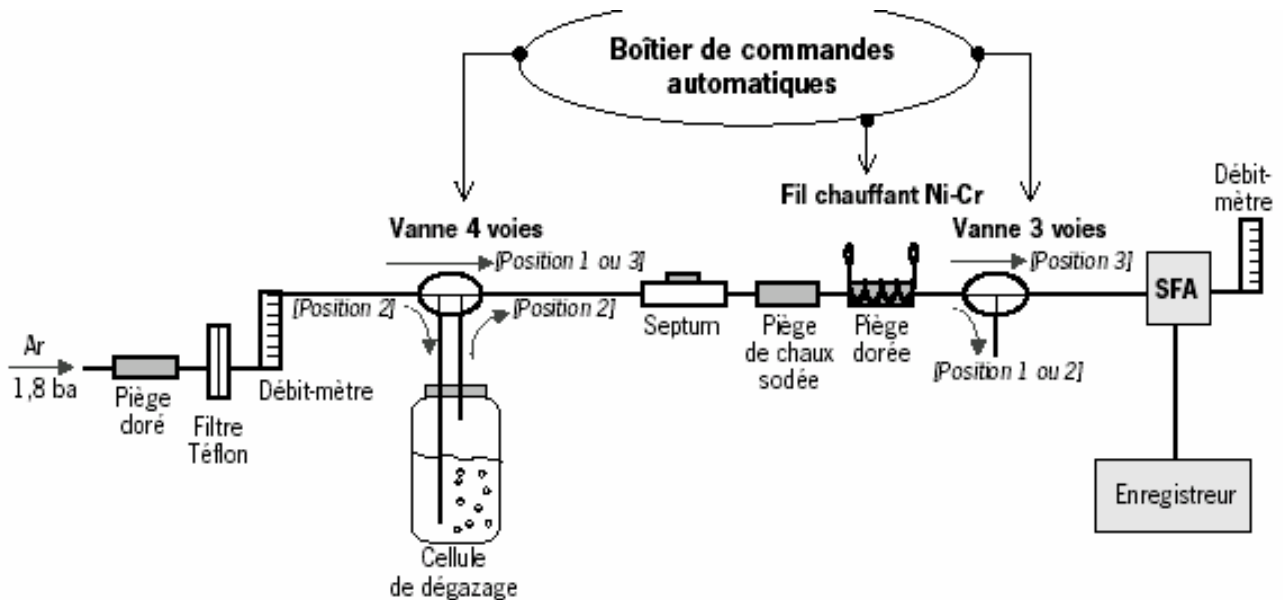


Figure 3.2. Diagram of analytical assembly of dosage of gaseous, "reactive", and total dissolved mercury in seawater

3.8.6. Analytical sequences

The semi-automatic apparatus used at Ifremer is piloted by a control box (Figure 3) specifically developed by the CREA-Automatisme company (crea.automatisme@wanadoo.fr). Before proceeding with analysis, it is recommended that the detector be stabilised for approximately an hour beforehand.

- Phase 1: golden trap sweeping (duration 5 sec)

Depending on the chemical species to be measured, a suitable volume of a sample is introduced into the degassing cell. The argon circulates for a few seconds in the circuit of the three-way and four-way valves in Position 1 (see Figure 3.3).

- Phase 2 : *Degassing* (duration 6 to 60 min)

The four-way valve moves to allow the bubbling of argon in the cell (Position 2 as per Figure 3). As this involves dosages of HgR or HgT, 0.5 mL of the SnCl₂ solution will have been introduced beforehand. Depending on the volume of the sample bubbling will be programmed to last from 6 mins (for 50 mL) to 60 mins (for 600 mL).

- *Phase 3: golden trap sweeping* (duration 10 sec)

During this sequence, the golden trap is dried before heating, the four-way valve returning to Position 1.

- *Phase 4: Measurement* (duration 1 min)

At this stage the golden trap is heated and mercury vapour liberated from its amalgamation on the gold. The three-way valve moves to Position 3 (Figure 3.3), enabling the argon stream to reach the fluorescence detector; the trap is then heated by the Ni-Cr resistor to 550°C. The transmission peak corresponds to the passing of Hg⁰ vapour and takes place approximately 30 seconds after the beginning of heating and drops to the baseline after one minute.

- *Phase 5: Cooling the trap* (duration 2 min)

The trap cools down with the valves in Position 1 for two minutes. At the end of the phase, the system is ready for its next analysis.

3.9. QUALITY CONTROL

Performance evaluation criteria for the method have been borrowed from Taylor (1987).

3.9.1. Accuracy

Due to the absence of reference marine water for the determination of dissolved mercury, the accuracy of the method has not been established with any certainty. Participation in intercomparison exercises indicates a level of reliability. During the last exercise organised with the universities of Connecticut (Groton, USA) and Maryland (Solomon, USA) as well as with Frontier Geosciences (Seattle, USA) and the Centre Saint-Laurent (Montréal, Canada), the consensus value was $1.0 \pm 0.08 \text{ ng L}^{-1}$ (Quémerais et al., 1998) and the average of measurements was $0.8 \pm 0.1 \text{ ng L}^{-1}$.

3.9.2. Precision

The repeatability of the method expressed by the variation coefficient (defined as the ratio between standard deviation and the average) varies with average concentrations. It varies by 5 % for a concentration of around 1 ng L^{-1} to 15 % for values of around 0.1 ng L^{-1} . These values have been obtained for test samples of 50 mL and six replications.

3.9.3. Detection limit

The detection limit is defined as 3.29 times the standard deviation of whites; the multiplication factor of 3.29 takes into account errors of Types I and II (Taylor, 1987). The detection limit calculated on a daily basis varies from 0.02 to 0.06 ng L^{-1} for a sample of 100 mL.

3.9.4. Analytical rhythm

The analytical sequence allows approximately 6 dosages per hour. However, due to the low concentrations encountered (more often than not less than 1 ng L^{-1}) particular care must be given in order to obtain the lowest whites possible. This requires many consecutive analytical cycles on the same "white". This process progressively "cleans" the analytical line. Given the requirements for frequent calibration (approximately every five samples), the specific conditions for the dosage of trace elements make it difficult, to carry out more than 25 analyses per day.

3.9.5. Domain of validity

Due to the possible adjustment of the sample analysed, the range of measurable concentrations above the measurable detection limit is wide. In the case of the determination of total dissolved mercury, for waters that are highly concentrated in dissolved organic matter it may be necessary to increase the quantity of BrCl solution (the maintenance of the yellow colour is a criterion that shows a sufficient quantity of BrCl) to be added to the sample. Furthermore, a reaction time for the BrCl longer than one hour may be necessary in certain cases.

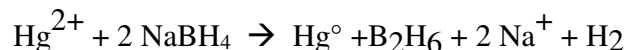
3.10. DOSAGE OF DISSOLVED METHYLMERCURY

3.10.1. Principle

The dosage of dimethylmercury, a volatile species, consists simply of bubbling the sample through a helium stream and trapping the volatile species on a chromatographic column in liquid nitrogen. The column is then heated, and the volatile compounds (Hg and DMHg) that are freed quantified by AFS (e.g., Bloom, 1989). Different dosage methods for monomethylmercury (MMHg) in waters have been investigated by Horvat (1996). Those where the sensitivity and the obtaining of sufficiently low whites enables an application to dosage of MMHg in marine waters use alkyl compounds or hydrides (e.g., Bloom, 1989; Tseng et al., 1998). In the first group the methods are based on ethylation, used for the determination of MMHg in biological matrices and sediments (e.g. Cossa et al., 2002), presenting the inconvenience of interference with chlorides and therefore the need for prior extraction. For this reason, the preference was for the method via hydriding which is here described. This allows very low detection limits ($< 5 \text{ pg L}^{-1}$) to be achieved, but is only applicable to seawater with very low concentrations of dissolved organic substances. In the case of waters rich in organic substances we adapted the ethylation method after extraction by organic solvent, as described in a previous pamphlet (Cossa et al., 2002).

The volatile hydrides of metals are known to be formed from the addition of sodium tetrahydroboride (NaBH_4). This volatility is used as a pre-concentration and separation technique.

The reactions applied are the following:



In 1998, Tseng et al. proposed a protocol enabling a quantitative recuperation of the formed hydrides . The dosage method for methylmercury proposed is made up of different stages: (1) the formation of volatile hydrides with NaBH₄, (2) their preconcentration by cryogenics (-196°C), (3) their separation by gas chromatography, and (4) their detection by atomic absorption spectrometry (AAS), or atomic fluorescence (AFS) or then again by mass spectrometry after ionisation by argon plasma (ICPMS). We have chosen AFS for its sensitivity and its low cost. Thus hydrides formed are concentrated and then separated by cryogenic chromatography before being atomised (800°C) and drawn in vapour form by a helium stream to a detector.

The modifications to the method of Tseng et al. are minimal, but have enabled an improvement in the detection threshold by a factor of 20. This concerns the reduction and stabilisation of the white by the use of a minimal quantity of reagents and a very sensitive AFS detector (absolute detection from 0.1 to 0.3 pg of Hg).

The conceptual diagram for dosage and the analytical device are respectively described in Figures 3.3 and 3.4.

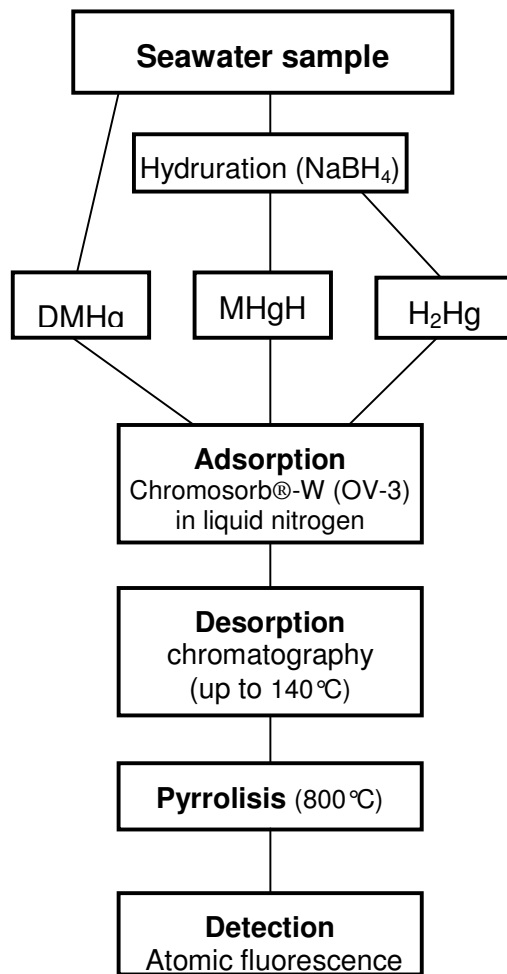


Figure 3.3. Analytical diagram for the determination of methylmercury in seawater

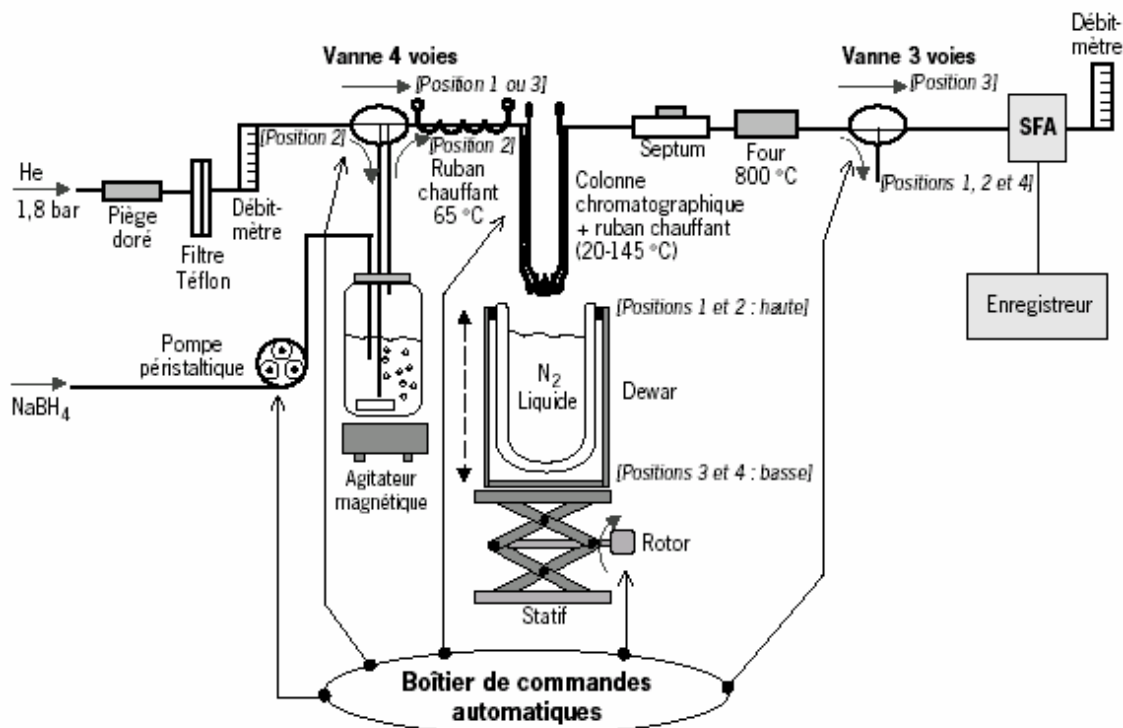


Figure 3.4. Diagram showing analytical assembly of determination of methylmercury in seawater.

3.10.2. Analytical system

The analytical system is described in Figure 3.4. It comprises a helium line made up of a Teflon tube (PFA) that passes through the following:

- A gas vector purification device comprising a golden sand trap and a Teflon filter (Vacu-Gard L3749, Whatman),
- A flowmeter (the Aalborg or Platon type)
- A four-way solenoid valve that allows the orientation of the argon either to a 250 mL borosilicated glass hydriding cell, decontaminated at 450°C, then silanised (5% DMDCS in the toluene), or directly to the chromatographic column made up of a U-shaped silanised borosilicated glass tube ($\varnothing_{\text{ext.}}$ 6 mm and $\varnothing_{\text{int.}}$ 4 mm) and filled with Chromosorb WAW-DMCS (60/80 mesh impregnated with 15% OV-3); 20 Ω a resistor in Ni-Cr, supplied with 24 volts, wrapped around the column enabling it to reach 145°C (the number turns on can be programmed on the control box)
- A septum holder

- A pyrolysis oven at a temperature of 800°C comprising a quartz wool in a quartz tube 10 cm long and with an interior diameter of 0.8 cm
- A three-way solenoid valve cutting off the AFS
- An atomic fluorescence detector and a data acquisition system on a PC (or a recorder)
- A flowmeter at the output of the AFS enabling the setting of helium flow at 35 and 30 mL⁻¹ for respective temperatures of the chromatographic column of 30°C and 145°C

A peristaltic pump (the Ismatec type) allowing the introduction of the sodium borohydride solution. An automatic control box controls the valves, the peristaltic pump, the retort stand engine and the heating of the resistor.

All the tubings are Teflon (PFA) as well as the attachments (PTFE). The connections between the different parts of the circuit must be as short as possible to avoid dead areas. The connection between the output of the column and the input of the last oven is enveloped by a heated resistor at 65°C in order to avoid condensation of elemental mercury on "cold spots" (Figure 5).

3.10.3. Analytical procedure

The aqueous sample is placed in the hydriding cell (Figure 3.5). On leaving the cylinder, the helium functions as a vector gas under 1.8 bars and is purified thanks to a golden sand trap that amalgamates the traces of Hg present in the gas and the Teflon filter. The four-way valve allows the helium stream to either cross directly to the chromatographic column submerged in liquid nitrogen, or to bubble through the sample (flow 1000 mL / min⁻¹) where the NaBH₄ solution is introduced with the help of a peristaltic pump with a flow of 0.35 mL / min⁻¹, for 5 minutes. Hydriding rate is identical to natural pH or to pH 2. The pH choice depends on the type of conservation of the sample. If analysis can be carried out shortly after sampling, it is preferable to work at natural pH. The hydrides formed are drawn by the helium and are trapped on the column in a liquid nitrogen bath. After having purged the circuit with a stream of helium to chase away the dihydrides that may have formed during the addition of NaBH₄, the column is removed from a nitrogen bath and slowly reheated until it reaches 145°C. The hydrides come out of the column at temperatures lower than 30°C, the water vapour is ejected at a much higher temperature; a three-way valve evacuates the helium stream to fresh air so that the water vapour does not reach the fluorescence detector. The helium flow during chromatography goes from 35 mL / min⁻¹ when the column is cold to 30 mL / min⁻¹ when the temperature reaches 140°C.

The identification of peaks is carried out according to their retention time. In the conditions described above, the respective retention times of inorganic mercury and monomethylmercury are 1 min 50 s and 3 min after the beginning of heating of the column (Figure 6).

A calibration curve is drawn every day from additions of MMHg in 200 mL of Milli-Q water. We can verify the linearity of the curve up to 500 pg L^{-1} , as well as the identity of the respective response factors of freshwater and seawater. The estimation of hydriding yield can be carried out by comparing the area of the peak obtained by the addition of a known quantity of MMHg in solution compared to the area of the peak obtained by injection of an equivalent quantity of gaseous mercury (Hg^0) at the septum (Figure 3.5).

The whites must be checked regularly and they are often negligible. The white determined from the dosage of MMHg in 200 mL of Milli-Q water has values of around one picogram of MMHg per litre. These traces can be partly from the Milli-Q water, in part from the NaBH_4 solution (we determine this proportion by doubling the quantity of reagent used). During calculation of concentrations the white part due to Milli-Q water is not deducted from measurements.

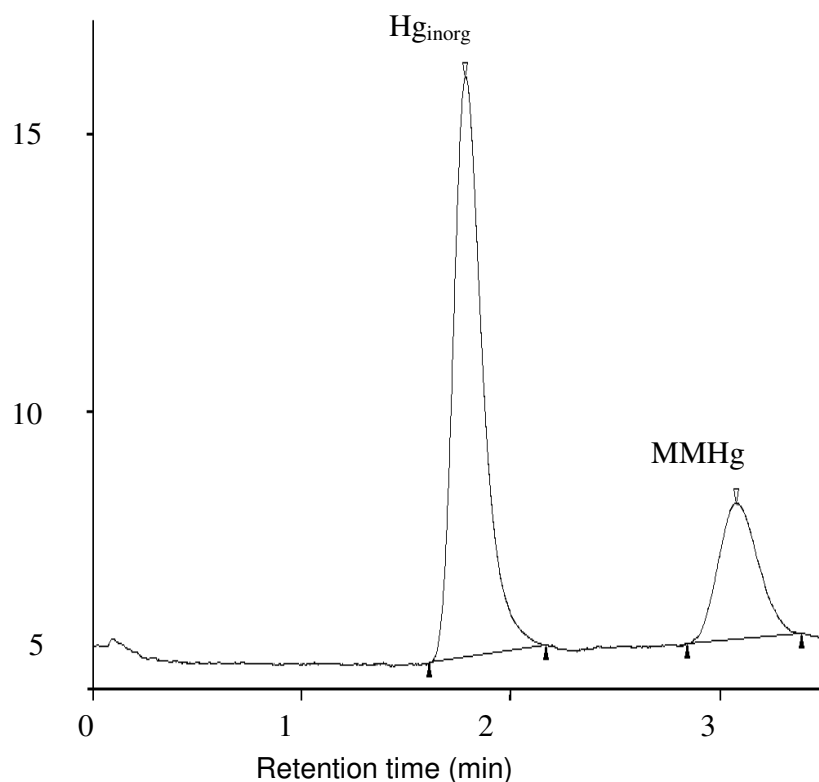


Figure 3.5. Typical chromatogram for a 20 pg quantity of MMHg.

3.10.4. Analytical sequences

The helium intake is permanently kept open. The semi-automatic apparatus used by Ifremer is controlled by a control box (Figure 5) developed specifically by the CREA-Automatisme company (crea.automatisme@wanadoo.fr). Before proceeding with analysis, it is recommended that the detector be stabilised for approximately one hour.

Phase 1: Cooling of the column (duration 1 min)

The column is submerged in liquid nitrogen. The four-way valve directs the helium directly to the column (Position 1, Figure 5) at $1000 \text{ mL} / \text{min}^{-1}$ whilst the effluents are emitted into the atmosphere through the three-way valve on oven output. During this time, 200 mL of sample are introduced to the hydriding cell and the circuit is then opened.

Phase 2: Formation of hydrides (duration 5 min)

The NaBH_4 solution at 1% is introduced into the sample with a flow of $0.35 \text{ mL} \text{ min}^{-1}$ for 5 min under magnetic agitation. At the same time, the helium purges the sample, due to switching of the four-way valve (Position 2, Figure 5) thus drawing the hydrides formed towards the column upon whose head they are trapped.

Phase 3: Complement to the extraction of hydrides of the sample (duration 2 min)

During this sequence we finish the purging of hydrides under a helium stream without adding NaBH_4 , with the valves remaining in Position 2 (Figure 5),

Phase 4: Elution and acquisition of data (duration 3 min 30 s)

This is the elution phase for mercury compounds. The Dewar vase support drops causing the exit of the column from the liquid nitrogen. Simultaneously, the vector gas is directed directly towards the chromatographic column due to the four-way valve (Position 3, Figure 5), shunting the hydriding cell, and downstream, due to the three-way valve, towards the AFS detector (Position 3, Figure 5). The helium flow goes to $35 \text{ mL} / \text{min}^{-1}$ thanks to the flow meters situated on the AFS output. After 2 minutes at ambient temperature the column is heated progressively to 30°C . The acquisition of data takes place during this phase.

Phase 5: Drying of the column (duration 6 min)

During de-gassing, the helium has drawn the water vapour that was trapped on the column. We eliminate this water by increasing the heating of the column to its maximum (approximately 145°C), and we evacuate the vapour formed into the atmosphere (three-way valve in Position 4, figure 5). At the end of the phase, the system is ready to carry out a new analysis.

3.10.5. *Quality control of dosages*

The performance evaluation criteria of the method have been borrowed from Taylor (1987).

Accuracy. The accuracy of the method has not been established due to the absence of a certified seawater reference for MMHg.

Precision. The repeatability of the method expressed by the variation coefficient (defined as the ratio between standard deviation and the average for six analyses of the same sample) varies from 15 % for a sample of a concentration close to the detection limit to 6 % for a sample of 100 pg L⁻¹ or more.

Detection limit. The detection limit is defined as 3.3 times the standard deviation of the concentration of a sample whose concentration is close to zero (in practice this refers to whites); the multiplication factor of 3.3 takes into account errors of Types I and II (Taylor, 1987). The detection limit calculated on a daily basis varies from 1 to 4 pg L⁻¹.

Analytical rhythm. Taking into account white and calibration, we can analyse approximately 15 samples every day.

Domain of validity. The linearity of the response has been verified from the detection limit at 500 pg L⁻¹ for seawater with a low concentration of dissolved organic matter (i.e., less than around 2 mg L⁻¹). In the case of water rich in organic matter we must proceed with the prior extraction of MMHg at pH 2 by a volume of methylene chloride equivalent to that of the aqueous sample. We proceed with a return extraction via evaporation of the solvent in the presence of 50 mL Milli-Q water. From this extracted aqueous phase we proceed with the dosing of MMHg via ethylation as per the procedure described in a previous sheet (Cossa et al., 2002). Due to the extraction of phase the variability of whites can be high and the detection limit is only 10 pg L⁻¹ in the best case.

4. DETERMINATION OF LABILE CADMIUM IN SEA WATER

The total dissolved metal concentration is not a pertinent information to assess the potential effect of their presence in the marine biota. The biological reactivity of metals is more linked to the total inorganic metal concentration (Morel et al., 1991, Morel et Hering, 1993, Bruland, 1989, Coale and Bruland, 1988 Gerringa et al., 1995,).

Lipophilic metal complexes also cross the cell wall by passive diffusion (Phinney & Bruland 1994).

A.S.V. (anodic stripping voltammetry) is well suited to estimate the “free hydrated” and “inorganically complexed” forms of Cd, which correspond to the so called “labile fraction” of the metal. Inorganically

complexed Cd is deposited on the mercury coated glassy carbon electrode at $-0.9V$ vs Ag/AgCl . Labile organic complexes are very scarce in sea water (Pizeta and Branica 1997) and may often be neglected.

Cadmium complexed by strong organic ligands is generally not detected by ASV in natural samples and needs a strong mineralization process (e.g.U.V. irradiation) to be detected.

4.1. PRINCIPLE OF DETERMINATION

The first step of the ASV analysis is an electrolysis of the raw (unacidified) filtered ($.45\mu m$) water on a mercury electrode (in this case, mercury coated glassy carbon electrode) at a potential of $-0.9V$. During this step free hydrated Cd^{2+} ion is reduced and amalgamated in mercury. Inorganic complexes are dissociated, and the resulting cadmium is reduced and amalgamated. These species constitute the "labile" fraction of Cd.

The second step of the analysis consists of a scan of potentials from the depositing potential towards more positive potentials (from $-0.9V$ to $-0.2V$).

During this scan amalgamated Cd is reoxidized at a potential close to $-0.7 V$. In the differential pulse mode this gives a peak the height of which is proportional to the concentration of Cd in the amalgam, and finally to the concentration of labile Cd in the seawater.

When spiking the sample with Cd^{++} ($Cd Cl_2$) ,if some unsaturated strong ligand is present in the sample, part of the spike may be complexed by this ligand, causing the increase of the height of the peak to be less than proportional to the spike. Upon spiking, this situation will last until all the ligand is saturated by the added cadmium. Then, new spikes will no longer be complexed and will be wholly detected by the ASV process. So the height of the Cd peak H will increase proportionally to the concentration of cadmium added in: $dH = P * dC$, where C is the concentration of Cd added in the cell. The graph of the Curve H vs C becomes then a straight line. The slope of this line, $p = dH/dC$, is called "sensitivity". dC is the differential increase of the Labile Cd concentration ion and dH the corresponding increase in Cd peak height.

From this the initial labile Cadmium concentration C_0 can be calculated using $C_0 = H_0/P$.

4.2. PROCEDURE

Preparation of the titration samples. 20 ml aliquots of the sample under study are put in fifteen 30 ml Teflon vials. Cd spiking solution ($25\mu g/l$) is added in each vial so as to reach the wanted cadmium

added concentrations for titrations (Typically between 20 and 500 ng/l) These vials are left to equilibrate overnight. Each vial content will be called a spiked titration sample.

Preparation of the mercury coated glassy carbon electrode. The glassy carbon electrode must be finely polished with alumina powder (0.05 μ) slurry .

Mercury film formation. A mercury plating solution is made with 20ml milliQ water 30 μ l Hg⁺⁺ solution (5000ppm) and 100 μ l ultrapur saturated KCl solution . The pH is set to 2 with suprapur HCl. This solution is put into a Teflon polarographic cell and adjusted on the polarographic stand. An ASV cycle is performed to coat the glassy carbon and test the cleanliness of the operating conditions

Deoxygenation. A gentle flow of N₂ is allowed to bubble through the cell for 10 min. No potential is applied to the working electrode.

Depositing: W.E.potential -0.9V vs Ag/AgCl. N₂ is forced through the sample during this stage. W.E. rotation speed:3000rpm

Rest. After deposit the sample is allowed to rest for 30sec. The W.E. potential is maintained at -0.9V, rotation speed 0rpm

Scan. Rotation speed 0rpm. Working electrode potential is scanned from - 0.9 to - 0.2 V (Vs Ag/AgCl) at a rate of 20 mV/sec.

20 mV potential pulses are superimposed to the potential increase ramp: pulse frequency 600Hz, pulse height:20mV. In satisfactory analytical conditions, no significant Cd peak should be observed.

During these two last stages, N₂ bubbling is stopped and replaced by N₂ blanketing of the sample in the cell.

4.3. ANALYSIS

If no significant peak has been detected during the precedent stage, a complete ASV cycle is run. The “zero spike titration sample” is put in the polarographic cell.

Deoxygenation is conducted in the same way as for mercury film formation.

A conditioning stage precedes the deposit . A potential of -0.15 V vs Ag/AgCl is applied to the working electrode. During this stage, N₂ is forced through the sample and the electrode rotation speed is fixed at 3000rpm. This is to strip residual metals from the mercury film

Depositing and scan are completed in the same way as for mercury film formation.

After the analytical cycle is finished, and the peak height H₀ has been recorded, the next spike titration sample is put in the cell, and so on , with bigger and bigger spikes. This ends when four consecutive

spikes give a linear trace on the graph H (peak height) vs added Cd concentration.. Then the slope P of this linear part is measured and the initial concentration C_0 is calculated : $C_0=H_0/P$.

The difference between “total “ Cd obtained by ICP MS or by ASV after UV irradiation and “labile” Cd obtained by this method is considered as the “organically complexed” Cd.

5. ANALYSIS OF DISSOLVED CADMIUM

The Method is described in Danielsson et al. (1982). First, cadmium is complexed using a mixture of ammonium pyrrolidine dithiocarbamate (APDC) and diethylammonium diethyldithiocarbamate (DDDC). The hydrophobic complex is extracted with freon. Addition of nitric acid to the organic phase causes cadmium to transfer to the aqueous phase. Cadmium concentration is measured in this phase by flameless atomic absorption spectrometry.

5.1. CLEANING OF THE SAMPLING DEVICE

Polyethylene gloves are used when handling samples. Ten percent HCl (analytical grade) is circulated through the whole pumping system for several days, then the system is thoroughly rinsed with deionised water. This equipment is stored wet in a thick plastic bag (tear-resistant).

Filter holders are immersed in 10% HCl for a week, then rinsed with deionised water, dried in a incubator (50°C), and packed in 2 individually closed polyethylene bags.

Polycarbonate filtration membranes are perfectly suited for metal analysis. They are immersed in 10% HCl for 2 weeks, then thoroughly rinsed (until pH is neutral), dried, weighed, and then individually stored in clean petri dishes that are put in a plastic bag. Prior to their use, crystal polystyrene petri dishes are cleaned by immersion in a 10% HCl solution at 40°C for 3 days and rinsed with deionised water.

Distinction of dissolved and particulate material is operational and carried out by in-line filtration of raw water.

5.2. FIELD OPERATIONS

An inflatable boat will be used to collect samples. The engine must be switched off while samples are being collected.

Special attention will be given to the boat's angle to the wind. We will make sure that the wind blows eventual dust emanating from the boat or personnel away from the sampling device (output of the filter

holder and mouth of the bottle). These important parts will be protected by a polyethylene bag. The drift path of the boat will also be taken into account: water from areas where the boat has just passed must not be sampled. This is even more true for surface samples. The drift path is not such an important issue when samples are collected at greater depths (> 2 m). Samples are collected using a pump (ASTI[®] Teflon pump, polyethylene tubing). In-line filtration is performed using a nuclepore[®] polycarbonate membrane filter (47 mm in diameter, 0.4- μ m pore size). Filtration is continued until the membrane is saturated, however, pressure must never exceed 1.5 atm. The total volume of water that has been filtrated is recorded. When the suspended particulate matter (SPM) content is calculated, we ensure that the volume of the sample that will be analyzed, which is not recorded when samples are collected, is taken into account..

Back in the field laboratory, filtered water is acidified under a class 100 laminar flow hood (HCl 1.5 %). Filter membranes loaded with particulate matter are kept frozen in plastic boxes (millipore[®]) until analysis by atomic absorption spectrometry.

5.3. MATERIALS AND REAGENTS

- polypropylene volumetric flasks are used to prepare the chelating solution and buffer.
- 500-ml Teflon separatory funnels for extraction
- 100-ml Teflon separatory funnels for back extraction
- polypropylene vials to recover the aqueous extract
- purified freon in a quartz dispenser
- high-grade, i.e., suprapur[®], nitric acid, acetic acid, and ammonia (Merck)

Preliminary precautions:

All materials are thoroughly rinsed with either deionised water (volumetric flasks, separatory funnels for the extraction solution, small separatory funnels to recover the acid phase after extraction, vials used to recover the acid phase), purified freon (quartz dispenser containing the freon used for the extraction), or the sample (large separatory funnels used for the extraction).

5.4. PREPARATION OF REAGENTS

Chelating solution

Pour 22.5 ml of ammonia and 11.8 ml of acetic acid in a 100-ml volumetric flask “A”, adjust volume to 100 ml with deionised water.

Weigh out 0.5 g of APDC and 0.5 g of DDDC in a volumetric flask “B” and adjust volume to 100 ml

with deionised water.

Pour the contents of flasks A and B in a 500-ml separatory funnel and adjust pH to 5.5 with one or the other component of the buffer. Pour 20 ml of freon in the funnel and stir for 3 minutes. Let stand for 5 minutes then drain off the freon by opening the stopcock at the bottom of the funnel (until the interface between the two phases is no longer visible). Wash again with freon twice, then add 50 ml of purified freon. This freon will not be eliminated until all extractions are done. The separatory funnel will be shaken for 3 minutes and left to settle for 5 minutes before each series of extractions of the chelation solution.

Freon

Pour 400 ml of freon and 5 ml of ultra-pure nitric acid in a 500-ml separatory funnel. Stir for 3 minutes, let stand for 10 minutes, rinse the quartz dispenser with a few tens of millilitres of purified freon then collect the remaining freon into the quartz dispenser.

5.5. EXTRACTION PROCEDURE

A series of extractions is performed with consecutive subseries of six 500-ml separatory funnels (SF500). Each subseries includes a blank. Each series includes three certified reference standards, whose matrix composition and metal content are presumably close to that of samples to analyze.

Weigh out exactly 100 to 500 g of sample in each SF500 but for the blank. In each of the 6 SF500, add 3 ml of chelating solution. Stir for a few seconds, then collect 10 μ l of the sample at the surface, using a micropipette, to control pH. If needed, adjust pH to 4.0-5.5. Add 20 ml of freon to each SF500. Degas with caution in order not to waste solution, then stir for 3 minutes and let stand for 5 minutes. Collect freon in 100-ml separatory funnels (SF100) and leave the last microliters in the SF500.

Add 10 ml of freon in each SF500. Stir for 1 minute, then let stand for 5 minutes. Collect freon in the same SF100 and leave, once again, the last few microlitres in the SF500. In each SF100, add 1 ml of nitric acid and stir for 3 minutes. Let stand for 10 minutes then add 4 ml of deionised water. Stir for a few seconds, then let stand for 5 minutes. Eliminate the organic phase as well as the interface between the two phases (until drops that drain out of the SF100 change in size). Collect the aqueous phase in 15-ml tubes.

N.B. it is sometimes advisable to highly concentrate the sample. Concentration may be increased by a factor of 2 by adding only 0.5 ml of nitric acid and 2 ml of deionised water to the organic phase.

Extraction of blanks + Production of metal-free deionised water

Pour approximately 100 ml of deionised water in a SF500. Include this separatory funnel in the first analytical subseries, then do the extraction the same way as you did for samples. The extract in the freon phase contains all of the metals present in the deionised water and reagents. This extract is not used for the remainder of the analysis.

Generation of the “reagent blank”

After this first extraction, the content of the SF500, i.e., deionised water without trace metals, is combined to the second analytical subseries. The final extract from this funnel only contains metals from reagents. This will be the first « reagent blank ». Water which has undergone two extractions is kept to determine reagent blanks for further analytical subseries.

5.6. ATOMIC ABSORPTION SPECTROMETRY ANALYSIS

Cadmium content is analyzed by electrothermal atomic absorption spectrometry with Zeeman background correction. Although not indispensable, the use of a matrix modifier helps getting a better definition of absorption peaks and counteract the accidental presence of salt in the final extract. This matrix modifier includes the following components:

High-grade 85% orthophosphoric acid,	1.21 g
High-grade 25% ammonia,	1.07 g
High-grade magnesium nitrate: $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$,	86.4 mg
Freshly deionized water	QS to 50 ml

Graphite tubes placed in the furnace can be equipped with a L'vov platform.

5.7. ASSESSMENT OF THE METHOD

5.7.1. Sensitivity

The sensitivity of a method reflects its ability to detect and/or measure with precision small amounts of elements. It is expressed by 2 measures: the *detection limit*, which is the smallest concentration that can be detected with certainty, and the *quantification limit*, i.e., the smallest measurable concentration. The detection limit corresponds to 3 times the standard deviation, σ , of the method blank, as the latter integrates the whole procedure, i.e., from extraction to final analysis, while the quantification limit corresponds to 10 times this standard deviation. Between the years 1990 and 2000, approximately 500 blank measurements were performed and allowed precise identification of the value of these blanks and

their variability, both within a series of analyses and between series. The value of the Cd blanks for final extracts as well as their standard deviation are :Blank 0.03 $\mu\text{g}/\text{kg}$ σ ($\mu\text{g}/\text{kg}$) 0.023

With this method, detection and quantification limits are a function of the concentration factor:

$$\text{det. lim.} = 3 \times \sigma \times V / P \quad \text{and} \quad \text{quant. lim.} = 10 \times \sigma \times V / P$$

They can be modified if this factor is changed. Thus, with a **concentration factor of 20** (test portion of 100 g and final volume of 5 ml), the detection limit is 0.003 $\mu\text{g}/\text{kg}$ and the quantification limit is 0.011 $\mu\text{g}/\text{kg}$. These limits are divided by 10 if the concentration factor increases to 200.

5.7.2. Reproducibility

The reproducibility of a method is its ability to always provide the same result for the same sample. It is expressed by the coefficient of variation of the concentration (expressed in %), and equal to the ratio of the standard deviation of the series of concentrations to the mean concentration. The **accuracy** of a method is its ability to provide the true sample concentration. The use of certified reference samples whose analyte content is accurately known allows these parameters to be evaluated.

The method used to determine cadmium content is very reproducible (coefficients of variation of measurements range from 8% to 12%) for all matrices studied. This is notably due to the fact that the values of extraction blanks are low and that the determination limits are much lower than the cadmium content of the certified reference standards.

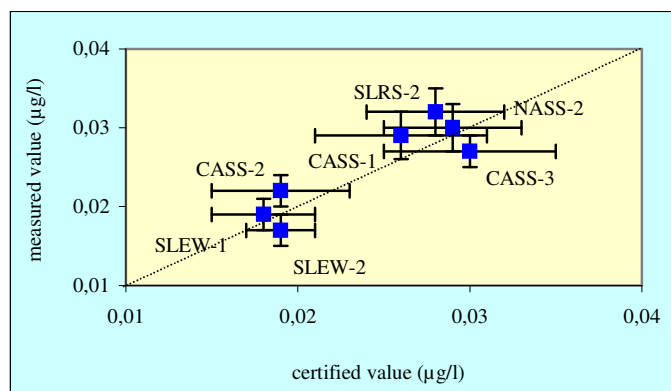


Figure 5.1. Measured and certified (true) cadmium content of standard water samples from the National Research Council of Canada (NRCC).

This method is very precise for cadmium as the mean concentration measured for each type of standard systematically falls within the certified range of values for cadmium content (fig. 5.1).

5.7.3. *Validity range*

Results obtained with this method suggest that it can be used with natural waters (freshwaters, estuarine and marine waters) more or less rich in organic matter. However, it has not been tested on solutions rich in strong complexing agents which could compete with the chelators used during the extraction phase (APDC and DDDC).

The extraction yield is independent of the amount of starting sample (test portion) within the 20-500 ml range. It is, therefore, possible to adapt this test portion to the expected metal content of the sample. Direct analysis of samples by electrothermal atomic absorption spectrometry after dilution is recommended for samples that are highly concentrated in trace metals to avoid the undesirable effects of salt. In this case, the prior concentration step is unnecessary.

This method is therefore valid for all types of natural waters, contaminated or not.

5.8. **QUALITY ASSURANCE CRITERIA**

Quality assurance requires a competent and organized personnel, adapted rooms and equipment, suitable management of equipment and reagents, a good metrology system, a well-defined analytical protocol. In order to control result quality, it is necessary to use reference materials, to regularly participate in interlaboratory comparisons, and monitor a few indicators of stability (blank values, slope of calibration curves, results obtained with standards) on a daily basis.

5.8.1. *Certified reference materials*

Certified reference materials are natural water samples collected under ultraclean conditions—to preserve their characteristics — that are filtered and stabilized in an acid medium and kept in high-quality packaging. Long-term stability tests are performed to guarantee their use for many years, and an inter-sample variability study is carried out to ensure a good statistical distribution and homogeneity of reference standard samples. Certification examinations are generally realized by several laboratories acknowledged as experts in this field, using at least two independent methods.

Certified reference materials of different types (freshwater, estuarine and coastal water, oceanic water), are available on the market. It is, therefore, always possible to find a standard that matches the type of sample to under study. It is preferable to use reference standard samples of the same type and with an analyte concentration of the same order of magnitude as that of samples to be analyzed.

In a series of analyses, it is necessary to have at least 2 certified standards of the same type and comparable test portions.

5.8.2. *Interlaboratory comparisons*

An important aspect of quality assurance, which complements the systematic use of certified standards, is a regular participation in interlaboratory comparisons. Such studies are used to test analytical methods without presumption, in order to report objective results.

Many organizations perform national or international comparisons on a regular basis (or not) in the field of metals dissolved in water. These studies, however, concern different types of laboratories (industrial or academic research) and include different types of samples.

As far as the analysis of trace metals in the marine environment is concerned, however, the Conseil International pour l'Exploration de la Mer (CIEM) has conducted seven interlaboratory comparisons since the end of the seventies, the last of which was performed in 1996 (Berman *et al.*, 2000). Since that date, the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring) program, coordinated by the Marine Laboratory Aberdeen (Scotland), includes the conduct of comparison studies on a regular basis.

5.8.3. *Quality charts*

Monitoring stability indicators is essential to a quality assurance protocol. It allows real-time measurement of the performance of the method (sensitivity, reproducibility, accuracy, and identification of anomalies or drifts). These indicators include the slope of the calibration curve, values of the blanks and certified standards.

6. ANALYSIS OF CADMIUM IN SUSPENDED PARTICULATE MATTER

The analysis must be performed on dry samples. Each Petri dish containing a filter loaded with particulate matter is placed in a clean incubator with its lid half open. The filters are dried at 70°C for 12 hours. Petri dishes are then closed, allowed to cool down in a dessicator, and the dry loaded filter is weighed.

Teflon bombs used for mineralization are cleaned with nitric acid (immersed in 30% HNO₃ for 1 week, rinsed with demineralised water, immersed in 10% HNO₃ for 1 week, rinsed with demineralised water again, dried in a clean incubator, bagged in two polyethylene bags). Immediately before use, bombs are rinsed with milli-Q® water.

Consumables (pipette tips autosampler micro-vials) and polypropylene collecting vials are all analytical grade and are not cleaned in the laboratory. They are stored dry in closed plastic bags and rinsed 3 times with the solution that they will contain before use.

6.1. MINERALIZATION

It may technically be difficult to collect large amounts of particulate matter on a filter. For example, in slightly turbid waters with SPM concentration below 0.1 mg/l, filtration of 20 l will only provide 2 mg of particulate matter on the filter. For this type of sample, we must adjust the reagent quantity and the final dilution volume in order not to fall below the analytical quantification limit (3.1.1).

6.1.1. Mode of operation

A) Samples:

In each 30-ml Teflon bomb, place the filters loaded with particulate matter as close as possible to the bottom. Add the following, according to the mass of particulate matter on the filter (m) :

	$m \leq 5 \text{ mg}$	$5 \text{ mg} \leq m \leq 25 \text{ mg}$	$25 \text{ mg} \leq m$
Nitric acid	25 μl	50 μl	250 μl
Hydrochloric acid	75 μl	150 μl	750 μl
Hydrofluoric acid	600 μl	1.2 ml	6 ml

Shake well, then heat at 130°C for 2h30, while bombs are closed. Allow to cool down at room temperature.

In 50-ml polypropylene vials, add the following, according to the mass of the test portion:

	$m \leq 5 \text{ mg}$	$5 \text{ mg} \leq m \leq 25 \text{ mg}$	$25 \text{ mg} \leq m$
Boric acid	270 mg	540 mg	2.7 g
Deionized water	2 ml	4 ml	20 ml

Shake, then pour the content of the cooled bombs into the corresponding tubes. Rinse bombs and collect rinse water into the polypropylene vials, while being careful not to exceed the following final dilution volume:

	$m \leq 5 \text{ mg}$	$5 \text{ mg} \leq m \leq 25 \text{ mg}$	$25 \text{ mg} \leq m$
Final volume	5 ml	10 ml	50 ml

Shake until solid matter has dissolved. Adjust final volume as indicated above with deionised water.

B) Analysis blanks, certified standards:

In each series of analyses, 3 blanks will be prepared by pouring in 3 Teflon bombs the same amount and quality of acids and deionised water as in the related samples. These samples will undergo the same mineralization protocol as other samples.

Moreover, quality assurance requires that reference standards with a certified analyte concentration be used (see below). Each series of analyses will therefore include at least 2 certified standard samples whose type and an analyte content are close to that of samples to be analyzed. The test portion for these standard samples will be of the same order of magnitude as that used for the other samples, and the mineralization protocol will be identical.

6.2. INSTRUMENTAL ANALYSIS

Analyses are systematically done by electrothermal atomic absorption spectrometry with a Zeeman background correction system.

The use of a matrix modifier helps improve definition of absorption peaks and prevents signal saturation due to non-specific absorption. This matrix modifier includes the following components :

85% orthophosphoric acid	1.21 g
25% ammonia	1.07 g
magnesium nitrate: $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$	86.4 mg
deionized water	QS to 50 ml

Graphite tubes placed in the furnace can be equipped with a L'vov platform. This apparatus ensures a more homogenous distribution of temperature during atomization. It is well suited for relatively volatile elements such as cadmium, as atomization of the latter is not prevented or decreased by the temperature of the platform, which is lower than that of the tube that holds it.

6.3. PERFORMANCE OF THE METHOD

6.3.1. Sensitivity

It is expressed by two measures: the *detection limit*, which is the smallest concentration that can be detected with certainty, and the *quantification limit*, i.e., the smallest measurable concentration.

The detection limit corresponds to 3 times the standard deviation, σ , of the method blank, as the latter integrates the whole procedure, while the quantification limit corresponds to 10 times this standard deviation. Between the years 1980 and 2000, up to 800 blank measurements were performed by the IFREMER laboratory which measures the concentration of metals in sediments.

Detection and quantification limits of an element with a method are a function of the parameters of this method (test portion, final volume) :

$$\text{det. lim.} = 3 \times \sigma \times V / P \quad \text{and} \quad \text{quant. lim.} = 10 \times \sigma \times V / P$$

These limits can be high when the amount of collected matter on the filter is low, hence the necessity to filter as much water as possible to have a suitable detection limit.

Table Detection	m = 200 mg ; FV = 50 ml		(m = 2 mg ; FV = 5 ml)	
	det. lim.	quant. lim.	det. lim.	quant. lim.
Cd (µg/g)	0.01	0.04	0.1	0.4

quantification limits for Cd (m: test portion, FV: final volume)

This method is sensitive enough to allow precise measurement of metal contaminant content of marine sediment samples. When the load of particulate matter is very low, however, it is difficult to quantify cadmium, which is present in low concentration. In such a rare case, it is advisable to concentrate the final solution in a volume of 2 ml.

6.3.2. Precision (reproducibility and accuracy)

The reproducibility of a method is its ability to always provide the same result for the same sample. It is expressed by the coefficient of variation of the concentration (expressed in %), and equal to the ratio of the standard deviation of the series of concentrations to the mean concentration. The **accuracy** of a method is its ability to provide the true sample concentration. In this study, accuracy was evaluated by the analysis of certified reference standards, namely, BCSS-1, MESS-1, MESS-2, and MESS-3, which were purchased from the National Research Council of Canada (Ottawa, ON Canada).

Certified values presented in the next part of this section correspond to means of average concentrations reported by acknowledged laboratories (each of them used a different method) \pm

standard deviation of the distribution of means. **Measured values** reported therein, however, correspond to mean concentrations measured by the Department of chemical pollutants at IFREMER (using the same method) \pm standard deviation of the distribution of mean concentrations.

The method used to determine metal content proved very reproducible for cadmium (coefficients of variation ranged between 3% and 7%) for all standards used.

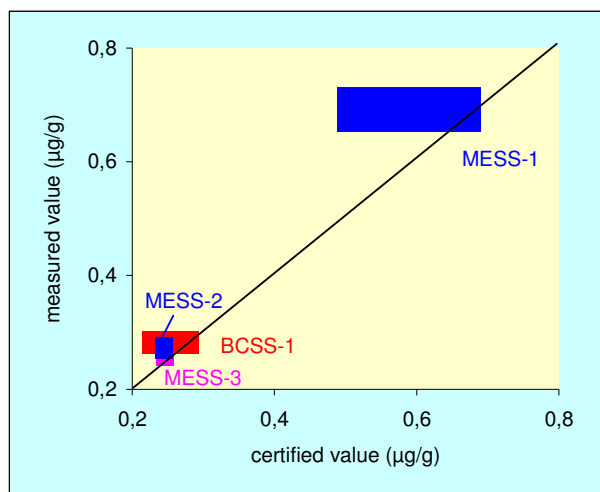


Figure 6.1. Measured and certified cadmium content of standard water samples from the National Research Council of Canada (Ottawa, ON, Canada).

This method is very precise for cadmium, although the cadmium content measured for each type of standard systematically falls at the maximum end of the certified range of values for cadmium content (fig.6.1.).

6.3.3. *Validity range*

Results obtained with this method suggest that it is suitable for a wide range of cadmium concentrations. Various international interlaboratory comparisons demonstrated the validity of this method when used with clayey as well as sandy sediments (CIEM, QUASIMEME, AIEA). Moreover, the test portion can range from a few milligrams to a few hundred milligrams, provided that the amount of each reagent used is adjusted. It is, as a matter of fact, always possible to dilute the acid leachate whose concentration in a particular element is too high. This means that this method is suitable for the analysis of samples that are either slightly to highly contaminated with metals, and more generally, all types of marine sediments and particles.

On the other hand, it is more difficult to measure low amounts of Cd, e.g. in suspended particulate matter. Where it can be found in low concentrations, of the same magnitude as the quantification limit of the method.

6.3.4. Analytical rate

A series of analyses includes 25 samples, at least 2 blanks, and at least 2 certified reference standards. It takes half a day to weigh and solubilize such a series of samples.

The analysis of a metal element by flameless atomic absorption spectrometry for a series of extractions requires approximately 4 hours.

6.4. QUALITY ASSURANCE CRITERIA

Quality assurance requires a competent and organized personnel, adapted rooms and equipment, suitable management of equipment and reagents, a good metrology system, a well-defined analytical protocol. In order to control result quality, it is necessary to use reference materials, to regularly participate in interlaboratory comparisons, and monitor a few indicators of stability (blank values, slope of calibration curves, results obtained with standards) on a daily basis.

6.4.1. Certified reference materials

Certified reference materials are natural water samples collected under ultraclean conditions to preserve their characteristics. They are lyophilized, crushed to powder, homogenized, distributed in a series of identical high-quality vials. Long-term stability tests are performed to guarantee their use for many years, and an inter-sample variability study is carried out to ensure the good homogeneity of the batch and, thereby, ensure that the different vials of standard samples are comparable. Certification examinations are realized by several laboratories acknowledged as experts in this field, using at least two independent methods.

Different types of certified reference materials (sandy or clayey sediments, sediments of harbours) are available on the market. It is, therefore, always possible to find a standard that matches the type of sample to analyze. It is preferable to use reference standard samples of the same type and with an analyte concentration of the same order of magnitude as that of samples that are being analyzed.

In a series of analyses, it is necessary to have at least 2 certified standards of the same type and comparable test portions.

6.4.2. Interlaboratory comparisons

An important aspect of quality assurance, which complements the systematic use of certified standards, is a regular participation in interlaboratory comparisons. Such studies are used to test analytical methods without presumption, in order to report objective results.

Many organizations perform national or international comparisons on a regular basis (or not) in the field of metals dissolved in water. These studies, however, concern different types of laboratories (industrial or academic research) and include different types of samples.

As far as the analysis of trace metals in the marine environment is concerned, however, the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring) program, coordinated by the Marine Laboratory Aberdeen (Scotland), includes the conduct of comparison studies on a regular basis.

6.4.3. *Quality charts*

Monitoring stability indicators is essential to a quality assurance protocol. It allows real-time measurement of the performance of the method (sensitivity, reproducibility, accuracy, and identification of anomalies or drifts). These indicators include the slope of the calibration curve, values of the blanks and certified standards.

6.5. APPLICATIONS OF THIS METHOD

This method is successfully used at IFERMER as part of the RNO sediment program (Boutier and Claisse, 1995) and has been included in a number of research programs for over 15 years. This method was used to show the distribution of certain metals in the suspended particulate matter of the main French estuaries (Boutier *et al.*, 1993 ; Kraepiel *et al.*, 1997 ; Chiffoleau *et al.*, 2001) and in the marine water column (Pempkowiak *et al.*, 2000).

For example, Chiffoleau *et al.* (2001) studied cadmium distribution in the Seine estuary. By studying the suspended particulate matter, they identified the anthropogenic inputs in the Rouen area (France) and the natural dissolution process of cadmium in brackish water, as well as the mixing of river and marine particles in the downstream estuary (figure 6.2.).

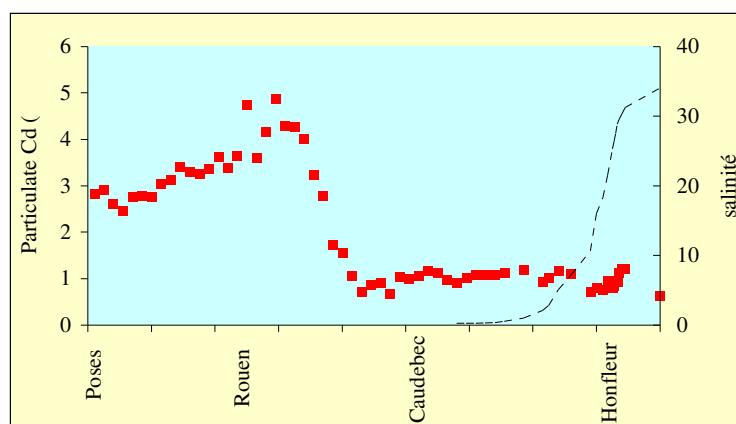


Figure 6.2. Distribution of cadmium in suspended particulate matter of the Seine estuary in June 1996 (Chiffoleau *et al.*, 2001). Dashed line: salinity.

7. CONCLUSIONS

The objective of this deliverable is the description and assessment of analytical methodologies that are being used to analyze the three families of organic compounds (PCBs, PAHs, PBDEs), and the two metals: mercury and cadmium in several environmental matrices. These are the compounds selected in the Thresholds project to study (see Milestone 4.2.1).

In all cases, priority has been given to determine low concentrations of these compounds in complex matrices as well as to the quality control analysis and how to proceed with validation exercises in view of the needed to provide highly accurate results.

The analytical protocols developed or adapted within Thresholds will be used throughout the project experimental campaigns to provide highly accurate data to be used to determine the concentration of relevant contaminants in the marine environment. This data will then be used in the development of models and techniques to compare and assess the existence of thresholds in coastal and transitional aquatic ecosystems.

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